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Medical Genetics Summaries

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Medical Genetics Summaries is a growing collection of summaries which describe the impact that specific sequence variations have on health. The summaries review genetic variants that underlie inherited conditions, affect the risk of developing a disease in the future, or influence how an individual may respond to a specific drug.

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Table of Contents

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Introduction

Laura Dean, MD[1]

Created: September 15, 2016.

Medical Genetics Summaries (MGS) is a collection of articles that feature conditions with a genetic component, for which information useful at the point of care is limited. Topics fall into two broad categories: diseases and drug responses.

The intended audience of *MGS* is clinicians who seek practical, evidence-based information to use in clinical care settings. The summaries are guideline-driven, drawn from authoritative sources, undergo a formal review process, and are regularly updated.

Genetic variants and disease

Pitt-Hopkins syndrome has a clear genetic component. A variant in the TCF4 gene results in the syndrome, and genetic testing of the *TCF4* gene confirms the diagnosis. However, for many other diseases, the underlying genetics is complex. For example, although schizophrenia is highly heritable, many genes have been implicated as contributing to the disease, and genetic testing is not currently available.

A person's blood group is determined by genetics—the four common blood groups (A, B, AB, and O) are encoded by *ABO* alleles. Serological testing is commonly used to determine an individual's blood type, e.g., before receiving a blood transfusion. However, in other settings, genetic testing may be used to determine an individual's ABO genotype, such as in the research setting, e.g., investigating the associations between ABO blood groups and the risk of diseases such as pancreatic cancer and thromboembolic disease.

Genetic variants and drug responses

There is often a wide variability in how different individuals respond to standard doses of the same drug. This is because a drug response can be influenced by age, gender, drug-drug interactions, drug-food interactions, comorbidity, liver and renal function, pregnancy, and genetic factors. For an increasing number of drugs, genetic testing (also known as pharmacogenetic testing) can be used to optimize drug therapy.

Currently, about 10% of drug labels approved by the U.S. Food and Drug Administration (FDA) contain pharmacogenetic information. However, actionable information on genetic variants can be hard to find, and sources often differ in their recommendations. *MGS* draws together information from different authoritative sources to one place, and includes a summary—thus providing accessible information at the point of care.

To avoid confusion, only generic drug names are used. Nomenclature tables include both the official and commonly used terms for alleles, and phenotypes are termed “drug responses”, e.g., omeprazole drug response. Finally, each summary links to the NIH’s Genetic Testing Registry, which provides information about laboratories that offer genetic tests and details about the tests, including ordering information.

Genetic testing to ensure the drug has a therapeutic target

A small number of drugs are prescribed after genetic testing has been performed. One reason for this is that the drug is effective for specific genotypes. These drugs include trastuzumab—a chemotherapy agent only indicated for specific tumors that overexpress HER2, and maraviroc—an antiviral agent that is only indicated for a specific strain of the HIV virus (CCR-5 trophic HIV-1).

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Genetic testing can help avoid idiosyncratic drug reactions

Another reason for genetic testing is to avoid severe, and potentially fatal, drug reactions. A category of drug reactions are idiosyncratic—they are unpredictable, severe, and not related to the dose and duration of the drug therapy.

The FDA recommends that all individuals be screened for the *HLA-B*57:01* allele before starting treatment with abacavir, a drug used in the treatment of HIV. This is because around 6% of Caucasians of European origin carry this variant allele, placing them at high risk of abacavir-induced hypersensitivity reaction. Symptoms include fever, rash, and acute respiratory symptoms.

An individual's ancestry may be important

For the epilepsy drug carbamazepine, the FDA states that patients with ancestry in "genetically at-risk populations" should be screened for the presence of *HLA-B*15:02* prior to initiating treatment. Carriers of this variant, which is most commonly found in individuals of Han Chinese descent, are at a high risk of developing Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN)—both potentially fatal conditions—during carbamazepine therapy.

Also common in individuals with Han Chinese ancestry is the *HLA-B*58:01* allele, which is strongly associated with severe cutaneous adverse reactions (SCAR) triggered by allopurinol therapy, which is used to treat gout.

A wide range of gene variants are associated with idiosyncratic drug reactions

Idiosyncratic drug reactions are not just limited to variant *HLA-B* alleles. For the antibiotic gentamicin, genetically predisposed individuals who carry a variant in a mitochondrial gene (*MT-RNR1*) may suffer from irreversible hearing loss after just a single dose of gentamicin. And for individuals who require treatment with thiopurines (e.g., azathioprine), the FDA recommends *TPMT* genotyping or phenotyping prior to treatment. This is because patients who carry two non-functional *TPMT* alleles universally experience life-threatening myelosuppression when treated with thiopurines.

Genetic testing can help optimize the drug dose

Drug labels always provide standard dosing information. But a growing number of labels also include recommendations for adjusting the dose, or selecting an alternative drug, based on a patient's genotype (if known). Generally, dose adjustment is recommended for variants in genes that are known to influence drug metabolism, leading to altered plasma levels of active drugs and metabolites.

Cytochrome P450 (CYP) genes influence drug levels

The "CYP" gene family encodes enzymes that metabolize over a quarter of commonly prescribed drugs. One of these genes, *CYP2D6*, is particularly complex. Over 100 variants are known, many of which encode enzymes with different levels of activity. Depending on the level of CYP2D6 activity, individuals may respond poorly to the analgesics codeine and tramadol. A standard dose of codeine may provide inadequate pain relief in some, and severe toxicity, such as respiratory depression, in others.

In addition, standard doses of a wide range of drugs (e.g., atomoxetine—used in ADHD, venlafaxine—an antidepressant, clozapine—an antipsychotic, and tamoxifen—used to treat breast cancer) will lead to higher than expected active drug plasma levels in individuals who have low or absent CYP2D6 activity. This can increase the risk of side effects, and may contribute to non-compliance and treatment failure.

Barriers to genetic testing

Ordering a genetic test to help determine whether a particular drug will be effective or safe is a relatively new area for doctors and genetic counselors. The field is rapidly evolving, evidenced by an increasing panorama of

genetic tests becoming available. And there are potential legal concerns, such as a cause for liability in cases where the optimal dose of a drug was not given. Education and training are needed.

More prospective randomized trials are needed to investigate the clinical outcomes when drug therapy or a specific dose is selected on the basis of genotype. The effectiveness data can be used for cost-effectiveness analysis, and be summarized into actionable clinical guidelines with prescribing recommendations.

Sometimes, genetic testing has not been possible because of the acute nature of the clinical scenario (e.g., gentamicin and neonatal sepsis). However, as technology improves and turn-around time is reduced, the use of genetic testing can be expected to increase.

For example, clopidogrel is an antiplatelet agent that is used in patients presenting with acute coronary syndrome, and patients who may need to undergo percutaneous intervention. Because clopidogrel is a pro-drug, it must first be metabolized by CYP2C19 before it becomes effective. However, in the 3% of Caucasians and 15 to 20% of Asians who have low or absent CYP2C19 activity, clopidogrel will have a smaller or no effect on platelet function. Fortunately, the advent of "bedside testing" and a faster turn-around of results means that more of these patients can be identified and offered alternative antiplatelet agents.

The use of genetic testing is often not clear-cut

In the case of warfarin, the FDA-approved drug label provides a dosing table, allowing for the adjustment of initial doses of warfarin based on *CYP2C9* and *VKORC1* genotypes. Warfarin is an anticoagulant, given to prevent the formation of blood clots. If the dose of warfarin is too low, the risk of thrombosis remains, but if the dose is too high, there is an increased risk of bleeding. And both outcomes can be a cause of a stroke.

Despite the drug label's dosing table, it is thought that less than 1% of patients commence warfarin therapy with their *CYP2C9* and *VKORC1* genotypes known. Interestingly, however, the most recent evidence suggests that *CYP2C9* and *VKORC1* variants may have less of an effect on warfarin levels than previously thought, with many other clinical factors having more of an impact.

The future

Genetic testing is important—it can help avoid drug toxicity and help optimize drug efficacy. As the number of genetic tests grows, *Medical Genetics Summaries* will expand to help ensure that healthcare providers have the information they need to provide evidence-based care.

Genetic variants and drug responses

Abacavir Therapy and *HLA-B*57:01* Genotype

Laura Dean, MD[1]

Created: September 1, 2015; Updated: April 18, 2018.

Introduction

Abacavir (brand name Ziagen) is used in the treatment of human immunodeficiency virus (HIV) infection. Abacavir is a nucleoside (and nucleotide) reverse transcriptase inhibitor (NRTI), and is used in combination with other medications as part of highly active antiretroviral therapy (HAART) (1).

Hypersensitivity reactions associated with abacavir can be severe and potentially fatal. Symptoms include fever, rash, vomiting, and shortness of breath. They typically appear within the first 42 days of treatment (11 days median onset).

*HLA-B*57:01* significantly increases the risk of hypersensitivity reactions when abacavir is administered. Approximately 6% of Caucasians and 2-3% of African Americans carry this allele in the human leukocyte antigen B (*HLA-B*) gene. The *HLA-B* gene plays an important role in how the immune system recognizes and responds to pathogens, and mediates hypersensitivity reactions. *HLA-B*57:01* has been found to be associated with abacavir hypersensitivity across different ethnicities, including Caucasians, Hispanics, and individuals of African origin (2, 3).

Screening for the *HLA-B*57:01* allele before starting abacavir therapy is recommended for all patients according to the FDA drug label for abacavir (Table 1). Even if previously tolerated, screening should happen before restarting abacavir therapy if *HLA-B*57:01* status is unknown. Abacavir is contraindicated in *HLA-B*5701*-positive patients, and in patients with a prior hypersensitivity reaction to abacavir. Dosing guidelines from the professional societies, Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP), also recommend that *HLA-B*57:01* screening should be performed prior to initiation of abacavir therapy and an alternate drug be administered for patients with the allele (Table 2, Table 3)(1, 3-5).

Table 1. FDA (2017) Drug Label for Abacavir. Therapeutic Recommendations based on *HLA-B*57:01* Genotype. Warnings and Precautions.

Genotype	Hypersensitivity reactions
*HLA-B*5701*-positive patients	Due to the potential for severe, serious, and possibly fatal hypersensitivity reactions with abacavir sulfate: All patients should be screened for the *HLA-B*5701* allele prior to initiating therapy with abacavir tablets or reinitiation of therapy with abacavir tablets, unless patients have a previously documented *HLA-B*5701* allele assessment. Abacavir tablets are contraindicated in patients with a prior hypersensitivity reaction to abacavir and in *HLA-B*5701*-positive patients.

Please see 2017 Statement from the US Food and Drug Administration (FDA) for more information from the FDA. Table adapted from (1).

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Table 2. CPIC (2014) Recommended Therapeutic Use of Abacavir in relation to *HLA-B* Genotype[a]

Genotype	Implications for phenotypic measures	Recommendations for abacavir	Classification of recommendations[b]
"Negative" Noncarrier of *HLA-B*57:01*	Low or reduced risk of abacavir hypersensitivity	Use abacavir per standard dosing guidelines	Strong
"Positive" Carrier of *HLA-B*57:01*	Significantly increased risk of abacavir hypersensitivity	Abacavir is not recommended	Strong

HLA-B, *human leukocyte antigen B.*
[a] The 2014 update states that the recommendations shown in the table from the 2012 guideline remain the same. This table has been adapted from the 2012 guideline (3, 4).
[b] Rating scheme described in supplementary data online (3, 4).
Please see 2014 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC) for more information from CPIC. Table adapted from (3).

Table 3. DPWG (2017) Recommendations for Abacavir based on *HLA-B* Genotype

Genotype	Recommendation
*HLA-B*57:01*-positive	Abacavir is contraindicated for *HLA-B*57:01*-positive patients. Advise the prescriber to prescribe an alternative according to the current guidelines.

Please see 2017 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP) for more information from DPWG. Table adapted from (5).

Drug: Abacavir

Abacavir is an antiretroviral drug that belongs to the drug class of nucleoside (and nucleotide) reverse transcriptase inhibitors (NRTIs). The NRTIs, also known as nucleoside (or nucleotide) analogs, were the first type of drug available to treat HIV infection, and they remain effective today. In addition to abacavir, NRTIs include drugs such as AZT/zidovudine, emtricitabine, tenofovir, and lamivudine. Abacavir is always used in combination with other drugs.

Antiretroviral drugs, like abacavir, inhibit the activity of retroviruses, such as HIV. To replicate, retroviruses must convert their RNA genome into a DNA copy, which can then be inserted into the host cell's genome. Abacavir inhibits the conversion of viral RNA to DNA, preventing viral replication.

Abacavir is a pro-drug and its antiviral activity is facilitated by the drug's phosphorylation by intracellular enzymes to form carbovir triphosphate, a nucleoside analog. Carbovir triphosphate competes with the natural substrate of the HIV reverse transcriptase enzyme, to be incorporated into viral DNA. Once incorporated, the nucleoside analog terminates DNA chain elongation, preventing further synthesis of viral DNA (6).

Abacavir started to be used in the late 1990s, as part of a combination of therapies to treat HIV. However, the use of abacavir in the US was limited by a severe hypersensitivity reaction that occurred in approximately 5-8% of patients. Symptoms occurred during the first 6 weeks and included a constellation of symptoms presenting as rash, fever, fatigue, gastrointestinal symptoms (e.g., nausea, vomiting, abdominal pain), and acute respiratory symptoms (e.g., cough and dyspnea) (7). Life-threatening skin diseases, Stevens-Johnson syndrome and toxic-epidermal necrolysis, can occur in severe reactions.

Data from the PREDICT-1 study suggest that 100% of individuals with immunologically confirmed (abacavir patch test positive) abacavir hypersensitivity present within 3 weeks of initial dosing. The median onset of symptoms is 9-11 days (1, 7, 8).

Abacavir can trigger a hypersensitivity reaction in people who have the *HLA-B*57:01* allele. The frequency of the *HLA-B*57:01* allele varies by population; for example, approximately 6% of Caucasians, and 2-3% of African-American and admixed American populations carry at least one copy of this high-risk *HLA-B* allele (Table 4). *HLA-B*57:01*-postitive individuals have an increased risk of a hypersensitivity reaction to abacavir compared to *HLA-B*57:01*-negative individuals (8).

Table 4. CPIC (2014) Assignment of likely *HLA-B* Phenotypes based on Genotype

Likely phenotype	Genotype	Examples of diplotype
Very low risk of hypersensitivity (constitutes ~94%[a] of patients)	Absence of **57:01* alleles (reported as "negative" on a genotyping test)	*X/*X[b]
High risk of hypersensitivity (~6% of patients)	Presence of at least one **57:01* allele (reported as "positive" on a genotyping test)	*57:01/*X[b] *57:01/*57:01

HLA-B, human leukocyte antigen B.
[a] See supplementary data online for estimates of genotype frequencies among different ethnic/geographic groups.
[b] *X = any *HLA-B* genotype other than *57:01.
Table adapted from (3).

The FDA-approved label for abacavir states that all patients should be screened for the *HLA-B*57:01* allele prior to initiating therapy with abacavir, or when reinitiating therapy with abacavir, unless patients have a previously documented *HLA-B*57:01* allele assessment. The FDA also warns that abacavir must be discontinued immediately if a hypersensitivity reaction is suspected, regardless of *HLA-B*57:01* status and even when other diagnoses are possible (1).

Several studies have shown that routine genetic screening for *HLA-B*57:01* significantly reduces the incidence of abacavir-induced hypersensitivity, and is cost-effective. Because it is rare for individuals who do not carry the high-risk *HLA* variant to develop hypersensitivity, adhering to the screening guidelines can reduce the incidence of immunologically confirmed cases of abacavir hypersensitivity to nearly zero (9-12).

HLA Gene Family

The *HLA* genes are members of the major histocompatibility complex (*MHC)* gene family, which includes more than 200 genes. The *MHC* family has been subdivided into 3 subgroups based on the structure and function of the encoded proteins: class I, class II, and class III. The class I region contains the genes encoding the HLA molecules HLA-A, HLA-B, and HLA-C. These molecules are expressed on the surfaces of almost all cells and play an important role in antigen presentation. The HLA region also contains a variety of other genes, including genes involved in immunity and genes not known to be involved in immune function.

An important role of HLA class I molecules is to present peptides (processed fragments of antigens) to immune cells ($CD8^+$ T cells). Most of these peptides originate from the breakdown of normal cellular proteins ("self"). However, if foreign peptide fragments are presented, e.g., from a pathogen, $CD8^+$T cells will recognize the peptides as "non-self" and will be activated to release inflammatory cytokines and launch an immune response to dispose of the pathogen (or foreign body).

Because HLA molecules need to present such a wide variety of "self" and "non-self" peptides, the *HLA* genes are both numerous and highly polymorphic. More than 4,700 *HLA-B* alleles have been identified (6, 13).

HLA Allele Nomenclature

HLA allele nomenclature includes the HLA prefix, followed by the gene, an asterisk and a four (or six) digit number that corresponds to the assigned allele number (14). For example, the *HLA-B*15:02:* allele is composed of:

- HLA: the HLA prefix (the HLA region on chromosome 6)
- B: the B gene (a particular HLA gene in this region)
- 15: the allele group (historically determined by serotyping, i.e., a group of alleles that share the same serotype)
- 02: the specific HLA allele (a specific protein sequence; determined by genetic analysis).

Additional digits have been added to the nomenclature to discriminate between alleles that do not differ in the protein amino acid sequence but differ in their genetic sequence (i.e., due to synonymous and noncoding genetic variants).

Variation in *HLA* genes plays an important role in susceptibility to autoimmune disease and infections. These variations are also critical in the context of transplant surgery where better outcomes are observed if the donor and recipient are HLA-compatible.

HLA variants have also been associated with susceptibility to Type B adverse drug reactions. For example, as noted above, an *HLA-B* variant has been associated with severe hypersensitivity reactions to abacavir. Other *HLA-B* variants have been associated with severe reactions to allopurinol (used to treat gout), and carbamazepine and phenytoin (used to treat epilepsy).

Gene: *HLA-B*

The *HLA-B*57:01* allele is associated with an increased risk of hypersensitivity reaction to abacavir. Studies across ethnicities have reported that in immunologically confirmed cases of abacavir hypersensitivity, 100% of cases occurred in patients who were carriers of this HLA variant (7).

Other immune factors are also involved, however. For example, not everyone who carries the high-risk *HLA* allele will develop abacavir hypersensitivity - approximately 39% of individuals who are positive for *HLA-B*57:01* will tolerate abacavir treatment (8).

Cytotoxic (CD8+) T cells mediate the hypersensitivity reaction to abacavir. Abacavir is thought to form a non-covalent complex with *HLA-B*57:01* (15-18). Several theories have been proposed for how this drug peptide-HLA complex activates the T cell receptor, which then releases inflammatory cytokines, signaling the start of the hypersensitivity response (19-23). More than one immune mechanism may be involved (7). It has been shown that abacavir occupies a space below the region of HLA that presents peptides. This leads to altered peptide presentation (including the presentation of self-peptides to which the host has not been tolerized) and triggers an autoimmune-like reaction (19, 24).

The hypersensitivity reaction to abacavir is thought to be maintained over the lifetime of an individual. The reintroduction of abacavir to a sensitized individual may be fatal, presumably due to a rapid activation of a memory T cell population. Therefore, abacavir is contraindicated in individuals with a prior hypersensitivity reaction to abacavir (1, 25).

*HLA-B*57:01* also has an important role in HIV infection. In Caucasians with HIV, *HLA-B*57:01* has been linked to a lower viral load set point (the amount of viral RNA detected in blood during the asymptomatic phase of HIV infection) (26). In addition, *HLA-B*57:01* is overrepresented in a small group of individuals who have HIV which has not progressed to AIDs, despite lack of treatment with antiretroviral therapy. These individuals are known as “long-term non-progressors” (27).

The frequency of the *HLA-B*57:01* allele varies significantly by population. The allele is most common in Northern Thai and Indian populations (up to 20%). It is relatively common in European populations (6–7%), and is present but less common in African Americans, admixed American populations, and Middle Eastern populations (2-3%). *HLA-B*57:01* is uncommon in homogenous South-Asian and African populations, being mostly absent in the Japanese, and some African populations (2, 3, 28).

Genetic Testing

Pharmacogenetic testing is now routine in HIV clinical practice (28).The NIH's Genetic Testing Registry (GTR) provides examples of the genetic tests that are currently available for abacavir hypersensitivity, and the *HLA-B* gene.

The genotype results for an *HLA* allele such as *HLA-B*57:01* can either be "positive" or "negative". There are no intermediate phenotypes because the *HLA* genes are expressed in a codominant manner.

Abacavir is contradicted in patients with a "positive" result, and only one copy of the **57:01* allele is required for a positive result. Therefore, the positive result is either "heterozygous" or "homozygous", depending upon whether the patient is carrying one or 2 copies of the **57:01* allele, respectively.

A negative result indicates that the patient does not carry the *HLA-B*57:01* allele. However, a negative result does not rule out the possibility of a patient developing abacavir hypersensitivity. Therefore, clinicians should carefully monitor all patients according to standard practices (3).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2017 Statement from the US Food and Drug Administration (FDA)

Serious and sometimes fatal hypersensitivity reactions have occurred with abacavir sulfate. These hypersensitivity reactions have included multi-organ failure and anaphylaxis and typically occurred within the first 6 weeks of treatment with abacavir sulfate (median time to onset was 9 days); although abacavir hypersensitivity reactions have occurred any time during treatment. Patients who carry the *HLA-B*57:01* allele are at a higher risk of abacavir hypersensitivity reactions; although, patients who do not carry the *HLA-B*57:01* allele have developed hypersensitivity reactions. Hypersensitivity to abacavir was reported in approximately 206 (8%) of 2,670 patients in 9 clinical trials with abacavir-containing products where *HLA-B*57:01* screening was not performed. The incidence of suspected abacavir hypersensitivity reactions in clinical trials was 1% when subjects carrying the *HLA-B*57:01* allele were excluded. In any patient treated with abacavir, the clinical diagnosis of hypersensitivity reaction must remain the basis of clinical decision making.

Due to the potential for severe, serious, and possibly fatal hypersensitivity reactions with abacavir sulfate:

- All patients should be screened for the *HLA-B*57:01* allele prior to initiating therapy with abacavir tablets or reinitiation of therapy with abacavir tablets, unless patients have a previously documented *HLA-B*57:01* allele assessment.
- Abacavir tablet is contraindicated in patients with a prior hypersensitivity reaction to abacavir and in *HLA-B*57:01* -positive patients.
- Before starting abacavir tablets, review medical history for prior exposure to any abacavir-containing product. NEVER restart abacavir tablets or any other abacavir-containing product following a hypersensitivity reaction to abacavir, regardless of *HLA-B*57:01* status.
- To reduce the risk of a life-threatening hypersensitivity reaction, regardless of *HLA-B*57:01* status, discontinue abacavir tablets immediately if a hypersensitivity reaction is suspected, even when other

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

diagnoses are possible (e.g., acute onset respiratory diseases such as pneumonia, bronchitis, pharyngitis, or influenza; gastroenteritis; or reactions to other medications).
- If a hypersensitivity reaction cannot be ruled out, do not restart abacavir tablets or any other abacavir-containing products because more severe symptoms which may include life-threatening hypotension and death, can occur within hours.
- If a hypersensitivity reaction is ruled out, patients may restart abacavir tablets. Rarely, patients who have stopped abacavir for reasons other than symptoms of hypersensitivity have also experienced life-threatening reactions within hours of reinitiating abacavir therapy. Therefore, reintroduction of abacavir tablets or any other abacavir-containing product is recommended only if medical care can be readily accessed.
- A Medication Guide and Warning Card that provide information about recognition of hypersensitivity reactions should be dispensed with each new prescription and refill.

Please review the complete therapeutic recommendations that are located here: (1).

2014 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC)

We agree with others that *HLA-B*57:01* screening should be performed in all abacavir-naive individuals before initiation of abacavir-containing therapy (see Table 2); this is consistent with the recommendations of the FDA, the US Department of Health and Human Services, and the European Medicines Agency. In abacavir-naive individuals who are *HLA-B*57:01*-positive, abacavir is not recommended and should be considered only under exceptional circumstances when the potential benefit, based on resistance patterns and treatment history, outweighs the risk. *HLA-B*57:01* genotyping is widely available in the developed world and is considered the standard of care prior to initiating abacavir. Where *HLA-B*57:01* genotyping is not clinically available (such as in resource-limited settings), some have advocated initiating abacavir, provided there is appropriate clinical monitoring and patient counseling about the signs and symptoms of HSR [hypersensitivity reaction], although this remains at the clinician's discretion.

Please review the complete therapeutic recommendations that are located here (3, 4).

2017 Summary of Recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

*HLA-B*57:01*-positive patients have a strongly increased risk of a hypersensitivity reaction to abacavir.

Recommendation:

Abacavir is contraindicated for *HLA-B*57:01*-positive patients.

1 Advise the prescriber to prescribe an alternative according to the current guidelines.

Background information

Mechanism:

Although the mechanism of hypersensitivity reactions to abacavir is not fully known, experimental data suggest the following mechanism.

Abacavir metabolites (aldehydes and acids) form a covalent bond with cellular proteins. Peptides derived from these modified proteins bind to *HLA-B*5701* and are recognised on the cell surface as foreign by the immune cells, which triggers an immune response against cells containing abacavir. For more information about the

*HLA-B*57:01* genotype: see the general background information about HLA on the KNMP Knowledge Bank or on http://www.knmp.nl/ (search for HLA).

Other considerations:

If tests are performed for *HLA-B57* instead of *HLA-B*57:01*, some patients will incorrectly be denied treatment with abacavir. This is primarily the case in patients of African descent, where *HLA-B*57:03* is the most common *HLA-B57* sub-type and to a lesser extent for Caucasian patients, where *HLA-B*57:01* is the most common *HLA-B57* sub-type. If there are enough alternatives, it is not a problem that the patient is being denied abacavir incorrectly.

Clinical consequences:

*HLA-B*5701*-positive patients have a strongly increased risk of a hypersensitivity reaction to abacavir (OR [odds ratio] 7 to 960 for clinically diagnosed hypersensitivity reactions and 900 to 1945 for immunologically confirmed hypersensitivity reactions).

Exclusion of *HLA-B*5701*-positive patients from abacavir therapy reduced the number of clinically diagnosed hypersensitivity reactions in predominantly white populations by 56-96% and the number of immunologically confirmed hypersensitivity reactions by 100%.

Hypersensitivity reactions to abacavir generally disappear spontaneously after stopping abacavir, but can be fatal in severe cases.

Please review the complete therapeutic recommendations that are located here: (5).

Nomenclature

Nomenclature of Selected *HLA-B* alleles

Allele name	dbSNP reference identifier for allele location
*HLA-B*57:01*	rs2395029 is a tag SNP for *HLA-B*57:01*

For the MHC region, variations in genes such as *HLA-B* occur across the whole sequence of the gene, not a single locus. Therefore, the *HLA-B*57:01* allele is defined by its sequence (GenBank: AF196183.1) rather than single coding or protein variants. If there is strong linkage disequilibrium between one or more SNPs, the presence of these SNPs (tag SNPs) may be used for *HLA* typing (29). In the case of *HLA-B*, the presence of the rs2395029 allele (a SNP in the HLA complex P5 gene) is 99.9% predictive of the presence of an *HLA-B*57:01* allele (30).
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (4).
Guidelines on the naming of *HLA* genes are available from *HLA* Nomenclature.

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Version History

To view the 2015 version of this summary (Created: September 1, 2015) please click here.

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Allopurinol Therapy and *HLA-B*58:01* Genotype

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Introduction

Allopurinol is a xanthine oxidase inhibitor that decreases the production of uric acid. It is most commonly used in the management of gout and hyperuricemia (high levels of uric acid).

The human leukocyte antigen B (HLA-B) plays an important role in how the immune system recognizes and responds to pathogens. The variant *HLA-B*58:01* allele is strongly associated with severe cutaneous adverse reactions (SCAR) during treatment with allopurinol. This allele is most commonly found in Asian subpopulations, notably in individuals of Korean, Han Chinese, or Thai descent (1-3).

At this time, the FDA-approved drug label does not discuss *HLA-B* genotype (4). However, the Clinical Pharmacogenetics Implementation Consortium (CPIC) recommends that allopurinol should not be prescribed to patients who have tested positive for *HLA-B*58:01*, and that an alternative medication should be considered to avoid the risk of developing SCAR (see Table 1) (1, 2).

Table 1. *HLA-B* phenotypes and the therapeutic recommendations for allopurinol therapy, adapted from CPIC

Genotype	Examples of diplotypes	Phenotype	Therapeutic recommendations
Noncarrier of *HLA-B*58:01*	**X/*X*^{b}	Low or reduced risk of allopurinol-induced SCAR	Use allopurinol per standard dosing guidelines
Carrier of *HLA-B*58:01*	**5801/*X*^{b} **5801/*5801*	Significantly increased risk of allopurinol-induced SCAR	Allopurinol is contraindicated

The strength of therapeutic recommendations is "strong" (1).
HLA-B, human leukocyte antigen B
SCAR, severe cutaneous adverse reaction
**X*, any *HLA-B* genotype other than *HLA-B*58:01*
**X*^{b}, any *HLA-B* genotype other than *HLA-B*58:01*
Table is adapted from Hershfield M.S., Callaghan J.T., Tassaneeyakul W., Mushiroda T., Thorn C.F., Klein T.E., Lee M.T.Clinical pharmacogenetics implementation consortium guidelines for human leukocyte antigen-B genotype and allopurinol dosing. Clinical pharmacology and therapeutics. 2013;93(2):153–8 (1, 2).

Drug: Allopurinol

Allopurinol is a commonly prescribed drug for the management of gout and hyperuricemia. Uric acid is produced by the breakdown of purine nucleotides, and high concentrations of uric acid can lead to gout and uric acid kidney stones.

Allopurinol is an analogue of the purine hypoxanthine. Allopurinol decreases the production of uric acid by inhibiting xanthine oxidase, which catalyzes the conversion of hypoxanthine and xanthine to uric acid. In addition, allopurinol facilitates the incorporation of hypoxanthine and xanthine into DNA and RNA, and the resulting increase in nucleotide concentration leads to a feedback inhibition of *de novo* purine synthesis, which in turn leads to a decrease in uric acid levels (5).

Allopurinol is rapidly oxidized in the liver to the active metabolite oxypurinol, which also inhibits xanthine oxidase. Allopurinol has a short plasma half-life of ~1-2 hours, whereas oxypurinol has a half-life of ~15 hours. After the rapid oxidation of allopurinol, any remaining drug is promptly filtered and excreted by the kidneys.

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However, after oxypurinol is filtered by the kidneys, it is reabsorbed in a manner similar to how uric acid is reabsorbed. Therefore, it is thought that the effective inhibition of xanthine oxidase over a 24-hour period after a single dose of allopurinol is largely brought about by the effects of oxypurinol (4).

In general, allopurinol is well tolerated; however, allopurinol is one of the most common causes of severe cutaneous adverse reactions (SCAR), and the *HLA-B*58:01* allele is strongly associated with allopurinol-induced SCAR.

Allopurinol-induced Adverse Drug Reactions

In general, there are two categories of adverse drug reactions. Type A reactions account for up to 85-90% of all adverse drug reactions. They are predictable based on the known properties of the drug, and they can affect any individual, if their exposure to the drug is high enough. For allopurinol, one of the most common type A adverse effects is an acute attack of gout after starting allopurinol therapy (4).

Type B reactions account for the remaining 10-15% of adverse drug reactions. These include hypersensitivity reactions that occur in susceptible individuals. Such idiosyncratic hypersensitivity reactions can occur at any dose and develop through a mechanism that is unrelated to the mechanism of action of the drug. For this reason, it is difficult to predict in whom a drug-induced hypersensitivity reaction is likely to occur.

Severe cutaneous adverse reactions are type B reactions, which include Stevens-Johnson syndrome (SJS), or the more severe toxic epidermal necrolysis (TEN); as well as drug reaction with eosinophilia and systemic symptoms (DRESS), and allopurinol hypersensitivity syndrome (AHS).

Allopurinol is the most common cause of SJS/TEN in Europe (6). SJS /TEN are life-threatening conditions that are primarily characterized by lesions of the skin (detachment of the epidermis) and mucus membranes (severe erosions). SJS/TEN is also associated with fever, raised white cell count, hepatitis, and acute renal failure.

The underlying mechanisms for allopurinol-induced SCARs remain unclear, but cytotoxic T cells (CD8+ T cells) are involved. In the case of allopurinol, although the presence of *HLA-B*58:01* substantially increases the risk of SCAR, it is not an absolute requirement, indicating that other variables also contribute to its etiology (1, 7).

One theory, known as the p-I concept, is that there is a direct pharmacological reaction of the drug (e.g., allopurinol) with the immune receptors (activated drug-specific T cells) and this provides an initial signal to induce T-cell activation and trigger a T cell–mediated hypersensitivity reaction. The signal may be strengthened by the additional interaction with HLA molecules (e.g., *HLA-B*58:01*) (7-11).

Although allopurinol induced-SCAR is rare (the risk is estimated to be 0.1–0.4%), allopurinol is one of the most serious causes of SCAR, which carries a mortality rate of up to 25% (1, 2).

The FDA-approved dose of allopurinol for the management of gout or hyperuricemia is to start with a daily dose of 100 mg, and titrate the dose upwards to a maximum daily dose of 800 mg, until the uric acid concentrations are less than 6.0 mg/dl. Allopurinol is often prescribed in doses that may be too low to achieve a therapeutic goal, an approach taken in part to reduce the risk of drug hypersensitivity (12). One study has found that a lower starting dose of allopurinol may reduce the risk of allopurinol hypersensitivity syndrome (13).

HLA Gene Family

The human leukocyte antigen (HLA) genes are members of the MHC gene family, which includes more than 200 genes. The MHC family has been subdivided in to 3 subgroups based on the structure and function of the encoded proteins: Class I, Class II, and Class III.

The class I region contains the genes encoding the HLA molecules HLA-A, HLA-B, and HLA-C. These molecules are expressed on the surfaces of almost all immune cells and play an important role in processing and

presenting antigens. The class I gene region also contains a variety of other genes, many of which are not known to be involved in immune function.

An important role of HLA class I molecules is to present peptide fragments to immune cells (CD8+ T cells). Most of these peptides originate from the breakdown of normal cellular proteins ("self"). However, if foreign peptide fragments are presented (e.g., from a pathogen), CD8+T cells will recognize the peptides as "non-self" and will be activated to release inflammatory cytokines and launch an immune response to dispose of the pathogen or foreign body (14).

Because HLA molecules need to present such a wide variety of "self" and "non-self" peptides, the HLA genes are both numerous and highly polymorphic. More than 1,500 *HLA-B* alleles have been identified. Each HLA allele has a name that is prefixed by HLA, followed by the gene name, an asterisk and a two digit number that corresponds to antigen specificity, and the assigned allele number (15). For example, the *HLA-DRB1*13:01* allele is composed of:

- HLA: the HLA prefix (the HLA region on chromosome 6)
- DRB1: the DRB1 gene (a particular HLA gene in this region)
- 13: the allele group (historically determined by serotyping, i.e., a group of alleles that share the same serotype)
- 01: the specific HLA allele (a specific protein sequence; determined by genetic analysis).

Additional digits have recently been added to the nomenclature to discriminate alleles that do not differ in the protein amino acid sequence, but differ in their genetic sequence (i.e., due to synonymous and noncoding genetic variants).

Variation in the HLA genes plays an important role in the susceptibility to autoimmune disease and infections and they are also critical in the context of transplant surgery where better outcomes are observed if the donor and recipient are HLA-compatible (1, 2). More recently, specific HLA variants have been associated with susceptibility to adverse drug reactions, including allopurinol-induced hypersensitivity reactions.

Gene: *HLA-B*

The *HLA-B*58:01* allele is associated with an increased risk of severe hypersensitivity reactions to allopurinol, such as SJS/TEN. The allele is codominant, so an individual needs to carry only one copy of the *HLA-B*58:01* allele to be at increased risk.

The association between *HLA-B*58:01* and allopurinol-induced adverse effects was first discovered in the Han Chinese population, where a study found that all patients who had allopurinol-induced SJS/TEN (51/51, 100%) carried *HLA-B*58:01*, compared with only 15% of the allopurinol-tolerant patients (20/135, 15%) (16).

Further studies also found an association with *HLA-B*58:01* and severe allopurinol-induced adverse effects in other populations, including Thai, Korean, European, and Japanese populations (17-19). The association is stronger in the Han Chinese than in European and Japanese populations, which is most likely due to differences in *HLA-B*58:01* allele frequencies between racial and ethnic populations (20).

The *HLA-B*58:01* allele is most common in individuals of Asian descent, with a frequency of ~10-15% in the Han Chinese, ~12% in Koreans, and ~6-8% in individuals of Thai descent (3, 21-25). The risk allele is less common among Europeans and Japanese with a frequency of only ~1-2% (26, 27).

Although the risk of SCAR due to allopurinol is generally low (0.1–0.4%) and certain populations have a low frequency of the *HLA-B*58:01* risk allele (e.g., Europeans), the risk of allopurinol-induced SCAR is substantially elevated in *HLA-B*58:01* carriers. The odds ratio for allopurinol-induced SCAR among *HLA-B*58:01* carriers in a meta-analysis was 73 using healthy controls and 165 using allopurinol-tolerant controls (5).

Genetic Testing

Genetic testing is available for several *HLA-B* alleles, including *HLA-B*58:01*. The genotype results are either "positive" (*HLA-B*58:01* being present in one or both copies of the *HLA-B* gene) or "negative" (no copies of *HLA-B*58:01* are present). There are no intermediate phenotypes because *HLA-B* is expressed in a codominant manner (1, 2).

Several studies have looked in to the cost-effectiveness of *HLA-B*58:01* testing to guide urate-lowering therapy (ULT). A 2012 American College of Rheumatology guideline recommended that prior to treatment with allopurinol, the *HLA-B*58:01* genotype of gout patients at high risk for SCARs, including Korean patients with chronic renal insufficiency, should be determined (3). One study reported that in Korean patients with kidney disease, ULT guided by *HLA-B58:01* genotyping was less costly and more effective than treatment without genotyping, and that *HLA-B*58:01* genotyping could considerably reduce the occurrence of allopurinol-induced SCARs and related deaths (28). Cost-effectiveness analysis of treating patients with chronic gout (without additional risk factors) in Singapore and in Portugal found that *HLA-B*58:01*-guided ULT was not cost-effective at this time.

A potential alternative to costly HLA genotyping, may be to test for single nucleotide variants that are tightly associated with *HLA-B*58:01*. A number of variants have been found to be in linkage disequilibrium (LD) with *HLA-B*58:01*, for example, the rs9263726 variant in the *PSORS1C1* gene is strongly associated with *HLA-B*58:01* in the Japanese population (20).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2015 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC): Given the high specificity for allopurinol-induced SCAR, allopurinol should not be prescribed to patients who have tested positive for *HLA-B*58:01*. Alternative medication should be considered for these patients to avoid the risk of developing SCAR. For patients who have tested negative, allopurinol may be prescribed as usual (see Table 1). However, testing negative for *HLA-B*58:01* does not totally eliminate the possibility of developing SCAR, especially in the European population.

Please review the complete therapeutic recommendations that are located here (1, 2).

2012 Statement from the American College of Rheumatology (ACR): Prior to initiation of allopurinol, rapid polymerase chain reaction-based *HLA-B*5801* screening should be considered as a risk management component in subpopulations where both the *HLA-B*5801* allele frequency is elevated and the *HLA-B*5801*-positive subjects have a very high hazard ratio ("high risk") for severe allopurinol hypersensitivity reaction (e.g., Koreans with stage 3 or worse chronic kidney disease and all those of Han Chinese and Thai descent).

Please review the complete therapeutic recommendations that are located here (3).

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

Nomenclature

Allele name	Other name(s)	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*HLA-B*58:01*		Not applicable*	Not applicable*	Not applicable*

* For the MHC region, variations in genes such as *HLA-B* occur across the whole sequence of the gene, not a single locus. Therefore, the *HLA-B*58:01* allele is defined by its sequence (GenBank: EU499350.1) rather than single coding or protein variants.

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

Guidelines on nomenclature of the HLA system are available from HLA Nomenclature: http://hla.alleles.org/

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Amitriptyline Therapy and *CYP2D6* and *CYP2C19* Genotype

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Introduction

Amitriptyline is a tricyclic antidepressant used in the treatment of several psychiatric disorders, including major depression, obsessive-compulsive disorder, panic attacks, generalized anxiety disorder, post-traumatic stress disorder, and bulimia. Amitriptyline also has different off-label uses, including migraine prevention, neuropathic pain management, fibromyalgia, and enuresis (bedwetting) (1).

Tricyclic antidepressants (TCAs) primarily mediate their therapeutic effect by inhibiting the reuptake of both serotonin and norepinephrine, leaving more neurotransmitter in the synaptic cleft stimulating the neuron. Because tricyclics can also block different receptors (H1 histamine, alpha 1 α1-adrenergic, and muscarinic receptors), side effects are common. As such, more specific selective serotonin reuptake inhibitors (SSRIs) have largely replaced the use of them. However, TCAs still have an important use in specific types of depression and other conditions.

Amitriptyline is metabolized mainly via CYP2C19 and CYP2D6 pathways. Metabolism by CYP2C19 results in active metabolites, including nortriptyline, which is also a tricyclic antidepressant. Metabolism catalyzed by CYP2D6 results in the formation of the less active 10-hydroxy metabolite. Individuals who are "CYP2D6 ultrarapid metabolizers" carry more than two normal function alleles (i.e., multiple copies) (Table 1, 2), whereas "CYP2C19 ultrarapid metabolizers" carry two increased function alleles (Table 3, 4). Individuals who are CYP2D6 or CYP2C19 "poor metabolizers" carry two no function alleles for *CYP2D6* or *CYP2C19*, respectively.

The FDA-approved drug label for amitriptyline states that CYP2D6 poor metabolizers have higher than expected plasma concentrations of tricyclic antidepressants when given usual doses. The FDA recommendations also include monitoring tricyclic antidepressant plasma levels whenever a tricyclic antidepressant is going to be co-administered with another drug known to be an inhibitor of CYP2D6 (1).

In 2016, the Clinical Pharmacogenetics Implementation Consortium (CPIC) made dosing recommendations for tricyclic antidepressants based on *CYP2C19* and *CYP2D6* genotypes (2). For CYP2D6 ultrarapid metabolizers, CPIC recommends avoiding the use of a tricyclic due to the potential lack of efficacy, and to consider an alternative drug not metabolized by CYP2D6. If a TCA is still warranted, CPIC recommends considering titrating the TCA to a higher target dose (compared to normal metabolizers) and using therapeutic drug monitoring to guide dose adjustments. For CYP2D6 intermediate metabolizers, CPIC recommends considering a 25% reduction of the starting dose, and for CYP2D6 poor metabolizers, to avoid the use of tricyclics because of the potential for side effects. If a tricyclic is still warranted for CYP2D6 poor metabolizers, CPIC recommends considering a 50% reduction of the starting dose while monitoring drug plasma concentrations to avoid side effects.

For CYP2C19 ultrarapid metabolizers, CPIC recommends avoiding the use of tertiary amines (e.g., amitriptyline) due to the potential for a sub-optimal response, and to consider an alternative drug not metabolized by CYP2C19, such as the secondary amines nortriptyline or desipramine. For CYP2C19 poor metabolizers, CPIC recommends avoiding tertiary amine use due to the potential for sub-optimal response, and to consider an alternative drug not metabolized by CYP2C19. If a tertiary amine is still warranted for CYP2C19 poor metabolizers, CPIC recommends considering a 50% reduction of the starting dose while monitoring drug plasma concentrations while monitoring plasma concentrations to avoid side effects (2).

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Drug Class: Tricyclic Antidepressants

Tricyclic antidepressants (TCAs) are mixed serotonin-norepinephrine reuptake inhibitors. They increase the amount of neurotransmitter in the synaptic cleft, thought to mediate their antidepressant effects.

From the 1960s to the 1980s, tricyclics were the first-line treatment for depression, until the introduction of SSRIs, which have fewer side effects and are safer. The common side effects of tricyclics include anticholinergic side effects (e.g., blurred vision, dry mouth, constipation, and sedation), cardiac effects, and orthostatic hypotension.

Today, the main therapeutic use of tricyclics is chronic pain management, such as neuropathic pain. However, tricyclics are still used in the treatment of depression as well as other psychiatric disorders, including obsessive–compulsive disorder, panic attacks, generalized anxiety disorder, post-traumatic stress disorder, bulimia nervosa, smoking cessation, and enuresis (bedwetting).

Tricyclics are named after their chemical structure of three central rings and a side chain important for its function and activity. Its structure determines whether a drug is classified a tertiary amine (amitriptyline, clomipramine, doxepin, imipramine, and trimipramine) or secondary amine (desipramine and nortriptyline).

Whereas tertiary amines are generally more potent in blocking reuptake of serotonin, the secondary amines are more potent in blocking the reuptake of norepinephrine. Secondary amines are better tolerated and are also associated with fewer anticholinergic side effects.

The CYP2C19 enzyme metabolizes tertiary amines to active metabolites, which include desipramine (the active metabolite of imipramine) and nortriptyline (the active metabolite of amitriptyline). Both the tertiary and secondary amines are metabolized by CYP2D6 to less active metabolites.

The effectiveness and tolerability of tricyclics are affected by CYP2D6 metabolism and partially by CYP2C19 metabolism. Individuals who carry *CYP2D6* or *CYP2C19* variants that influence enzyme activity may be at an increased risk of treatment failure (if plasma drug levels are decreased) or drug toxicity (if plasma drug levels are increased).

Drug: Amitriptyline

Amitriptyline is used to relieve the symptoms of depression, with endogenous depression being more likely to respond to treatment than other depressive states (e.g., reactive depression) (1). Off-label uses of amitriptyline include migraine prevention, and the treatment of neuropathic pain, fibromyalgia, and enuresis (bedwetting).

Amitriptyline blocks the uptake of both serotonin and norepinephrine, but more potently blocks the reuptake of serotonin. Amitriptyline also has strong affinities for histamine (H1), alpha-1 adrenergic, and muscarinic (M1) receptors, which account for its side effects, including sedation, weight gain, blurred vision, dry mouth, and constipation. The intensity of these side effects tends to be greater for amitriptyline compared to other tricyclics (3).

Amitriptyline is metabolized by CYP2C19 to the active metabolite, nortriptyline, which is also a tricyclic antidepressant thought to be approximately twice as potent as other TCAs. In contrast to amitriptyline, nortriptyline blocks the reuptake of norepinephrine more potently than serotonin (3).

Because both the parent drug (amitriptyline) and the CYP2C19 metabolite (nortriptyline) are pharmacologically active compounds, the plasma levels of both drugs should monitored (4). The sum of amitriptyline plus nortriptyline plasma levels may correlate with an individual's response to amitriptyline therapy (5).

The optimal therapeutic range for amitriptyline has been well-defined (6). Most individuals display an optimal response to amitriptyline when combined serum levels of amitriptyline and nortriptyline are between 80 and 200 ng/mL. Higher levels are associated with an increased risk of adverse events. At levels greater than 300 ng/ml, cardiac toxicity occurs. This is characterized by ECG changes (widening of QRS), which may lead to potentially fatal ventricular tachycardia. In some individuals, cardiac toxicity may occur at lower concentrations or even when they are within the recommended therapeutic range (7, 8).

Nortriptyline is metabolized by CYP2D6 to hydroxyl metabolites, which have been associated with cardiac toxicity. Safe levels of hydroxyl metabolites have not yet been defined (4).

Individuals who are carriers of certain *CYP2D6* and/or *CYP2C19* variants may have drug levels that are outside the therapeutic range after treated with standard doses of amitriptyline. As a result, they may have an increased risk of toxicity (if the level of amitriptyline and its active metabolites are too high) or treatment failure (if drug levels are too low).

Gene: *CYP2D6*

The cytochrome P450 superfamily (CYP) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP genes are very polymorphic and can result in reduced, absent, or increased drug metabolism.

CYP2D6 is responsible for the metabolism of many commonly prescribed drugs, including antipsychotics, analgesics, beta-blockers, and TCAs such as amitriptyline.

CYP2D6 is highly polymorphic, with over 100 star (*) alleles described and currently catalogued at the Human Cytochrome P450 (CYP) Allele Nomenclature Database (9).

CYP2D6 is a particularly complex gene that is difficult to genotype, partly because of the large number of variants, but also because of the presence of gene deletions, duplications, and its neighboring pseudogenes. The complexity of genetic variation at this locus complicates the ability to interrogate *CYP2D6*.

There is substantial variation in *CYP2D6* allele frequencies among different populations (10). *CYP2D6*1* is the wild-type allele and is associated with normal enzyme activity and the "normal metabolizer" phenotype. The *CYP2D6* alleles **2*, **33*, and **35* are also considered to have normal activity.

Other alleles include no function variants that produce a non-functioning enzyme (e.g., **3*, **4*, **5*,**6*, **7*, **8*, and **12*) or an enzyme with decreased activity (e.g., **10*, **17*, *29, and **41*) (see Table 1) (11) . There are large inter-ethnic differences in the frequency of these alleles, with **3*, **4*, **5*, **6*, and **41* being more common in the Caucasian population, **17* more common in Africans, and **10* more common in Asians (12).

Table 1: 2016 Assignment of *CYP2D6* phenotypes by CPIC

Phenotype	Activity Score	Genotypes	Examples of diplotypes
CYP2D6 ultrarapid metabolizer (approximately 1–20% of patients)[a]	Greater than 2.0	An individual carrying duplications of functional alleles	*(*1/*1)xN* *(*1/*2)xN* *(*2/*2)xN*[b]

Table 1 continued from previous page.

Phenotype	Activity Score	Genotypes	Examples of diplotypes
CYP2D6 normal metabolizer (approximately 72–88% of patients)	1.0 – 2.0[c]	An individual carrying two normal function alleles or two decreased function alleles or one normal and no function allele or one normal function and decreased function allele or combinations of duplicated alleles that result in an activity score of 1.0 to 2.0	**1/*1* **1/*2* **2/*2* **1/*9* **1/*41* **41/*41* **1/*5* **1/*4*
CYP2D6 intermediate metabolizer (approximately 1–13% of patients)	0.5	An individual carrying one decreased function and one no function allele	**4/*41* **5/*9* **4/*10*
CYP2D6 poor metabolizer (approximately 1–10% of patients)	0	An individual carrying two no function alleles	**4/*4* **4/*4xN* **3/*4* **5/*5* **5/*6*

[a] For population-specific allele and phenotype frequencies, please see (2).
[b] Where *xN* represents the number of *CYP2D6* gene copies (N is 2 or more).
[c] Patients with an activity core of 1.0 may be classified as intermediate metabolizers by some reference laboratories.
For more information about activity scores, please see the Genetic Testing section.
This table has been adapted from Hicks J.K., Sangkuhl K., Swen J.J., Ellingrod V.L., Müller D.J., Shimoda K., Bishop J.R., Kharasch E.D., Skaar T.C., Gaedigk A., Dunnenberger H.M., Klein T.E., Caudle K.E. Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC®) for *CYP2D6* and *CYP2C19* Genotypes and Dosing of Tricyclic Antidepressants: 2016 Update. Clinical pharmacology and therapeutics. 2016 Dec 20 [Epub ahead of print] (2).

Individuals who are intermediate or poor metabolizers carry copies of decreased or no function *CYP2D6* alleles, respectively (Table 1). Approximately 30% of Asians and individuals of Asian descent are intermediate metabolizers. In these populations, only half of *CYPD6* alleles are fully functional, with the reduced function **10* variant being very common (~40%, compared to ~2% in Caucasians) (13). As a result, Asians are more likely to be intermediate metabolizers than Caucasians (14). Similarly, in Africans and African Americans, only half of *CYPD6* alleles are functional. However, a wider range of variants account for the remaining alleles (14-16).

Approximately 6–10% of European Caucasians and their descendants are poor metabolizers, mainly due to no function **4* and **5* alleles (14). Notably, less than 40% are homozygous normal metabolizers (carrying two copies of **1* allele) (17-19).

Individuals who are CYP2D6 poor metabolizers have higher plasma levels of amitriptyline, compared to normal metabolizers, after standard doses of amitriptyline (20). Individuals who carry at least one non-functional *CYP2D6* variant have been found to be at medium to high risk of developing side effects (21).

Because standard doses of amitriptyline may lead to an increased risk of adverse events in individuals who are CYP2D6 poor metabolizers, CPIC recommends avoiding the use of amitriptyline or other tricyclic antidepressants, and to consider using an alternative drug that is not metabolized by CYP2D6. If a tricyclic is warranted, CPIC recommends considering a 50% reduction of the recommended starting dose, and they strongly recommend therapeutic drug monitoring to guide dose adjustments (4).

Individuals who have more than two copies of normal function *CYP2D6* alleles are *CYP2D6* ultrarapid metabolizers. The increased rate of metabolism of amitriptyline leads to less active drug being available and a poor therapeutic response. Because of the potential lack of efficacy, CPIC recommends considering an alternative drug to amitriptyline that is not metabolized by CYP2D6. If a tricyclic is warranted, CPIC

recommends increasing the starting dose and using therapeutic drug monitoring to guide dose adjustments (4) (Table 2).

Table 2. 2016 CPIC Dosing recommendations for tricyclic antidepressants based on *CYP2D6* phenotype

Phenotype	Implication	Therapeutic recommendation
CYP2D6 ultrarapid metabolizer	Increased metabolism of TCAs to less active compounds compared to normal metabolizers	Avoid tricyclic use due to potential lack of efficacy. Consider alternative drug not metabolized by CYP2D6
	Lower plasma concentrations of active drugs will increase probability of pharmacotherapy failure	If a TCA is warranted, consider titrating to a higher target dose (compared to normal metabolizers)[a]. Utilize therapeutic drug monitoring to guide dose adjustments.
CYP2D6 normal metabolizer	Normal metabolism of TCAs	Initiate therapy with recommended starting dose[b].
CYP2D6 intermediate metabolizer	Reduced metabolism of TCAs to less active compounds compared to normal metabolizers	Consider a 25% reduction of recommended starting dose[b]. Utilize therapeutic drug monitoring to guide dose adjustments[a].
	Higher plasma concentrations of active drug will increase the probability of side effects	
CYP2D6 poor metabolizer	Greatly reduced metabolism of TCAs to less active compounds compared to normal metabolizers	Avoid tricyclic use due to potential for side effects. Consider alternative drug not metabolized by CYP2D6
	Higher plasma concentrations will increase the probability of side effects	If a TCA is warranted, consider a 50% reduction of recommended starting dose[b]. Utilize therapeutic drug monitoring to guide dose adjustments[a].

TCAs: Tricyclic Antidepressants
Dosing recommendations only apply to higher initial doses of TCAs for treatment of conditions such as depression.
The therapeutic recommendations for amitriptyline are classified as "moderate" for intermediate CYP2D6 metabolizers and "strong" for ultrarapid, normal, and poor CYP2D6 metabolizers.
[a] Titrate dose to observed clinical response with symptom improvement and minimal (if any) side effects.
[b] Patients may receive an initial low dose of tricyclic, which is then increased over several days to the recommended steady-state dose. The starting dose in this guideline refers to the recommended steady-state dose.
Table has been adapted from Hicks J.K., Sangkuhl K., Swen J.J., Ellingrod V.L., Müller D.J., Shimoda K., Bishop J.R., Kharasch E.D., Skaar T.C., Gaedigk A., Dunnenberger H.M., Klein T.E., Caudle K.E. Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC®) for *CYP2D6* and *CYP2C19* Genotypes and Dosing of Tricyclic Antidepressants: 2016 Update. Clinical pharmacology and therapeutics. 2016 Dec 20 [Epub ahead of print] (2).

One issue with increasing the dose of amitriptyline dose for CYP2D6 metabolizers is increasing the level of hydroxyl-metabolites, which have been associated with cardiotoxicity (22, 23). Currently, the safe range of hydroxy-metabolite plasma concentrations is not known. In addition, there are few studies on how the combination of *CYP2D6* and *CYP2C19* phenotypes influences an individual's response to amitriptyline (4).

Gene: CYP2C19

The CYP2C19 enzyme contributes to the metabolism of a range of clinically important drugs, such as several proton pump inhibitors, clopidogrel, benzodiazepines, and several tricyclic antidepressants, including amitriptyline.

The *CYP2C19* gene is highly polymorphic as 35 variant star (*) alleles are currently catalogued at the Human Cytochrome P450 (CYP) Allele Nomenclature Database: (http://www.cypalleles.ki.se/cyp2c19.htm).

The *CYP2C19*1* wild-type allele is associated with normal enzyme activity and the "normal metabolizer" phenotype, whereas the *CYP2C19*17* allele is associated with increased enzyme activity and the "rapid" and "ultrarapid" metabolizer phenotypes (24).

The most common no function variant is *CYP2C19*2*, which is characterized by c.681G>A in exon 5 that results in an aberrant splice site and the production of a truncated and non-functioning protein. The *CYP2C19*2* allele frequencies are ~15% in Caucasians and Africans, and ~29–35% in Asians (24, 25).

Another commonly tested no function variant is *CYP2C19*3*, which is characterized by c.636G>A in exon 4 that causes a premature stop codon. The *CYP2C19*3* allele frequencies are ~2–9% in Asian populations, but rare in other racial groups. Other no function variants occur in less than 1% of the general population, and include *CYP2C19*4-*8* (24, 25).

"CYP2C19 intermediate metabolizers" carry one copy of an allele that encodes decreased or no function (e.g. *1/*2), whereas "poor metabolizers" are homozygous or compound heterozygous for two no function alleles (e.g., *2/*2, *2/*3) (Table 3).

Table 3: Assignment of *CYP2C19* phenotypes by CPIC

Phenotype	Genotypes	Examples of diplotypes
CYP2C19 ultrarapid metabolizer (approximately 2–35% of patients)[a]	An individual carrying two increased function alleles	**17/*17*
CYP2C19 rapid metabolizer (approximately 2–30% of patients)	An individual carrying one normal function allele and one increased function allele	**1/*17*
CYP2C19 normal metabolizer (approximately 35–50% of patients)	An individual carrying two normal function alleles	**1/*1*
CYP2C19 Intermediate metabolizer (approximately 18–45% of patients)	An individual carrying one normal function and one no function allele or one no function allele and one increased function allele	**1/*2* **1/*3* **2/*17*[b]
CYP2C19 Poor metabolizer (approximately 2–15% of patients)	An individual carrying two no function alleles	**2/*2* **2/*3* **3/*3*

[a] For population-specific allele and phenotype frequencies, please see (2).
[b] The predicted metabolizer phenotype for the *2/*17 genotype is a provisional classification.
Table has been adapted from Hicks J.K., Sangkuhl K., Swen J.J., Ellingrod V.L., Müller D.J., Shimoda K., Bishop J.R., Kharasch E.D., Skaar T.C., Gaedigk A., Dunnenberger H.M., Klein T.E., Caudle K.E. Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC®) for *CYP2D6* and *CYP2C19* Genotypes and Dosing of Tricyclic Antidepressants: 2016 Update. Clinical pharmacology and therapeutics. 2016 Dec 20 [Epub ahead of print] (2).

Individuals who are CYP2C19 poor metabolizers have a reduced rate of metabolism of amitriptyline compared to normal metabolizers. As a result, standard doses of amitriptyline lead to higher plasma levels of amitriptyline, lower levels of nortriptyline, and may increase the risk of side effects (20, 26-28). Therefore, for CYP2C19 poor metabolizers, CPIC recommends considering a 50% reduction of the recommended starting dose, and to use therapeutic drug monitoring to guide dose adjustments (4).

Individuals who are ultrarapid metabolizers may be at an increased risk of treatment failure and/or metabolites adverse effects. Being a carrier of the increased activity allele *CYP2C19*17* is not associated with an increased level of the sum of amitriptyline plus nortriptyline levels, but the ratio is altered. A higher level of nortriptyline is seen, which may be linked to increased side effects. Therefore, for ultrarapid metabolizers, CPIC have an optional recommendation of considering using an alternative drug to amitriptyline that is not metabolized by CYP2C19, or if a tricyclic is warranted, to use therapeutic drug monitoring to guide dose adjustments (4, 26) (Table 4, Table 5).

Table 4. 2016 CPIC Dosing recommendations for amitriptyline based on *CYP2C19* phenotype

Phenotype	Implication	Therapeutic recommendation
CYP2C19 ultrarapid metabolizer and CYP2C19 rapid metabolizer	Increased metabolism of tertiary amines as compared to normal metabolizers Greater conversion of tertiary amines to secondary amines may affect response or side effects	Avoid tertiary amine use due to potential for sub-optimal response. Consider alternative drug not metabolized by CYP2C19. TCAs without major CYP2C19 metabolism include the secondary amines nortriptyline and desipramine.
		If a tertiary amine is warranted, utilize therapeutic drug monitoring to guide dose adjustments[a].
CYP2C19 normal metabolizer	Normal metabolism of tertiary amines	Initiate therapy with recommended starting dose[b].
CYP2C19 intermediate metabolizer	Reduced metabolism of tertiary amines compared to normal metabolizers	Initiate therapy with recommended starting dose[b].
CYP2C19 poor metabolizer	Greatly reduced metabolism of tertiary amines compared to normal metabolizers	Avoid tertiary amine use due to potential for sub-optimal response. Consider alternative drug not metabolized by CYP2C19. TCAs without major CYP2C19 metabolism include the secondary amines nortriptyline and desipramine. For tertiary amines, consider a 50% reduction of recommended starting dose[b]. Utilize therapeutic drug monitoring to guide dose adjustments[a].
	Decreased conversion of tertiary amines to secondary amines may affect response or side effects	

Dosing recommendations apply only to higher initial doses of amitriptyline for treatment of conditions such as depression. The therapeutic recommendations for amitriptyline are classified as "strong" for normal and intermediate CYP2C19 metabolizers, "moderate" for poor metabolizers, and "optional" for ultrarapid metabolizers.

[a] Titrate dose to observed clinical response with symptom improvement and minimal (if any) side effects).

[b] Patients may receive an initial low dose of tricyclic, which is then increased over several days to the recommended steady-state dose. The starting dose in this guideline refers to the recommended steady-state dose.

Table has been adapted from Hicks J.K., Sangkuhl K., Swen J.J., Ellingrod V.L., Müller D.J., Shimoda K., Bishop J.R., Kharasch E.D., Skaar T.C., Gaedigk A., Dunnenberger H.M., Klein T.E., Caudle K.E. Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC®) for *CYP2D6* and *CYP2C19* Genotypes and Dosing of Tricyclic Antidepressants: 2016 Update. Clinical pharmacology and therapeutics. 2016 Dec 20 [Epub ahead of print] (2).

Table 5. 2016 CPIC Dosing recommendations for amitriptyline based on both CYP2D6 and CYP2C19 phenotypes [a,b]

Phenotype	CYP2D6 Ultrarapid metabolizer	CYP2D6 Normal metabolizer	CYP2D6 Intermediate metabolizer	CYP2D6 Poor metabolizer
CYP2C19 ultrarapid or rapid metabolizer	Avoid amitriptyline use[c] Classification of recommendation[d]: Optional	Consider alternative drug not metabolized by CYP2C19[c,e] Classification of recommendation[d]: Optional	Consider alternative drug not metabolized by CYP2C19 [c,e] Classification of recommendation[d]: Optional	Avoid amitriptyline use[c] Classification of recommendation[d]: Optional
CYP2C19 normal metabolizer	Avoid amitriptyline use. If amitriptyline is warranted, consider titrating to a higher target dose (compared to normal metabolizers)[f,g] Classification of recommendation[d]: Strong	Initiate therapy with recommended starting dose[h] Classification of recommendation[d]: Strong	Consider a 25% reduction of recommended starting dose[f,h] Classification of recommendation[d]: Moderate	Avoid amitriptyline use. If amitriptyline is warranted, consider a 50% reduction of recommended starting dose[f,h] Classification of recommendation[d]: Strong

Table 5. continued from previous page.

Phenotype	CYP2D6 Ultrarapid metabolizer	CYP2D6 Normal metabolizer	CYP2D6 Intermediate metabolizer	CYP2D6 Poor metabolizer
CYP2C19 intermediate metabolizer	Avoid amitriptyline use[c] Classification of recommendation[d]: Optional	Initiate therapy with recommended starting dose[h] Classification of recommendation[d]: Strong	Consider a 25% reduction of recommended starting dose[f,h] Classification of recommendation[d]: Optional	Avoid amitriptyline use. If amitriptyline is warranted, consider a 50% reduction of recommended starting dose[f,h] Classification of recommendation[d]: Optional
CYP2C19 poor metabolizer	Avoid amitriptyline use[c] Classification of recommendation[d]: Optional	Avoid amitriptyline use. If amitriptyline is warranted, consider a 50% reduction of recommended starting dose[f,h] Classification of recommendation[d]: Moderate	Avoid amitriptyline use[c] Classification of recommendation[d]: Optional	Avoid amitriptyline use[c] Classification of recommendation[d]: Optional

[a] Dosing recommendations only apply to higher initial doses of TCAs for treatment of conditions such as depression.
[b] The dosing recommendations are based on studies focusing on amitriptyline. Because tricyclic antidepressants have comparable pharmacokinetic properties, it may be reasonable to apply these guidelines to other tertiary amines including clomipramine, doxepin, imipramine and trimipramine (the classification of this recommendation is optional).
[c] If amitriptyline is warranted, utilize therapeutic drug monitoring to guide dose adjustment.
[d] The rating scheme for the recommendation classification is described in Supplementary Data (2). See CYP2D6 and CYP2C19 combined dosing recommendations for explanation of classification of recommendations for this table.
[e] TCAs without major CYP2C19 metabolism include the secondary amines nortriptyline and desipramine.
[f] Utilizing therapeutic drug monitoring if a tricyclic is prescribed to a patient with CYP2D6 ultrarapid, intermediate or poor metabolism in combination with CYP2C19 ultrarapid, intermediate or poor metabolism is strongly recommended.
[g] Titrate dose to observed clinical response with symptom improvement and minimal (if any) side effects.
[h] Patients may receive an initial low dose of TCAs, which is then increased over several days to the recommended steady-state dose. The starting dose in this guideline refers to the recommended steady-state dose.
Table has been adapted from Hicks J.K., Sangkuhl K., Swen J.J., Ellingrod V.L., Müller D.J., Shimoda K., Bishop J.R., Kharasch E.D., Skaar T.C., Gaedigk A., Dunnenberger H.M., Klein T.E., Caudle K.E. Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC®) for *CYP2D6* and *CYP2C19* Genotypes and Dosing of Tricyclic Antidepressants: 2016 Update. Clinical pharmacology and therapeutics. 2016 Dec 20 [Epub ahead of print] (2).

Genetic Testing

Clinical genotyping tests are available for many CYP2D6 and *CYP2C19* alleles. The NIH's Genetic Testing Registry (GTR) provides a list of test providers for "amitriptyline response," and the CYP2D6 and CYP2C19 genes.

Results are typically reported as a diplotype, such as *CYP2D6 *1/*1*. A result for copy number, if available, is also important when interpreting *CYP2D6* results (29). However, it is important to note that the number of variants tested can vary among laboratories, which can result in diplotype result discrepancies between testing platforms and laboratories (30).

If the test results include an interpretation of the patient's predicted metabolizer phenotype, this should be confirmed by checking the diplotype and assigning an activity score to each allele (e.g., 0 for nonfunctional, 0.5 for reduced function, and 1 for each copy of a functional allele). The phenotype is defined by the sum of the two scores:

- A normal (previously referred to as "extensive") metabolizer phenotype has an activity score of 1 to 2
- An intermediate metabolizer has an activity score of 0.5

- A poor metabolizer has an activity score of 0
- An ultrarapid metabolizer has an activity score greater than 2 (2, 31)

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2016 Statement from the US Food and Drug Administration (FDA): The biochemical activity of the drug metabolizing isozyme cytochrome P450 2D6 (debrisoquin hydroxylase) is reduced in a subset of the caucasian population (about 7 to 10% of Caucasians are so called "poor metabolizers"); reliable estimates of the prevalence of reduced P450 2D6 isozyme activity among Asian, African and other populations are not yet available. Poor metabolizers have higher than expected plasma concentrations of tricyclic antidepressants (TCAs) when given usual doses. Depending on the fraction of drug metabolized by P450 2D6, the increase in plasma concentration may be small, or quite large (8 fold increase in plasma AUC of the TCA).

In addition, certain drugs inhibit the activity of this isozyme and make normal metabolizers resemble poor metabolizers. An individual who is stable on a given dose of TCA may become abruptly toxic when given one of these inhibiting drugs as concomitant therapy. The drugs that inhibit cytochrome P450 2D6 include some that are not metabolized by the enzyme (quinidine; cimetidine) and many that are substrates for P450 2D6 (many other antidepressants, phenothiazines, and the Type 1C antiarrhythmics propafenone and flecainide). While all the selective serotonin reuptake inhibitors (SSRIs), e.g., fluoxetine, sertraline, and paroxetine, inhibit P450 2D6, they may vary in the extent of inhibition. The extent to which SSRI-TCA interactions may pose clinical problems will depend on the degree of inhibition and the pharmacokinetics of the SSRI involved. Nevertheless, caution is indicated in the coadministration of TCAs with any of the SSRIs and also in switching from one class to the other. Of particular importance, sufficient time must elapse before initiating TCA treatment in a patient being withdrawn from fluoxetine, given the long half-life of the parent and active metabolite (at least 5 weeks may be necessary).

Concomitant use of tricyclic antidepressants with drugs that can inhibit cytochrome P450 2D6 may require lower doses than usually prescribed for either the tricyclic antidepressant or the other drug. Furthermore, whenever one of these other drugs is withdrawn from co-therapy, an increased dose of tricyclic antidepressant may be required. It is desirable to monitor TCA plasma levels whenever a TCA is going to be coadministered with another drug known to be an inhibitor of P450 2D6.

Please review the complete therapeutic recommendations that are located here: (1).

2016 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC):

CYP2D6 dosing recommendations.

[…]. The recommended starting dose of amitriptyline or nortriptyline does not need adjustment for those with genotypes predictive of CYP2D6 normal metabolism. A 25% reduction of the recommended dose may be considered for CYP2D6 intermediate metabolizers. The strength of this recommendation is classified as "moderate" because patients with a CYP2D6 activity score of 1.0 are inconsistently categorized as intermediate or normal metabolizers in the literature, making these studies difficult to evaluate.

CYP2D6 ultrarapid metabolizers have a higher probability of failing amitriptyline or nortriptyline pharmacotherapy due to subtherapeutic plasma concentrations, and alternate agents are preferred. There are documented cases of CYP2D6 ultrarapid metabolizers receiving large doses of nortriptyline in order to achieve

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

therapeutic concentrations. However, very high plasma concentrations of the nortriptyline hydroxy-metabolite were present, which may increase the risk for cardiotoxicity. If a tricyclic is warranted, there are insufficient data in the literature to calculate a starting dose for a patient with CYP2D6 ultrarapid metabolizer status, and therapeutic drug monitoring is strongly recommended. Adverse effects are more likely in CYP2D6 poor metabolizers due to elevated tricyclic plasma concentrations; therefore, alternate agents are preferred. If a tricyclic is warranted, consider a 50% reduction of the usual dose, and therapeutic drug monitoring is strongly recommended.

CYP2C19 dosing recommendations.

[…]. The usual starting dose of amitriptyline may be used in CYP2C19 normal and intermediate metabolizers. Although CYP2C19 intermediate metabolizers would be expected to have a modest increase in the ratio of amitriptyline to nortriptyline plasma concentrations, the evidence does not indicate that CYP2C19 intermediate metabolizers should receive an alternate dose.

Patients taking amitriptyline who are CYP2C19 rapid or ultrarapid metabolizers may be at risk for having low plasma concentrations and an imbalance between parent drug and metabolites causing treatment failure and/or adverse events. Although the CYP2C19*17 allele did not alter the sum of amitriptyline plus nortriptyline plasma concentrations, it was associated with higher nortriptyline plasma concentrations, possibly increasing the risk of adverse events. For patients taking amitriptyline, extrapolated pharmacokinetic data suggest that CYP2C19 rapid or ultrarapid metabolizers may need a dose increase. Due to the need for further studies investigating the clinical importance of CYP2C19*17 regarding tricyclic metabolism and the possibility of altered concentrations, we recommend to consider an alternative tricyclic or other drug not affected by CYP2C19. This recommendation is classified as optional due to limited available data. If amitriptyline is administered to a CYP2C19 rapid or ultrarapid metabolizer, therapeutic drug monitoring is recommended.

CYP2C19 poor metabolizers are expected to have a greater ratio of amitriptyline to nortriptyline plasma concentrations. The elevated amitriptyline plasma concentrations may increase the chance of a patient experiencing side effects. Use an alternative agent not metabolized by CYP2C19 (e.g., nortriptyline and desipramine) or consider a 50% reduction of the usual amitriptyline starting dose along with therapeutic drug monitoring.

Please review the complete therapeutic recommendations that are located here: (2).

2011 Summary of recommendations from the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

For CYP2D6 ultrarapid metabolizers:

The genetic polymorphism leads to increased metabolic capacity of CYP2D6, which may cause a decrease in the plasma concentrations of amitriptyline and its active metabolite nortriptyline and increased plasma concentrations of the active metabolites E-10-OH-amitriptyline and E-10-OH- nortriptyline.

Recommendation:

1. Choose an alternative if possible. Antidepressants that are not metabolized via CYP2D6 - or to a lesser extent - include, for example, citalopram and sertraline.
2. If an alternative is not an option: increase the dose to 1.25 times the standard dose, monitor the plasma concentrations and be alert to potential therapy failure due to decreased amitriptyline plus nortriptyline plasma concentrations and to increased plasma concentrations of the potentially cardiotoxic, active hydroxy metabolites.

For CYP2D6 intermediate metabolizers:

The genetic polymorphism leads to decreased metabolic capacity of CYP2D6, which may cause an increase in the plasma concentrations of amitriptyline and its active metabolite nortriptyline and decreased plasma concentrations of the active metabolites E-10-OH-amitriptyline and E-10-OH- nortriptyline.

Recommendation:

1. Choose an alternative if possible. Antidepressants that are not metabolized via CYP2D6 - or to a lesser extent - include, for example, citalopram and sertraline.
2. If an alternative is not an option: use 60% of the standard dose and monitor the plasma concentrations of amitriptyline and nortriptyline

As side effects are related to nortriptyline plasma concentrations and the efficacy to amitriptyline plus nortriptyline plasma concentrations, which are influenced to a lesser extent by CYP2D6, it is not known whether it is possible to reduce the dose to such an extent that the side effects disappear, but the efficacy is maintained.

For CYP2D6 poor metabolizers:

The genetic polymorphism leads to decreased metabolic capacity of CYP2D6, which may cause an increase in the plasma concentrations of amitriptyline and its active metabolite nortriptyline and decreased plasma concentrations of the active metabolites E-10-OH-amitriptyline and E-10-OH- nortriptyline.

Recommendation:

1. Choose an alternative if possible. Antidepressants that are not metabolized via CYP2D6 - or to a lesser extent - include, for example, citalopram and sertraline.
2. If an alternative is not an option: use 50% of the standard dose and monitor the plasma concentrations of amitriptyline and nortriptyline

As side effects are related to nortriptyline plasma concentrations and the efficacy to amitriptyline plus nortriptyline plasma concentrations, which are influenced to a lesser extent by CYP2D6, it is not known whether it is possible to reduce the dose to such an extent that the side effects disappear, but the efficacy is maintained.

Please review the complete therapeutic recommendations that are located here: (32).

Nomenclature

Nomenclature for selected *CYP2D6* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*4*	1846G>A	NM_000106.5:c.506-1G>A	Not applicable - variant occurs in a non-coding region	rs3892097
*CYP2D6*5*	Not applicable - variant results in a whole gene deletion			
*CYP2D6*6*	1707 del T Trp152Gly	NM_000106.5:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655
*CYP2D6*10*	100C>T Pro34Ser	NM_000106.5:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
*CYP2D6*17*	Includes at least two functional variants*: 1023C>T (Thr107Ile) 2850C>T (Cys296Arg)	NM_000106.5:c.320C>T NM_000106.5:c.886T>C	NP_000097.3:p.Thr107Ile NP_000097.3:p.Cys296Arg	rs28371706 rs16947
*CYP2D6*41*	2988G>A	NM_000106.5:c.985+39 G>A	Not applicable – variant occurs in a non-coding region	rs28371725

* In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.

Nomenclature for selected *CYP2C19* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C19*2*	681G>A Pro227Pro	NM_000769.1:c.681G>A	NP_000760.1:p.Pro227=	rs4244285
*CYP2C19*3*	636G>A Trp212Ter	NM_000769.1:c.636G>A	NP_000760.1:p.Trp212Ter	rs4986893
*CYP2C19*17*	-806C>T	NM_000769.2:c.-806C>T	Not applicable—variant occurs in a non-coding region	rs12248560

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: http://www.cypalleles.ki.se/

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Aripiprazole Therapy and *CYP2D6* Genotype

Laura Dean, MD[1]

Created: September 22, 2016.

Introduction

Aripiprazole is an atypical antipsychotic used in the management of schizophrenia, bipolar disorder, major depressive disorder, irritability associated with autistic disorder, and treatment of Tourette's disorder.

The metabolism and elimination of aripiprazole is mainly mediated through two enzymes, CYP2D6 and CYP3A4. Approximately 8% of Caucasians, 3–8% of Black/African Americans and up to 2% of Asians cannot metabolize CYP2D6 substrates and are classified as "poor metabolizers" (1).

The FDA-approved drug label for aripiprazole states that in CYP2D6 poor metabolizers, half of the usual dose should be administered. In CYP2D6 poor metabolizers who are taking concomitant strong CYP3A4 inhibitors (e.g., itraconazole, clarithromycin), a quarter of the usual dose should be used (Table 1) (2).

Table 1. The FDA-recommended dose adjustments for aripiprazole in patients who are known CYP2D6 poor metabolizers and patients taking concomitant CYP2D6 inhibitors, CYP3A4 inhibitors, and/or CYP3A4 inducers (2016)

Factors	Dosage Adjustments for ABILIFY
Known CYP2D6 Poor Metabolizers	Administer half of usual dose
Known CYP2D6 Poor Metabolizers taking concomitant strong CYP3A4 inhibitors (e.g., itraconazole, clarithromycin)	Administer a quarter of usual dose
Strong CYP2D6 (e.g., quinidine, fluoxetine, paroxetine) or CYP3A4 inhibitors (e.g., itraconazole, clarithromycin)	Administer half of usual dose
Strong CYP2D6 and CYP3A4 inhibitors	Administer a quarter of usual dose
Strong CYP3A4 inducers (e.g., carbamazepine, rifampin)	Double usual dose over 1 to 2 weeks

Table is adapted from a FDA-approved drug label for aripiprazole (2).

Drug: Aripiprazole

Aripiprazole is an atypical antipsychotic primarily used in the treatment of schizophrenia and bipolar disorder. Aripiprazole may also be used as part of the management of major depressive disorder, irritability associated with autism, and treatment of Tourette's disorder (2).

The first antipsychotics to be discovered in the 1950s were haloperidol and chlorpromazine. Known as "first-generation" or "typical" antipsychotics, these drugs are used to treat psychosis (regardless of the cause), chronic psychotic disorders (e.g., schizophrenia), and other psychiatric conditions. However, prominent adverse effects included extrapyramidal side effects such as tardive dyskinesia, muscle rigidity, tremors, and Parkinsonian-like symptoms.

Newer antipsychotics, known as "second generation" or "atypical" antipsychotics, have a lower risk of extrapyramidal side effects such as tardive dyskinesia. However, many have serious metabolic effects. Aripiprazole is an atypical antipsychotic that is noted for having fewer metabolic side effects than other atypicals, such as clozapine, olanzapine, risperidone, and quetiapine. Other atypicals currently approved by the FDA include asenapine, brexpiprazole, cariprazine, lurasidone, paliperidone, and ziprasidone.

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The main action of both first-generation and second-generation antipsychotics is thought to be the post-synaptic blockade of D2 dopamine receptors. All antipsychotics, with the exception of aripiprazole, are D2 antagonists.

Aripiprazole is a partial D2 agonist. Aripiprazole binds to the D2 receptor with a high affinity similar to dopamine. However, because it has low intrinsic activity, it causes much lower activation of the receptor compared to dopamine.

The combination of a high affinity for the D2 receptor and its partial agonist activity may result in aripiprazole reducing the high-frequency firing of dopamine neurons in the brain's mesolimbic system. Overactivity in this region is thought to underlie psychosis and other positive symptoms of schizophrenia. In addition, the preservation of some D2 receptor activity in other dopamine-rich pathways in the brain (mesocortical and nigrostriatal areas) may provide more protection against extrapyramidal side effects (3, 4).

Aripiprazole also has a high affinity for the serotonin 5-HT_{2A} receptors, where it acts as an antagonist and it moderately blocks the alpha 1 adrenergic and histamine H1 receptors, which may account for the lower incidence of orthostatic hypotension and sedation compared to other antipsychotics (5).

Adverse events to aripiprazole include increased mortality in elderly patients with psychosis caused by dementia, suicidal thoughts and behavior in children and young adults, neuroleptic malignant syndrome, and tardive dyskinesia (2).

Aripiprazole is extensively metabolized in the liver by CYP450 enzymes, mainly CYP2D6 and CYP3A4. Aripiprazole activity is thought to be primarily due to the parent drug, and to a lesser extent its major metabolite, dehydro-aripiprazole. The mean elimination half-life is about 75 hours for aripiprazole, but in individuals who have no appreciable CYP2D6 activity (poor metabolizers), the mean elimination half-life for aripiprazole is about 146 hours.

Genetic variations in the *CYP2D6* gene have been found to impact serum levels of aripiprazole (6, 7). Because standard doses of aripiprazole lead to higher plasma levels of aripiprazole and dehydro-aripiprazole, the dose of aripiprazole should be adjusted in subjects carrying two nofunction alleles causing poor metabolizer status.

The FDA recommends that patients who are known to be CYP2D6 poor metabolizers should receive half the standard dose of aripiprazole, or a quarter of the standard dose if they are also taking medicines that strongly inhibit CYP3A4 (e.g., itraconazole, clarithromycin) (See Table 1).

A recent study substantiates the FDA recommendations by concluding that poor metabolizers should receive a reduced dose of aripiprazole (30–50% reduction). This study also suggested that individuals with increased CYP2D6 activity (ultrarapid metabolizers) may need to take an alternative antipsychotic not metabolized by CYP2D6 because of reduced drug levels (8).

The cytochrome P450 superfamily

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP450 genes are highly polymorphic and can result in no, decreased, normal or increased enzyme activity.

Gene: *CYP2D6*

CYP2D6 is highly polymorphic, with over 100 star (*) alleles described (9). *CYP2D6*1* is the reference (or wild-type) allele encoding enzyme with normal activity. The *CYP2D6*2*, **33*, and **35* alleles are also considered to confer normal activity (Table 2).

Table 2. Activity status of selected *CYP2D6* alleles

Allele type	*CYP2D6* Alleles
Increased	**1xN, *2xN* (*xN* denoting gene duplication or multiplication)
Normal	**1, *2, *35*
Decreased activity	**9, *10, *17, *29, *36, *41*
Inactive	**3-*8, *11-*16, *18-*21, *38, *40, *42*

For a detailed list of *CYP2D6* alleles, please see (9).

Individuals who have more than two normal function copies of the *CYP2D6* gene are "ultrarapid metabolizers," whereas individuals who carry two normal or one normal and one decreased function allele are classified as "normal metabolizers." Subjects with one normal and one no function allele or two decreased function alleles are categorized as "normal metabolizers" by CPIC guidelines, but have also been categorized as "intermediate metabolizers" in the literature. Subjects with one decreased and one no function allele are predicted to be intermediate metabolizers and those with two no function alleles, as mentioned above, are poor metabolizers.

The most common no function alleles include *CYP2D6*3*, **4*, **5*, and **6* (10, 11), and the most common decreased function alleles include *CYP2D6*9, *10, *17, *29* and **41* (Table 2). There are large inter-ethnic differences in the frequency of these alleles. For example, *CYP2D6*4* is the most common no function allele in Caucasians, but less abundant in subjects with African ancestry, and rare in Asians. In contrast, the decreased function allele *CYP2D6*10* is the most common allele in Asians, and *CYP2D6*17* is almost exclusively found in individuals with African ancestry (1). Consequently, the phenotype frequencies also vary substantially among the major ethnicities and may vary among populations. Approximately 6-10% of European Caucasians and their descendants are poor metabolizers, mainly due to the prevalent no function *CYP2D6*4* and **5* alleles (12, 13).

Gene: *CYP3A4*

In contrast to *CYP2D6*, genetic variation cannot explain *CYP3A4* variability. Although 26 allelic variants are currently described, the majority have not been shown to alter CYP3A4 activity (14, 15). To date, only three no function *CYP3A4* alleles, all being rare, have been identified (*CYP3A4*6, CYP3A4*20* and *CYP3A4*26*) (16, 17). The *CYP3A4*20* allele, for example, has been reported to have a frequency of about 0.2% in European Americans and 0.05% in African Americans, while it was observed at a frequency of 1.2% in Spain; notably, it reached up to 3.8% in specific Spanish regions (16). Although a decreased function allele, *CYP3A4*22*, has been associated with tacrolimus dose requirements (18), its clinical utility warrants further investigation.

Genetic Testing

Genetic testing for *CYP2D6* and *CYP3A4* is available. Test panels may include tests for additional genes involved in drug metabolism including aripiprazole. For tests available to predict CYP2D6 activity to optimize aripiprazole therapy (i.e., adjust dosage or opt for an alternative drug) please see the Genetic Testing Registry.

Results are typically reported as a diplotype, such as *CYP2D6 *1/*1*. A result for copy number, if available, is also important when interpreting *CYP2D6* results (19).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

2016 Statement from the US Food and Drug Administration (FDA): Dosage adjustments are recommended in patients who are known CYP2D6 poor metabolizers and in patients taking concomitant CYP3A4 inhibitors or CYP2D6 inhibitors or strong CYP3A4 inducers (see Table 1). When the coadministered drug is withdrawn from the combination therapy, aripiprazole dosage should then be adjusted to its original level. When the coadministered CYP3A4 inducer is withdrawn, aripiprazole dosage should be reduced to the original level over 1 to 2 weeks. Patients who may be receiving a combination of strong, moderate, and weak inhibitors of CYP3A4 and CYP2D6 (e.g., a strong CYP3A4 inhibitor and a moderate CYP2D6 inhibitor or a moderate CYP3A4 inhibitor with a moderate CYP2D6 inhibitor), the dosing may be reduced to one-quarter (25%) of the usual dose initially and then adjusted to achieve a favorable clinical response.

Please review the complete therapeutic recommendations that are located here: (2).

2011 Summary of recommendations from the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP): In CYP2D6 poor metabolizers, reduce the maximum dose of aripiprazole to 10 mg/day (67% of the maximum recommended daily dose).

Please review the complete therapeutic recommendations that are located here: (20).

Nomenclature

Nomenclature of selected *CYP2D6* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*4*	1846G>A	NM_000106.5:c.506-1G>A	Variant occurs in a non-coding region (splice variant causes a frameshift)	rs3892097
*CYP2D6*5*	Variant results in a whole gene deletion			
*CYP2D6*6*	1707 del T Trp152Gly • CYP2D6T	NM_000106.5:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655
*CYP2D6*10*	100C>T (Pro34Ser)	NM_000106.5:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
*CYP2D6*17*	1023C>T[1] (Thr107Ile)	NM_000106.5:c.320C>T	NP_000097.3:p.Thr107Ile	rs28371706
	2850C>T[1] (Cys296Arg)	NM_000106.5:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
*CYP2D6*41*	2850C>T[2] (Cys296Arg)	NM_000106.5:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
	2988G>A	NM_000106.5:c.985+39G>	Variant occurs in a non-coding region (impacts slicing).	rs28371725

[1] In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.
[2] In the literature, 2850C>T is also referred to as 2938C>.

Nomenclature of selected *CYP3A4* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP3A4*6*	17661_17662insA 277Frameshift	NM_017460.5:c.830_831insA	NP_059488.2:p.Asp277Glufs	rs4646438
*CYP3A4*20*	1461_1462insA 488Frameshift	NM_017460.5:c.1461_1462insA	NP_001189784.1:p.Pro487Thrfs	rs67666821

Nomenclature of selected continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP3A4*26*	17633C>T R268Stop			

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines
Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: http://www.cypalleles.ki.se/

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Atomoxetine Therapy and *CYP2D6* Genotype

Laura Dean, MD[1]

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Introduction

Atomoxetine was the first non-stimulant drug to be used in the treatment of attention-deficit hyperactivity disorder (ADHD). Atomoxetine is a selective noradrenaline reuptake inhibitor, and is part of a treatment plan for ADHD that may include other measures such as psychological, educational, and social support.

The CYP2D6 enzyme metabolizes a quarter of all prescribed drugs, including atomoxetine. Individuals who carry two nonfunctional copies of the *CYP2D6* gene are known as poor metabolizers and have higher plasma concentrations of atomoxetine compared with individuals who have two copies of normal activity alleles.

The FDA states that the dose of atomoxetine may need to be adjusted in patients known to be *CYP2D6* poor metabolizers (1). A guideline from The Dutch Pharmacogenetics Working Group includes the recommendation that poor metabolizers can be given the standard dose of atomoxetine, but physicians should be aware of adverse drug events. They also state that for individuals who have more than two functional gene copies of *CYP2D6*, i.e., individuals with so-called ultrarapid metabolizer status, physicians should either be alert to reduced efficacy with the standard dose of atomoxetine, or they should prescribe an alternative drug, such as methylphenidate or clonidine (Table 1) (2).

Table 1. *CYP2D6* phenotypes and the therapeutic recommendations for atomoxetine therapy

Phenotype	Genotype	Recommendations for atomoxetine therapy
Ultrarapid metabolizer	Three or more functional gene copies	Insufficient data to allow calculation of dose adjustment. Be alert to reduced efficacy or select alternative drug (e.g., methylphenidate, clonidine).
Extensive metabolizer	Two functional gene copies	No recommendations.
Intermediate metabolizer	One active allele and one inactive allele, or two decreased activity alleles, or one decreased activity allele and one inactive allele	No recommendations.
Poor metabolizer	Two inactive alleles	Standard dose. Dose increase probably not necessary; be alert to adverse drug events.

The level of evidence for the therapeutic (dose) recommendations is 3/4 (“moderate quality”) for poor metabolizers, and 4/4 (“good quality”) for intermediate metabolizers. There are no data for ultrarapid metabolizers. The Table is adapted from Swen J.J., Nijenhuis M., de Boer A., Grandia L. et al. Pharmacogenetics: from bench to byte - an update of guidelines. Clinical pharmacology and therapeutics. 2011;89(5):662–73 (2).

Table 2. Activity status of selected *CYP2D6* alleles

Allele type	Alleles
Normal function	**1, *2, *33, *35*
Decreased function	**9, *10, *17, *29, *41*
No function	**3, *4, *5, *6, *7, *8*

For a more detailed list of CYP2D6 alleles, please see (3).

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Drug: Atomoxetine

Atomoxetine is used in the treatment of attention-deficit hyperactivity disorder (ADHD), which is one of the most common childhood disorders. Symptoms include difficulty focusing and paying attention, difficulty controlling behavior, and hyperactivity. Symptoms may continue in to adulthood. Atomoxetine may be used alone or in combination with behavioral treatment, as an adjunct to psychological, educational, social, and other remedial measures.

Atomoxetine was the first non-stimulant drug to be approved for use in ADHD. Atomoxetine is a selective norepinephrine reuptake inhibitor and it is thought to exert its therapeutic effect by increasing the concentration of synaptic norepinephrine. Because it is a non-stimulant, atomoxetine has the advantages of having less potential for abuse, and it is not scheduled as a controlled substance (4).

Atomoxetine is primarily metabolized through the CYP2D6 enzymatic pathway. The main metabolite, 4-hydroxyatomoxetine, is equipotent to atomoxetine as an inhibitor of the norepinephrine transport, but is found at much lower levels in the plasma (5). In individuals who lack CYP2D6 activity (poor metabolizers), 4-hydroxyatomoxetine is formed by other CYP enzymes, but at a much slower rate (1).

CYP2C19, along other CYP enzymes, forms the metabolite N-Desmethylatomoxetine. Although this metabolite has substantially less pharmacological activity compared to atomoxetine, and is present at much lower plasma concentrations, one study found that genetic polymorphisms of the *CYP2C*19 gene also influenced the pharmacokinetics of atomoxetine (6).

Atomoxetine has a wide therapeutic window, but the risk of adverse effects may be increased by the presence of *CYP2D6* genetic variants (7-9). Common adverse effects of atomoxetine therapy include weight loss, headache, and irritability. Psychiatric side effects may also occur; these include anxiety, depression, and the development of suicidal thoughts.

The FDA-approved drug label for atomoxetine includes a boxed warning and additional warning statements regarding the increased risk of suicidal thinking in children and adolescents treated with atomoxetine. The warning includes: "Children and teenagers sometimes think about suicide, and many report trying to kill themselves. Results from atomoxetine clinical studies with over 2200 child or teenage ADHD patients suggest that some children and teenagers may have a higher chance of having suicidal thoughts or actions. Although no suicides occurred in these studies, 4 out of every 1000 patients developed suicidal thoughts."(1)

Gene: *CYP2D6*

The cytochrome P450 superfamily (CYP) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The *CYP* genes are often polymorphic and can result in no decreased or increased activity impacting drug metabolism.

CYP2D6 is responsible for the metabolism of many commonly prescribed drugs, including antidepressants, antipsychotics, analgesics, and beta-blockers. The *CYP2D6* gene is highly polymorphic—more than 100 alleles have been described (10).

*CYP2D6*1* is the wild-type allele and is associated with normal enzyme activity and the normal "extensive metabolizer" phenotype. The *CYP2D6* alleles **2*, **33*, and **35*, among others, are also considered to have normal activity (11, 12).

Individuals who have multiple functional copies of the *CYP2D6* gene are known as "ultrarapid metabolizers" (UM) (Table 1). Because each *CYP2D6* allele contributes to the metabolism and inactivation of atomoxetine, atomoxetine may have decreased efficacy in UM individuals (2). The UM phenotype is estimated to be present in

up to 28% of North Africans, Ethiopians, and Arabs; up to 10% in Caucasians; 3% in African Americans, and up to 1% in Hispanics, Chinese, and Japanese (13).

The Dutch Pharmacogenetics Working Group recommendations state that for ultrarapid metabolizers, there are insufficient data to allow for an adjusted dose to be calculated, and therefore, the physician should be alert to reduced efficacy of a standard dose of atomoxetine, or prescribe an alternative drug, such as methylphenidate or clonidine.

The most common non-functional and reduced function *CYP2D6* alleles include *CYP2D6*3*, **4*, **5*, and **6* (2, 10, 11, 13-16) and *CYP2D6*10*, **17* and **41*) (4, 12, 17-19) (Table 2). There are large inter-ethnic differences in the frequency of these alleles, with **3*, **4*, **5*, **6*, and **41* being more common in Caucasians, **17* more common in Africans, and **10* more common in Asians (20).

Individuals who are intermediate or poor metabolizers carry copies of reduced-activity or non-functioning *CYP2D6* alleles (see Table 1 and 2). In these individuals, the metabolic capacity of CYP2D6 is decreased which may result in higher levels of atomoxetine. The FDA-approved drug label for atomoxetine states that poor metabolizers of CYP2D6 have a higher exposure to atomoxetine (10-fold higher area under the cover and a 5 fold-higher peak concentration) compared to extensive metabolizers who received the same dose. The label also states that in individuals who are known to be poor metabolizers, the dose of atomoxetine should be adjusted—treatment should be initiated at 0.5mg/kg/day and only increased to the usual target dose of 1.2 mg/kg/day if symptoms fail to improve after 4 weeks and the initial dose is well tolerated (see Therapeutic Recommendations) (1). However, the Dutch Pharmacogenetics Working Group recommendations state that for poor metabolizers, "a standard dose of atomoxetine is recommended. An increase in dose is probably not necessary, but the physician should be alert to adverse drug events. (2)"

One small study of 100 children with ADHD receiving atomoxetine therapy found that the presence of at least one nonfunctional or reduced function *CYP2D6* allele led to an increase in adverse effects, such as gastrointestinal problems and sleep disorders, and a late response to treatment (longer than 9 weeks). The study concluded that *CYP2D6* genotyping before atomoxetine treatment may be beneficial in preventing overdosing or early cessation of treatment because of initial adverse effects (21). However, another study found genotyping to be unnecessary, because during the routine clinical management of ADHD, investigators were able to adjust the dose of atomoxetine in children and adolescents who had normal or reduced CYP2D6 activity—so that their treatment was comparable in safety and efficacy—without knowing what their *CYP2D6* genotype was (22).

Poor metabolizers are commonly found in European Caucasians and their descendants (6-10%). The most common alleles in this population are the functional *CYP2D6*1* and **2* alleles (70%); the remaining alleles include *CYP2D6*10* and **41* conveying decreased function and the nonfunctional *CYP2D6*3, *4, *5* and **6* variants that largely account for the poor metabolizer phenotype in these populations (12). About 2-5% of African Americans are poor metabolizers, due to the presence of *CYP2D6*4 and *5* and a number of other nonfunctional alleles (1, 11, 15, 18, 20).

Approximately 30% of Asians and individuals of Asian descent are intermediate metabolizers. In these populations, 40-60% of individuals carry *CYP2D6*10, a* decreased function variant (only ~2-3% of Caucasians have this allele) (23, 24). As a result, Asians are more likely to have decreased CYP2D6 activity compared to Caucasians (12). Neither the FDA-approved drug label of the Dutch Pharmacogenetic Working Group gives dosing recommendations for subjects with decreased function alleles, often classified as intermediate metabolizers.

Genetic Testing

CYP2D6 genetic testing is available. Usually a patient's result is reported as a diplotype, such as *CYP2D6*1/*1* or **2/*4*. A result for copy number is also important when interpreting results for this gene. However, it needs to be

noted that the number of variants tested varies substantially among laboratories and there is no standardized way to report results (25).

If the test results include an interpretation of the patient's predicted metabolizer phenotype, this should be confirmed by checking the diplotype and assigning an activity score to each allele (e.g., 0 for nonfunctional, 0.5 for reduced function, and 1 for each copy of a functional allele). The phenotype is defined by the sum of the two scores:

- An extensive (normal) metabolizer phenotype has an activity score of 1 to 2
- An intermediate metabolizer has an activity score of 0.5
- A poor metabolizer has an activity score of 0
- An ultrarapid metabolizer has an activity score greater than 2 (3, 19, 26).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

Statement from the US Food and Drug Administration (FDA):

Dosing adjustment for use with a strong CYP2D6 inhibitor or in patients who are known to be CYP2D6 PMs[2] — In children and adolescents up to 70 kg body weight administered strong CYP2D6 inhibitors, e.g., paroxetine, fluoxetine, and quinidine, or in patients who are known to be CYP2D6 PMs, atomoxetine should be initiated at 0.5 mg/kg/day and only increased to the usual target dose of 1.2 mg/kg/day if symptoms fail to improve after 4 weeks and the initial dose is well tolerated.

In children and adolescents over 70 kg body weight and adults administered strong CYP2D6 inhibitors, e.g., paroxetine, fluoxetine, and quinidine, atomoxetine should be initiated at 40 mg/day and only increased to the usual target dose of 80 mg/day if symptoms fail to improve after 4 weeks and the initial dose is well tolerated. **Please review the complete therapeutic recommendations that are located here:** (1)

Summary of recommendations from the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP): For individuals who are poor metabolizers, a standard dose of atomoxetine is recommended. An increase in dose is probably not necessary, but the physician should be alert to adverse drug events. For individuals who are ultrarapid metabolizers, there are insufficient data to allow for an adjusted dose to be calculated. The physician should be alert to reduced efficacy of a standard dose of atomoxetine, or prescribe an alternative drug, such as methylphenidate or clonidine.

Please review the complete therapeutic recommendations that are located here: (2)

Nomenclature

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*4*	1846G>A	NM_000106.4:c.506-1G>A	Not applicable—variant occurs in a non-coding region	rs3892097

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labelled all formulations containing the generic drug.

[2] PMs: Poor metabolizers

Table continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*5*	CYP2D6,DEL	NC_000022.10:g.(42534124_42531353)_(42521970_42519196)del	Not applicable—variant results in a whole gene deletion	
*CYP2D6*6*	1707 del T Trp152Gly	NM_000106.4:c.454delT	NP_000097.2:p.Trp152Glyfs	rs5030655
*CYP2D6*10*	100C>T Pro34Ser	NM_000106.4:c.100C>T	NP_000097.2:p.Pro34Ser	rs1065852
*CYP2D6*17*	Includes at least two functional variants*: 1023C>T (Thr107Ile) 2850C>T (Cys296Arg)	NM_000106.4:c.320C>T NM_000106.4:c.886T>C	NP_000097.2:p.Thr107Ile NP_000097.2:p.Cys296Arg	rs28371706 rs16947
*CYP2D6*41*	2988G>A	NM_000106.4:c.985+39G>A	Not applicable—variant occurs in a non-coding region	rs28371725

* In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: http://www.cypalleles.ki.se/

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Azathioprine Therapy and *TPMT* Genotype

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Introduction

Azathioprine is an immunosuppressant that belongs to the drug class of thiopurines. It is used in combination with other drugs to prevent kidney transplant rejection and in the management of rheumatoid arthritis when other treatments have not been effective (1). In addition, off-label uses include the treatment of inflammatory bowel disease (2).

Azathioprine is a prodrug that must first be activated to form thioguanine nucleotides (TGNs), the major active metabolites. Thiopurine S-methyltransferase (TPMT) inactivates azathioprine, leaving less parent drug available to form TGNs.

An adverse effect of azathioprine therapy is bone marrow suppression, which can occur in any patient, is dose-dependent, and may be reversed by reducing the dose of azathioprine. However, patients who carry two nonfunctional *TPMT* alleles universally experience life-threatening myelosuppression when treated with azathioprine, due to high levels of TGNs. Patients who carry one nonfunctional *TPMT* allele may also be unable to tolerate conventional doses of azathioprine (3, 4).

The FDA recommends *TPMT* genotyping or phenotyping before starting treatment with azathioprine. This allows patients who are at increased risk for toxicity to be identified and for the starting dose of azathioprine to be reduced, or for an alternative therapy to be used (1).

The Clinical Pharmacogenetics Implementation Consortium (CPIC) has published recommendations for *TPMT* genotype-based azathioprine dosing. These recommendations include:

Consider an alternate agent or extreme dose reduction of azathioprine for patients with low or deficient TPMT activity. Start at 30-70% of target dose for patients with intermediate enzyme activity (see Table 1) (2-4).

Table 1. *TPMT* phenotypes and the therapeutic recommendations for azathioprine therapy, adapted from CPIC

Phenotype	Phenotype details	*TPMT* Genotype	Examples of diplotypes	Therapeutic recommendations for azathioprine
Homozygous wild-type ("normal")	High enzyme activity. Found in ~86–97% of patients.	Two or more functional *TPMT* alleles	**1/*1*	Start with normal starting dose (e.g., 2–3 mg/kg/d) and adjust doses of azathioprine based on disease-specific guidelines. Allow 2 weeks to reach steady state after each dose adjustment.
Heterozygous	Intermediate enzyme activity. Found in ~3–14% of patients.	One functional *TPMT* allele plus one nonfunctional *TPMT* allele	**1/*2* **1/*3A* **1/*3B* **1/*3C* **1/*4*	If disease treatment normally starts at the "full dose", consider starting at 30–70% of target dose (e.g., 1–1.5 mg/kg/d), and titrate based on tolerance. Allow 2–4 weeks to reach steady state after each dose adjustment.

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Table 1. continued from previous page.

Phenotype	Phenotype details	*TPMT* Genotype	Examples of diplotypes	Therapeutic recommendations for azathioprine
Homozygous variant	Low or deficient enzyme activity. Found in ~1 in 178 to 1~3736 patients.	Two nonfunctional *TPMT* alleles	**3A/*3A* **2/*3A* **3C/*3A* **3C/*4* **3C/*2* **3A/*4*	Consider alternative agents. If using azathioprine start with drastically reduced doses (reduce daily dose by 10-fold and dose thrice weekly instead of daily) and adjust doses of azathioprine based on degree of myelosuppression and disease-specific guidelines. Allow 4–6 weeks to reach steady state after each dose adjustment. Azathioprine is the likely cause of myelosuppression.

The strength of therapeutic recommendations is "strong" for all phenotypes.
Table is adapted from Relling M.V. et al. Clinical Pharmacogenetics Implementation Consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing. Clinical pharmacology and therapeutics. 2011;89(3):387–91 (3, 4).

Drug Class: Thiopurines

Thiopurines are used as anticancer agents and as immunosuppressants in inflammatory bowel disease, rheumatoid arthritis, and other autoimmune conditions. Three thiopurines are used clinically: thioguanine, mercaptopurine, and azathioprine (a prodrug for mercaptopurine). All three agents have similar effects but are typically used for different indications. Thioguanine is most commonly used in the treatment of myeloid leukemias, mercaptopurine is used for lymphoid malignancies, and mercaptopurine and azathioprine are used for immune conditions.

Thiopurines are either activated to form TGNs (the major active metabolite) or deactivated by TPMT. Individuals who carry two non-functional *TPMT* alleles ("*TPMT* homozygotes") universally experience life-threatening bone marrow suppression because of high levels of TGNs when treated with conventional doses. Individuals who carry one non-functional *TPMT* allele ("*TPMT* heterozygotes") may also be unable to tolerate conventional doses of thiopurines due to increased levels of TGNs.

Drug: Azathioprine

Azathioprine is an immunosuppressive agent that is used in combination with other drugs to prevent the rejection of kidney transplants. It is also used in the management of active rheumatoid arthritis (1).

An off-label use of azathioprine is in the treatment of inflammatory bowel disease (IBD). Along with the closely related drug mercaptopurine (azathioprine is metabolized to mercaptopurine), azathioprine is used as an "immunomodulator" and as a "steroid-sparing agent" in the treatment of Crohn's disease and ulcerative colitis (2).

Azathioprine is a slow-acting drug and for IBD, it typically takes at least three months of therapy before a therapeutic effect is observed. Therefore, azathioprine is used for the induction and maintenance of IBD remission rather than as a monotherapy for acute relapses (5). Because the discontinuation of azathioprine is associated with a high rate of relapse of IBD, azathioprine is usually continued long-term if there are no adverse effects (6, 7).

The use of azathioprine or the related drug mercaptourine has been associated with a 4-fold increased risk of developing lymphoma, which does not persist after discontinuation of therapy (8, 9).

The increased risk of malignancy led to the following boxed label on the FDA-approved drug label for azathioprine:

Malignancy: Patients receiving immunosuppressants, including azathioprine, are at increased risk of developing lymphoma and other malignancies, particularly of the skin. Physicians should inform patients of the risk of malignancy with azathioprine. As usual for patients with increased risk for skin cancer, exposure to sunlight and ultraviolet light should be limited by wearing protective clothing and using a sunscreen with a high protection factor (1).

Like all thiopurines, azathioprine is a purine analogue, and acts as an antimetabolite by interfering with nucleic acid synthesis and inhibiting purine metabolism. Azathioprine is first metabolized to mercaptopurine, which is then activated via HPRT1 (hypoxanthine phosphoribosyltransferase). This is followed by a series of reactions to form TGNs. The cytotoxicity of azathioprine is due, in part, to the incorporation of TGNs into DNA.

Inactivation of azathioprine occurs via two different pathways, via methylation (by TPMT) or via oxidation (by xanthine oxidase). TPMT activity is highly variable in patients because of genetic polymorphism in the *TPMT* gene.

One of the most frequent adverse reactions to azathioprine is myelosuppression, which can occur in any patient, and can usually be reversed by decreasing the dose of azathioprine. However, all patients who carry two nonfunctional *TPMT* alleles (approximately 0.3%) experience life-threatening myelosuppression after starting treatment with conventional doses of azathioprine due to high levels of TGNs.

Individuals who are heterozygous for nonfunctional *TPMT* alleles (approximately 10%) are at a significantly higher risk for toxicity than individuals with two functional alleles. However, some of these individuals, approximately 40–70%, can tolerate the full dose of azathioprine. This may be because heterozygous-deficient individuals have lower concentrations of less active metabolites, such as MeMPN (methylmercaptopurine nucleotides), than homozygous-deficient individuals (3, 4).

Approximately 90% of individuals have normal TPMT activity with two functional alleles; however, all individuals receiving azathioprine require close monitoring (3, 4, 10, 11). One study reports that in patients with IBD receiving thiopurine therapy, TPMT polymorphisms are associated with the overall incidence of adverse reactions and with bone marrow toxicity, but not with other adverse reactions, such as liver damage and pancreatitis. Therefore, although determining *TPMT* genotype is helpful before initiating therapy, regular blood tests to monitor for side effects are needed during therapy (12, 13).

The other azathioprine inactivation pathway is via oxidation, which is catalyzed by xanthine oxidase. If this pathway is inhibited, for example, in patients taking allopurinol (an inhibitor of xanthine oxidase), the decreased break down of azathioprine can lead to azathioprine toxicity (13). However, some studies have found that the co-administration of allopurinol, with a reduced dose of azathioprine (or mercaptopurine), can help optimize the treatment response in patients with IBD (14, 15).

Gene: *TPMT*

The *TPMT* gene encodes one of the important enzymes of phase II metabolism, thiopurine S-methyltransferase. TPMT is one of the main enzymes involved in the metabolism of thiopurines, such as azathioprine. TPMT activity is inherited as a co-dominant trait, as the *TPMT* gene is highly polymorphic with over 40 reported variant alleles (16-19).

The wild-type *TPMT*1* allele is associated with normal enzyme activity. Individuals who are homozygous for *TPMT*1* (TPMT normal metabolizers) are more likely to have a typical response to azathioprine and a lower risk of myelosuppression. This accounts for the majority of patients (~86–97%) (3, 4).

Individuals who are TPMT poor (approximately 0.3%) or intermediate (approximately 3–14%) metabolizers carry variant *TPMT* alleles that encode reduced or absent enzyme activity. Three variant *TPMT* alleles account for over 90% of the reduced or absent activity *TPMT* alleles (16, 17):

- *TPMT*2* (c.238G>C)
- *TPMT*3A* (c.460G>A and c.719A>G)
- *TPMT*3B* (c.460G>A)
- *TPMT*3C* (c.719A>G)

The frequency of *TPMT* alleles varies among different populations. In the United States, the most common low-activity allele in the Caucasian population is *TPMT*3A* (~5%). This allele is also found in individuals who originate from India and Pakistan, but less frequently (18, 19).

In East Asian, African-American, and some African populations, the most common variant is *TPMT*3C* (~2%), although *TPMT*8* may be more common in African populations than previously thought (~2%). In general, *TPMT*2* occurs much less commonly, and *TPMT*3B* occurs rarely (18, 20).

Genetic Testing

Genetic testing is available for several *TPMT* variant alleles, which most commonly includes *TPMT*2*, **3A*, and **3C* as they account for >90% of inactivating alleles. Of note, rare and/or previously undiscovered variants will not be detected by variant-specific genotyping methods (3, 4, 21-24).

TPMT phenotype enzyme activity testing is also available by measuring TPMT activity in red blood cells directly. However, the results will not be accurate in patients who have received recent blood transfusions (13) and TPMT activity will also be falsely low in patients with leukemia, because of atypical hematopoiesis (25).

One study reported that *TPMT* genotyping was more reliable than phenotyping in identifying patients at risk of adverse reactions from thiopurine treatment (26). In addition, several studies report that the *TPMT* genotype is a better indicator than TPMT activity for predicting TGN accumulation or treatment outcome (11, 27-29).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2015 Statement from the US Food and Drug Administration (FDA): TPMT TESTING CANNOT SUBSTITUTE FOR COMPLETE BLOOD COUNT (CBC) MONITORING IN PATIENTS RECEIVING AZATHIOPRINE. TPMT genotyping or phenotyping can be used to identify patients with absent or reduced TPMT activity. Patients with low or absent TPMT activity are at an increased risk of developing severe, life threatening myelotoxicity from azathioprine if conventional doses are given. Physicians may consider alternative therapies for patients who have low or absent TPMT activity (homozygous for non-functional alleles). Azathioprine should be administered with caution to patients having one non-functional allele (heterozygous) who are at risk for reduced TPMT activity that may lead to toxicity if conventional doses are given. Dosage reduction is recommended in patients with reduced TPMT activity. Early drug discontinuation may be considered in patients with abnormal CBC results that do not respond to dose reduction.

Please review the complete therapeutic recommendations that are located here: (1).

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labelled all formulations containing the generic drug.

2013 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC): Testing for *TPMT* status is recommended prior to starting azathioprine therapy so that the starting dosages can be adjusted accordingly—see Table 1 for dosing recommendations. In homozygous variant individuals, either an alternative agent should be used, or the doses of azathioprine should be drastically reduced. In heterozygous individuals, depending on the disease being treated, starting doses should be reduced. In both patient groups, a longer period of time should be left after each dose adjustment to allow for a steady state to be reached.

Please review the complete therapeutic recommendations that are located here: (3, 4).

Nomenclature

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*TPMT*2*	238G>C Ala80Pro	NM_000367.2:c.238G>C	NP_000358.1:p.Ala80Pro	rs1800462
*TPMT*3A*	This allele contains two variants in cis: c.460G>A and c.719A>G			
*TPMT*3B*	460G>A Ala154Thr	NM_000367.2:c.460G>A	NP_000358.1:p.Ala154Thr	rs1800460
*TPMT*3C*	719A>G Tyr240Cys	NM_000367.2:c.719A>G	NP_000358.1:p.Tyr240Cys	rs1142345

The TPMT Nomenclature Committee defines the nomenclature and numbering of novel TPMT variants: http://www.imh.liu.se/tpmtalleles

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

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Brivaracetam Therapy and CYP2C19 Genotype

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Introduction

Brivaracetam (brand name Briviact) is an antiseizure drug used in the treatment of partial-onset (focal) epilepsy in adults. It is thought to act by binding to a synaptic vesicle glycoprotein, SV2A, and reducing the release of neurotransmitters.

Brivaracetam is primarily metabolized by hydrolysis, via amidase enzymes, to an inactive metabolite. To a lesser extent, it is also metabolized by a minor metabolic pathway via CYP2C19-dependent hydroxylation.

Individuals who have no CYP2C19 enzyme activity, "CYP2C19 poor metabolizers", will have a greater exposure to standard doses of brivaracetam. Because they are less able to metabolize the drug to its inactive form for excretion, they may have an increased risk of adverse effects. The most common adverse effects of brivaracetam therapy include sedation, fatigue, dizziness, and nausea.

The recommended starting dosage for brivaracetam monotherapy or adjunctive therapy is 50 mg twice daily (100 mg per day). Based on how the individual responds, the dose of brivaracetam may be decreased to 25 mg twice daily (50 mg per day) or increased up to 100 mg twice daily (200 mg per day) (1).

The FDA-approved drug label for brivaracetam states that patients who are CYPC19 poor metabolizers, or are taking medicines that inhibit CYP2C19, may require a dose reduction (Table 1). Approximately 2% of Caucasians, 4% of African Americans, and 14% of Chinese are CYP2C19 poor metabolizers (1).

Table 1. FDA (2017) Drug Label for Brivaracetam. Recommendations for CYP2C19 Phenotype: Pharmacokinetics.

Phenotype	Recommendations
CYP2C19 poor metabolizer	CYP2C19 poor metabolizers and patients using inhibitors of CYP2C19 may require dose reduction.

This table is adapted from (1).

Drug: Brivaracetam

Brivaracetam is an antiseizure drug that is used in the treatment of partial-onset (focal) seizures in patients aged 16 years or older. Brivaracetam can be used as monotherapy, or more commonly, is used in combination with other antiseizure drugs (1). There is also some evidence to suggest that brivaracetam may be useful in the treatment of generalized seizures (2).

Brivaracetam displays a high and selective affinity for SV2A in the brain, which is thought to contribute to the antiseizure effect (3).

In a neuron, at the synapse, vesicles store various neurotransmitters. The neurotransmitters are released and then refilled in a process regulated by voltage-dependent calcium channels. These synaptic vesicles are essential for propagating nerve impulses between neurons, and the SV2A protein is a major component of the vesicle (4).

Levetiracetam was the first antiseizure drug that was found to bind to SV2A, among other targets. Brivaracetam is an analogue of levetiracetam and was designed to selectively target SV2A with a much higher affinity (5-7).

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Over 50 million people worldwide suffer from epilepsy. Epilepsy is characterized by spontaneous recurrent epileptic seizures, which may be classified as focal or generalized. Generalized seizures appear to originate in all regions of the cortex simultaneously and include absence seizures (sudden impaired consciousness and staring) and general tonic-clonic seizures (loss of consciousness, stiffening of limbs in the tonic phase, and twitching or jerking muscles in the clonic phase). In contrast, symptoms of focal seizures depend upon where the focus of the seizure originates in the brain; e.g., jerking of a limb indicates a focus in the contralateral motor cortex.

Most antiseizure drugs currently available target sodium channels (e.g., carbamazepine, phenytoin), calcium channels (e.g., ethosuximide), or the GABA pathway (e.g., clobazam). However, up to one-third of patients may not achieve seizure control or they may not be able to tolerate the side effects. Newer antiseizure drugs have unconventional targets, such as SV2A (8-10).

Brivaracetam was licensed in 2016, and in phase III trials and with long term follow up, brivaracetam was reported to be well tolerated with good efficacy (11, 12). Compared with the addition of placebo to a treatment regime, the addition of brivaracetam reduced the frequency of focal seizures by approximately half (13-16).

The most common side effects associated with brivaracetam therapy include dizziness, fatigue, somnolence, nausea and vomiting. Psychiatric symptoms such as irritability, insomnia and depression, and behavioral effects have also been reported, but some studies suggest these may be less likely to occur with brivaracetam compared with levetiracetam (17-20).

Brivaracetam is primarily metabolized (approximately 60%) by cytochrome P450 (CYP)-independent hydrolysis (via amidase) to inactive metabolites. Minor metabolic pathways include hydroxylation by CYP2C19.

One study found that co-administration of rifampin (a strong enzyme inducer) decreased exposure to brivaracetam by 45%, this is probably through an induction of the CYP2C19 pathway which clears brivaracetam metabolites from the blood (21). One small study (n=79) found that individuals who lacked CYP2C19 activity had increased exposure to brivaracetam (22). It is therefore possible that genetic variations associated with a loss of CYP2C19 function may reduce brivaracetam metabolism leading to increased levels of active drug levels in the plasma, and possibly increases the probability of side effects. However, the recommended therapeutic dose is 50–200 mg/day, and patients are individually titrated to optimal efficacy, safety, and tolerability.

Gene: CYP2C19

The CYP superfamily is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP genes are very polymorphic and can result in reduced, absent, or increased drug metabolism.

The CYP2C19 enzyme contributes to the metabolism of a range of clinically important drugs, such as antidepressants (23), benzodiazepines, several proton pump inhibitors, the antifungal agent voriconazole (24), the antiplatelet agent clopidogrel (25), and antiseizure drugs such as brivaracetam, clobazam, diazepam, lacosamide, phenytoin, and phenobarbital.

The *CYP2C19* gene is highly polymorphic—35 variant star (*) alleles are cataloged at the Pharmacogene Variation (PharmVar) Consortium. The *CYP2C19*1* is the wild type allele and is associated with normal enzyme activity and the "normal metabolizer" phenotype.

The *CYP2C19*17* allele is associated with increased enzyme activity and, depending on the number of alleles present, is associated with the "rapid" (one **17* allele) and "ultrarapid" (two **17* alleles) metabolizer phenotypes.

Nonfunctional alleles include *CYP2C19*2* and **3*. The *CYP2C19* "intermediate" metabolizers carry one copy of an allele that encodes a nonfunctional allele (e.g., **1/*2*), whereas "poor" metabolizers carry 2 nonfunctional alleles (e.g., **2/*2*, **2/*3*) (Table 2).

Table 2. CPIC (2016) *CYP2C19* Functional Status and Phenotypes

Phenotype	Genotype	Examples of diplotypes
CYP2C19 ultrarapid metabolizer (~2–5% of patients)[a]	An individual carrying 2 increased function alleles.	**17/*17*
CYP2C19 rapid metabolizer (~2–30% of patients)	An individual carrying one normal function allele and one increased function allele.	**1/*17*
CYP2C19 normal metabolizer (~35–50% of patients)	An individual carrying 2 normal function alleles.	**1/*1*
CYP2C19 intermediate metabolizer (~18–45% of patients)	An individual carrying one normal function allele and one no function allele, or one no function allele and one increased function allele.	**1/*2* **1/*3* **2/*17*[b]
CYP2C19 poor metabolizer (~2–15% of patients)	An individual carrying 2 no function alleles.	**2/*2* **2/*3* **3/*3*

[a] CYP2C19 metabolizer status frequencies are based on average multiethnic frequencies. See the *CYP2C19* Frequency Tables for population-specific allele and phenotype frequencies (23).
[b] The predicted metabolizer phenotype for the **2/*17* genotype is a provisional classification. The currently available evidence indicates that the *CYP2C19*17* increased function allele is unable to completely compensate for the *CYP2C19*2* nonfunctional allele.
This table is adapted from (23).

Approximately 2% of Caucasians, 4% of African Americans, and 14% of Chinese are CYP2C19 poor metabolizers; and up to 45% of patients are CYP2C19 intermediate metabolizers.

The most common nonfunctional variant is *CYP2C19*2*, which contains a NM_000769.1:c.681G>A variant in exon 5 that results in an aberrant splice site that produces a truncated and nonfunctioning protein. The *CYP2C19*2* allele frequencies are ~15% in Caucasians and Africans, and ~29–35% in Asians.

Another commonly tested nonfunctional variant is *CYP2C19*3*, which contains a NM_000769.1:c.636G>A variant in exon 4 that causes a premature stop codon. The *CYP2C19*3* allele frequencies are ~2-9% in Asian populations, but rare in other racial groups. Other nonfunctional variants occur in less than 1% of the general population and include *CYP2C19*4-*8* (25).

Linking Gene Variation with Treatment Response

One small study (n=79) reports that *CYP2C19* allele status influences the pharmacokinetics of brivaracetam, but that this is unlikely to be clinically relevant because of the minor role of CYP-dependent hydroxylation in the metabolism of brivaracetam (22). However, the FDA does state that CYP2C19 poor metabolizers may require a reduction in the dose of brivaracetam (1).

Genetic Testing

Clinical genotyping tests are available for several *CYP2C19* alleles. The NIH's Genetic Testing Registry (GTR) provides examples of the genetic tests that are currently available for the *CYP2C19* gene.

Usually a patient's result is reported as a diplotype, such as *CYP2C19 *1/*1*, and may also include an interpretation of the patient's predicted metabolizer phenotype (ultrarapid, rapid, normal, intermediate, or poor).

The *CYP2C19*2* and **3* alleles are most commonly tested for, and Table 2 summarizes common CYP2C19 phenotypes. Less common nonfunctional alleles (e.g., *CYP2C19*4-*8*) may also influence drug response

similarly to *2* and *3* but they may not be tested for, and data are lacking on their effects on the brivaracetam drug response.

To facilitate *CYP2C19* genetic testing and improve genotyping concordance across laboratories, the Pharmacogenetics Working Group of the Association for Molecular Pathology Clinical Practice Committee (AMP PGx) has recommended a minimum set of *CYP2C19* alleles, referred to as "tier 1", which should be included in clinical *CYP2C19* pharmacogenomic tests. As of 2018, the tier 1 alleles are *CYP2C19*2*, *CYP2C19*3*, and *CYP2C19*17* (Table 3) (26).

In addition, AMP PGx have defined a list of tier 2 *CYP2C19* alleles that do not meet the criteria for inclusion in tier 1 and are thus considered optional (Table 4) (26).

Table 3. AMP PGx (2018) *CYP2C19* Tier 1 Variant Alleles.

Allele	Allele functional status[a]	Defining functional variant	Multiethnic allele frequency, %
*CYP2C19*2*	No function	rs4244285	12-54
*CYP2C19*3*	No function	rs4986893	0.3-15
*CYP2C19*17*	Increased function	rs12248560	4-21

[a] Citations for assignment of function can be found at PharmGKB Gene-specific Information for *CYP2C19*.
Note that the defining *2* variant (rs4244285) is most likely linked with the defining variant of the *35* allele (rs12769205); however, the *35* definition includes rs12769205 without rs4244285.

Table 4. AMP PGx (2018) *CYP2C19* Tier 2 Variant Alleles.

Genotype	Allele functional status*	Defining functional variant	*Multiethnic allele frequency, %
*CYP2C19*4*	No function	rs28399504	0.1-0.3
*CYP2C19*4B*	No function	rs28399504; rs12248560	0-0.2
*CYP2C19*5*	No function	rs56337013	0
*CYP2C19*6*	No function	rs72552267	0-0.1
*CYP2C19*7*	No function	rs72558186	0
*CYP2C19*8*	No function	rs41291556	0.1-0.3
*CYP2C19*9*	Decreased function	rs17884712	0.1-4.2
*CYP2C19*10*	Decreased function	rs6413438	0.1-6
*CYP2C19*35*	No function	rs12769205	0.8-3.1

* Multiethnic allele frequency from PharmVar.org (last accessed June 20, 2017.)
Both Table 3 and Table 4 are adapted from (26).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2017 Statement from the US Food and Drug Administration (FDA)

Brivaracetam is primarily metabolized by hydrolysis of the amide moiety to form the corresponding carboxylic acid metabolite, and secondarily by hydroxylation on the propyl side chain to form the hydroxy metabolite. The hydrolysis reaction is mediated by hepatic and extra-hepatic amidase. The hydroxylation pathway is mediated

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

primarily by CYP2C19. In human subjects possessing genetic variations in CYP2C19, production of the hydroxy metabolite is decreased 2-fold or 10-fold, while the blood level of brivaracetam itself is increased by 22% or 42%, respectively, in individuals with one or both mutated alleles. CYP2C19 poor metabolizers and patients using inhibitors of CYP2C19 may require dose reduction. An additional hydroxy acid metabolite is created by hydrolysis of the amide moiety on the hydroxy metabolite or hydroxylation of the propyl side chain on the carboxylic acid metabolite (mainly by CYP2C9). None of the 3 metabolites are pharmacologically active.

Please review the complete therapeutic recommendations that are located here: (1).

Nomenclature of selected *CYP2C19* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C19*2*	681G>A Pro227Pro	NM_000769.1:c.681G>A	NP_000760.1:p.Pro227=	rs4244285
*CYP2C19*3*	636G>A Trp212Ter	NM_000769.1:c.636G>A	NP_000760.1:p.Trp212Ter	rs4986893
*CYP2C19*17*	-806C>T	NM_000769.1:c.-806C>T	Not applicable - variant occurs in a non-coding region	rs12248560

Note: the normal "wild type" allele is *CYP2C19*1*.
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (27).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for Cytochrome P450 enzymes is available from the Pharmacogene Variation (PharmVar) Consortium.

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Capecitabine Therapy and *DPYD* Genotype

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Created: September 15, 2016.

Introduction

Capecitabine is a chemotherapy agent that belongs to the drug class of fluoropyrimidines. It is widely used in the treatment of colon cancer, metastatic colorectal cancer, and metastatic breast cancer. Capecitabine is a prodrug that is enzymatically converted to its active form, fluorouracil, which acts as an antimetabolite to slow tumor growth.

The *DPYD* gene encodes dihydropyrimidine dehydrogenase (DPD), an enzyme that catalyzes the rate-limiting step in fluorouracil metabolism. Individuals who are carriers of non-functional *DPYD* variants, such as *DPYD*2A*, may not be able to metabolize capecitabine at normal rates, and are at risk of potentially life-threatening capecitabine toxicity, such as bone marrow suppression and neurotoxicity. The prevalence of DPD deficiency in Caucasians is approximately 3%-5%.

The FDA-approved drug label for capecitabine states that no capecitabine dose has been proven safe in patients with absent DPD activity, and that there is insufficient data to recommend a specific dose in patients with partial DPD activity as measured by any specific test (1).

The Clinical Pharmacogenetics Implementation Consortium (CPIC) has published dosing recommendations for fluoropyrimidines (capecitabine, fluorouracil, and tegafur) based on *DPYD* genotype (2) (Table 1). CPIC recommends using an alternative drug for patients who are "poor metabolizers". These individuals carry two copies of non-functional *DPYD* variants and typically have complete DPD deficiency. CPIC also recommends considering a 50% reduction in starting dose for "intermediate metabolizers". These individuals carry a combination of a normal-function and a non-functional variant and typically have reduced DPD activity (approximately 50% reduced) (2, 3).

Drug Class: Fluoropyrimidines

Fluoropyrimidines are a class of antimetabolite drugs that are widely used in the treatment of cancer. Currently, there are three types of fluoropyrimidines in clinical use: capecitabine, fluorouracil, and tegafur. Capecitabine and tegafur are both prodrugs of fluorouracil.

Fluoropyrimidines are thought to exert their chemotherapeutic effects in a number of ways, through several active metabolites. The main mechanism of action is thought to be the inhibition of thymidylate synthase, which plays an important part in the folate-homocysteine cycle, and purine and pyrimidine synthesis pathways. Also, active metabolites can be incorporated into RNA and DNA, ultimately leading to cell death (4).

Approximately 10-40% of patients develop severe and potentially life-threatening toxicity early during treatment with fluoropyrimidines (5). This typically leads to an interruption or discontinuation of potentially effective anticancer therapy, and often requires hospitalization (6).

The inter-individual variation in the occurrence and severity of adverse events in patients receiving fluoropyrimidines can be partly explained by clinical factors, such as age and sex. However, much of the variability in adverse events remains unexplained (7).

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Of the genetic factors thought to contribute to fluoropyrimidine intolerance, the *DPYD* gene has been the most studied. This gene encodes the primary enzyme involved in breaking down fluoropyrimidines to inactive metabolites. Individuals who have a deficiency of the DPD enzyme have a significantly increased risk of suffering from severe fluoropyrimidine toxicity, and the stratification of patients on the basis of the *DPYD* genotype may help to prevent such adverse events (8-13)

The Clinical Pharmacogenetics Implementation Consortium (CPIC) has published genetics-based dosing recommendations for fluoropyrimidines based on *DPYD* genotype (Table 1).

Table 1. 2013 Recommended dosing of Fluoropyrimidines by DPD phenotype, from Clinical Pharmacogenetics Implementation Consortium (CPIC)

Phenotype	Implications for phenotypic measures	Dosing recommendations	Classification of recommendations[a]
Normal metabolizer	Normal DPD activity and "normal" risk for fluoropyrimidine toxicity	Use label-recommended dosage and administration	Moderate
Intermediate metabolizer	Decreased DPD activity (leukocyte DPD activity at 30–70% that of the normal population) and increased risk for severe or even fatal drug toxicity when treated with fluoropyrimidine drugs	Start with at least a 50% reduction in starting dose, followed by titration of dose based on toxicity[b] or pharmacokinetic test (if available)	Moderate
Poor metabolizer	Complete DPD deficiency and increased risk for severe or even fatal drug toxicity when treated with fluoropyrimidine drugs	Select alternative drug	Strong

Fluoropyrimidines: 5-fluorouracil, capecitabine, and tegafur.
DPD, dihydropyrimidine dehydrogenase.
[a] Rating scheme is described here (2)
[b] Increase the dose in patients experiencing no or clinically tolerable toxicity to maintain efficacy; decrease the dose in patients who do not tolerate the starting dose to minimize toxicities.
Table is adapted from Caudle KE, Thorn CF, Klein TE, Swen JJ, McLeod HL, Diasio RB, Schwab M. Clinical Pharmacogenetics Implementation Consortium guidelines for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing. Clinical pharmacology and therapeutics.2013:94(6):640-5 (2)
Note: The nomenclature used in this table reflects the standardized nomenclature for pharmacogenetic terms proposed by CPIC in a 2016 paper, "Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC)" (14).

Drug: Capecitabine

Capecitabine is a form of chemotherapy used as an adjunct treatment for colon cancer, and as either monotherapy or part of combination therapy for metastatic colorectal cancer and metastatic breast cancer (1).

Capecitabine is an orally administered prodrug—it is converted to its active form, fluorouracil, by thymidine phosphorylase—an enzyme that tends to be found in higher concentrations in tumors compared to normal tissue and plasma. Fluorouracil is structurally similar to pyrimidines, and the enzyme that catalyzes the rate-limiting step in the breakdown of pyrimidines (DPD, dihydropyrimidine dehydrogenase) also catalyzes the rate-limiting step in 5-fluorouracil catabolism. DPD catalyzes the conversion of fluorouracil to the non-cytotoxic dihydrofluorouracil (DHFU) (15).

Symptomatic DPD deficiency is a rare autosomal recessive disorder with a wide range of symptoms, ranging from no symptoms or signs, to severe neurological problems. In affected individuals, the absent or greatly reduced DPD activity results in uracil and thymine accumulating in the blood, urine, and cerebrospinal fluid.

Neurological symptoms typically manifest in early childhood and include seizures, small head size, and delayed cognitive and motor development (16).

Symptomatic DPD deficiency is typically caused by homozygous inactivation of *DPYD*; whereas individuals who are heterozygotes tend to be asymptomatic. However, all patients with less than 70% DPD activity are considered at risk for the development of severe drug toxicity when treated with fluoropyrimidines (17). Signs of capecitabine toxicity include severe diarrhea, severe mucositis, neutropenia, hand-foot syndrome, and neurotoxicity (1).

Approximately 3-5% of Caucasians have partial DPD deficiency and 0.2% have complete DPD deficiency (18). Currently, most patients are not screened for DPD deficiency before starting capecitabine therapy (19).

Gene: *DPYD*

The *DPYD* gene encodes the enzyme dihydropyrimidine dehydrogenase (DPD), which catalyzes the first and the rate-limiting step in the breakdown of the pyrimidine nucleotides thymine and uracil. DPD also catalyzes the rate-limiting step in the breakdown of fluoropyrimidines.

Many *DPYD* variants have been described, although only a few have been demonstrated to influence DPD enzyme activity. *DPYD*1* is the wild-type allele and is associated with normal enzyme activity. Individuals who carry two copies of *DPYD* alleles with normal activity are known as "normal metabolizers" and have fully functional DPD enzyme activity (Table 2 and Table 3). Next to *DPYD*1*, the *DPYD* alleles **4*, **5*, **6*, and **9A* are also considered to have normal activity (20).

Table 2. Activity status of selected *DPYD* Alleles

Allele type	Alleles
Functional	**1, *4, *5, *6, *9A*
Nonfunctional	**2A, *13*, rs67376798

Table is adapted from (12, 15) For the nomenclature of human DPYD alleles, please see (21)

The nonfunctional *DPYD* variants which have been associated with low DPD activity and an increased risk of toxicity with fluoropyrimidines include **2A*, **13*, and rs67376798 (15). The most well studied variant is *DPYD*2A*, in which a single nucleotide substitution at the invariant splice donor site of intron 14 leads to translation skipping exon 14, resulting in the production of a truncated protein with virtually no enzyme activity.

Individuals who carry combinations of normal function, decreased function, and/or no function *DPYD* alleles are known as "intermediate metabolizers". They have partial DPD deficiency and are at increased risk of capecitabine toxicity. And individuals who carry a combination of nonfunctional *DPYD* alleles and/or decreased function *DPYD* alleles are known as "poor metabolizers". They have complete DPD deficiency and are at an even higher risk of capecitabine toxicity. Overall, the prevalence of individuals who are heterozygous for nonfunctional variant *DPYD* alleles (partially DPD deficient) that place them at risk of severe drug reactions is estimated to be as high as 3-5%, but this varies in different populations (5, 17, 22-25). For example, in the Dutch population, the *DPYD*2A* had an allele frequency of 0.91% in Caucasians (17).

Table 3 Assignment of likely phenotype based on *DPYD* genotypes

Likely phenotype	Functional definition	Genetic definition	Example diplotypes
Normal metabolizer	Fully functional DPD enzyme activity	Combinations of normal function and decreased function alleles	*DPYD*1/*1*
Intermediate metabolizer (~3–5% of patients)	Decreased DPD enzyme activity (activity between normal and poor metabolizer)	Combinations of normal function, decreased function, and/or no function alleles	**1/*2A*; **1/*13*; or *1/ rs67376798

Table 3 continued from previous page.

Likely phenotype	Functional definition	Genetic definition	Example diplotypes
Poor metabolizer (~0.2% of patients)	Little to no DPD enzyme activity	Combination of no function alleles and/ or decreased function alleles	*2A/*2A; 13/*13; *2/*13*; or rs67376798/ rs67376798

Table is adapted from Caudle KE, Thorn CF, Klein TE, Swen JJ, McLeod HL, Diasio RB, Schwab M. Clinical Pharmacogenetics Implementation Consortium guidelines for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing. Clinical pharmacology and therapeutics.2013:94(6):640-5 (2)
Note: The nomenclature used in this table reflects the standardized nomenclature for pharmacogenetic terms proposed by CPIC in the 2016 paper, "Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC)" (14).

A recent study proposed distinguishing between the various *DPYD* alleles and their functionality by assigning gene activity scores. The use of such scores could result in differentiated individualized dosing advice for fluororpyrimidines, which is essential for reducing toxic side effects while maintaining efficacy (12).

Genetic Testing

The NIH Genetic Testing Registry, GTR, displays genetic tests that are currently available for the *DPYD* gene and the capecitabine drug response. The *DPYD*2A* variant is the most commonly tested.

Biochemical genetic tests may also be used, which assess the level of activity of the DPD enzyme. These tests include biochemical assays such as analyte testing (e.g., measuring the amount of thymine and uracil in the urine or blood) or an enzyme assay (e.g., directly measuring the activity of DPD using RNA extracted from blood cells and measuring the DPD mRNA copy number) (2, 26, 27).

GTR provides a list of biochemical tests that assess the levels of thymine and uracil analytes, and the activity of the enzyme dihydropyrimidine dehydrogenase.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2016 Statement from the US Food and Drug Administration (FDA): Based on postmarketing reports, patients with certain homozygous or certain compound heterozygous mutations in the *DPD*[2] gene that result in complete or near complete absence of DPD activity are at increased risk for acute early-onset of toxicity and severe, life-threatening, or fatal adverse reactions caused by capecitabine (e.g., mucositis, diarrhea, neutropenia, and neurotoxicity). Patients with partial DPD activity may also have increased risk of severe, life-threatening, or fatal adverse reactions caused by capecitabine.

Withhold or permanently discontinue capecitabine based on clinical assessment of the onset, duration and severity of the observed toxicities in patients with evidence of acute early-onset or unusually severe toxicity, which may indicate near complete or total absence of DPD activity. No capecitabine dose has been proven safe for patients with complete absence of DPD activity. There is insufficient data to recommend a specific dose in patients with partial DPD activity as measured by any specific test.

Please review the complete therapeutic recommendations that are located here: (1).

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

[2] Note: the official gene symbol is DYPD. DPD is an alternate gene symbol.

2013 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC): [...] Furthermore, patients who are heterozygous for the nonfunctional *DPYD* variants mostly demonstrate partial DPD deficiency (leukocyte DPD activity at 30–70% that of the normal population).Thus, our recommendation is to start with at least a 50% reduction of the starting dose; followed by an increase in dose in patients experiencing no or clinically tolerable toxicity, to maintain efficacy; and a decrease in dose in patients who do not tolerate the starting dose, to minimize toxicities. An alternative is pharmacokinetic-guided dose adjustment (if available). Patients who are homozygous for *DPYD**2A, *13, or rs67376798 may demonstrate complete DPD deficiency, and the use of 5-fluouracil or capecitabine is not recommended in these patients. Because capecitabine and tegafur are converted to 5-fluorouracil and then metabolized by DPD, the clearance of and exposure to 5-fluorouracil, in addition to its toxic effects, are similar in patients with these variants.

Please review the complete therapeutic recommendations that are located here: (2).

Nomenclature

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*DPYD**2A	IVS14+1G>A c.1905+1G>A	NM_000110.3:c.1905+1G>A	Not applicable—deletion of exon 14 leads to the production of a truncated protein	rs3918290
*DPYD**13	1679T>G Ile560Ser	NM_000110.3:c.1679T>G	NP_000101.2:p.Ile560Ser	rs55886062
rs67376798	2846A>T Asp949Val	NM_000110.3:c.2846A>T	NP_000101.2:p.Asp949Val	rs67376798

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

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Carbamazepine Therapy and *HLA* Genotype

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Introduction

Carbamazepine (brand names include Carbatrol, Epitol, Equetro, and Tegretol) is an effective antiseizure drug that is often used as a first-line agent in the treatment of epilepsy. Carbamazepine is also used to treat bipolar disorder and to relieve pain in trigeminal neuralgia.

Hypersensitivity reactions associated with carbamazepine can occur in up to 10% of patients, and typically affect the skin. Some of these reactions are mild, as in the case of maculopapular exanthema (MPE); however, conditions such as Stevens–Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and drug reaction with eosinophilia and systemic symptoms (DRESS) are potentially life-threatening.

The risk of hypersensitivity is increased by the presence of specific human leukocyte antigen (*HLA)* alleles. The *HLA-B*15:02* allele is strongly associated with carbamazepine-induced SJS/TEN in populations where this allele is most common, such as in Southeast Asia.

According to the FDA-approved drug label for carbamazepine, testing for *HLA-B*15:02* should be done for all patients with ancestry in populations with increased frequency of *HLA-B*15:02*, prior to initiating carbamazepine therapy (Table 1). The label states that greater than 15% of the population is reported *HLA-B*15:02* positive in Hong Kong, Thailand, Malaysia, and parts of the Philippines, compared to about 10% in Taiwan and 4% in North China. The label states that South Asians, including Indians, appear to have intermediate prevalence of *HLA-B*15:02*, averaging 2 to 4%, but higher in some groups. In Japan and Korea, the *HLA- B*15:02* is present in less than 1% of the population. In individuals not of Asian origin (e.g., Caucasians, African-Americans, Hispanics, and Native Americans), the *HLA-B*15:02* allele is largely absent. These prevalence rates of *HLA-B*15:02* may be used to guide which patients should be screened. However, the FDA cautions to keep in mind the limitations of prevalence rate data when deciding which patients to screen. This is because of the wide variability in *HLA-B*15:02* rates (even within ethnic groups), the difficulty in ascertaining ethnic ancestry, and the likelihood of mixed ancestry (1).

The FDA label also states that carbamazepine should not be used in patients who are positive for *HLA-B*15:02* unless the benefits clearly outweigh the risks. Tested patients who are found to be negative for the allele are thought to have a low risk of SJS/TEN.

The *HLA-A*31:01* allele may also be a risk factor for SJS/TEN but is more strongly associated with other carbamazepine-induced reactions, such as DRESS and MPE. *HLA-A*31:01* is found in most populations, worldwide. *HLA-B*15:11* is another allele that has been linked with SJS/TEN. The FDA states that the risks and benefits of carbamazepine therapy should be weighed before considering carbamazepine in patients known to be positive for *HLA-A*31:01*, but does not discuss *HLA-B*15:11* (1).

Carbamazepine dosing guidelines based on *HLA* genotype have been published by the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP), the Clinical Pharmacogenetics Implementation Consortium (CPIC), and the Canadian Pharmacogenomics Network for Drug Safety (CPNDS) (2-5).

DPWG recommendations include avoiding the use of carbamazepine and selecting an alternative, if possible, for individuals positive for *HLA-B*15:02*, *HLA-A*31:01* and *HLA-B*15:11* (Table 2). CPIC recommendations

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include not using carbamazepine in carbamazepine-naïve patients who are positive for *HLA-B*15:02* and any *HLA-A*31:01* genotype (or *HLA-A*31:01* genotype unknown) (Table 3). CPNDS recommends genetic testing for all carbamazepine-naïve patients before they start treatment, with a moderate level of evidence for *HLA-A*31:01* testing, and strong to optional evidence for *HLA-B*15:02* testing (based on the frequency of *HLA-B*15:02* in the population the patient originates from, and if this is known or not) (Table 4).

Table 1. FDA (2018) Drug Label for Carbamazepine. Recommendations for *HLA-B*15:02* and *HLA-A*31:01* Genotype: Warnings.

Genotype	Recommendations
SJS/TEN and *HLA-B*15:02* Allele	Prior to initiating carbamazepine therapy, testing for *HLA-B*15:02* should be performed in patients with ancestry in populations in which *HLA-B*15:02* may be present. Carbamazepine should not be used in patients positive for *HLA-B*15:02* unless the benefits clearly outweigh the risks.
Hypersensitivity Reactions and *HLA-A*31:01* Allele	The risks and benefits of carbamazepine therapy should be weighed before considering carbamazepine in patients known to be positive for *HLA-A*31:01*.

Please see Therapeutic Recommendations based on Genotype for more information from the FDA. This table is adapted from (1).

Table 2. DPWG (2017) Recommendations for Carbamazepine and *HLA* Genotype.

Genotype	Recommendations
*HLA-B*15:02* positive	Choose an alternative if possible
*HLA-A*31:01* positive	1. carefully weigh the risk of DRESS and SJS/TEN against the benefits 2. if an alternative is an option, choose an alternative
*HLA-B*15:11* positive	1. carefully weigh the risk of SJS/TEN against the benefits 2. if an alternative is an option, choose an alternative

Please see Therapeutic Recommendations based on Genotype for more information from DPWG. This table is adapted from (2).

Table 3. CPIC (2016) Recommendations for Carbamazepine Therapy based on *HLA-B* and *HLA-A* Genotype.

Genotype[a]	Implication	Therapeutic recommendation	Classification of recommendations	Considerations for other aromatic anticonvulsants
*HLA-B*15:02* negative and *HLA-A*31:01* negative	Normal risk of carbamazepine-induced SJS/TEN, DRESS, and MPE	Use carbamazepine per standard dosing guidelines.[b]	Strong	N/A
*HLA-B*15:02* negative and *HLA-A*31:01* positive	Greater risk of carbamazepine-induced SJS/TEN, DRESS, and MPE	If patient is carbamazepine-naïve and alternative agents are available, do not use carbamazepine.	Strong	Other aromatic anticonvulsants[d] have very limited evidence, if any, linking SJS/ TEN, DRESS, and/or MPE with the *HLA-A*31:01* allele, and thus no recommendation can be made with respect to choosing another aromatic anticonvulsant as an alternative agent.

Table 3. continued from previous page.

Genotype[a]	Implication	Therapeutic recommendation	Classification of recommendations	Considerations for other aromatic anticonvulsants
		If patient is carbamazepine-naïve and alternative agents are not available, consider the use of carbamazepine with increased frequency of clinical monitoring. Discontinue therapy at first evidence of a cutaneous adverse reaction.	Optional	N/A
		The latency period for cutaneous adverse drug reactions is variable depending on phenotype; however, all usually occur within three months of regular dosing. Therefore, if the patient has previously used carbamazepine consistently for longer than three months without incidence of cutaneous adverse reactions, cautiously consider use of carbamazepine.	Optional	Previous tolerance of carbamazepine is not indicative of tolerance to other aromatic anticonvulsants.[d]
*HLA-B*15:02* positive[c] and any *HLA-A*31:01* genotype (or *HLA-A*31:01* genotype unknown)	Greater risk of carbamazepine-induced SJS/TEN	If patient is carbamazepine-naïve, do not use carbamazepine.	Strong	Other aromatic anticonvulsants[d] have weaker evidence linking SJS/TEN with the *HLA-B*15:02* allele; however, caution should still be used in choosing an alternative agent.

Table 3. continued from previous page.

Genotype[a]	Implication	Therapeutic recommendation	Classification of recommendations	Considerations for other aromatic anticonvulsants
		The latency period for drug-induced SJS/TEN is short with continuous dosing and adherence to therapy (4-28 days), and cases usually occur within three months of dosing; therefore, if the patient has previously used carbamazepine consistently for longer than three months without incidence of cutaneous adverse reactions, cautiously consider use of carbamazepine in the future.	Optional	Previous tolerance of carbamazepine is not indicative of tolerance to other aromatic anticonvulsants.[d]

DRESS, drug reaction with eosinophilia and systemic symptoms; MPE, maculopapular exanthema; N/A, not applicable; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis.
[a]If only *HLA-B*15:02* was tested, assume *HLA-A**31:01 is negative and vice versa.
[b]*HLA-B*15:02* has a 100% negative predictive value for carbamazepine-induced SJS/ TEN, and its use is currently recommended to guide the use of carbamazepine and oxcarbazepine only. Because there is a much weaker association and less than 100% negative predictive value of *HLA-B*15:02* for SJS/TEN associated with other aromatic anticonvulsants, using these drugs instead of carbamazepine or oxcarbazepine in the setting of a negative *HLA-B*15:02* test in Southeast Asians will not result in prevention of anticonvulsant-associated SJS/TEN.
[c]In addition to *HLA-B*15:02*, the risk for carbamazepine-induced SJS/TEN has been reported in association with the most common B75 serotype alleles in Southeast Asia, *HLA-B**15:08, *HLA-B*15:11*, and *HLA- B*15:21*. Although not described, the possibility of carbamazepine-induced SJS/TEN in association with less frequently carried B75 serotype alleles, such as *HLA- B**15*:30* and *HLA-B*15:31*, should also be considered.
[d]Aromatic anticonvulsants include carbamazepine, oxcarbazepine, eslicarbazepine, lamotrigine, phenytoin, fosphenytoin, and phenobarbital.
This table is adapted from Phillips EJ, Sukasem C, Whirl-Carrillo M, Müller DJ, Dunnenberger HM, Chantratita W, Goldspiel B, Chen YT, Carleton BC, George ALJ, Mushiroda T, Klein T, Gammal RS, and Pirmohamed M. Clinical Pharmacogenetics Implementation Consortium Guideline for HLA Genotype and Use of Carbamazepine and Oxcarbazepine: 2017 Update. Clinical pharmacology and therapeutics (4).

Table 4. CPNDS (2014) Recommendations for Carbamazepine and *HLA* Genotype.

Genotype	Recommendation 1.1
*HLA-B*15:02*	Genetic testing for *HLA-B*15:02* is recommended for all CBZ-naive patients before initiation of CBZ therapy (Level A – strong in patients originating from populations where *HLA-B*15:02* is common, its frequency unknown or whose origin is unknown; Level C – optional in patients originating from populations where *HLA-B*15:02* is rare).
*HLA-A*31:01*	Genetic testing for *HLA-A*31:01* is recommended for all CBZ-naive patients before initiation of CBZ therapy (Level B – moderate in all patients; Table 7).

CBZ: Carbamazepine.
Please see Therapeutic Recommendations based on Genotype for the all the recommendations from CPNDS, and the grading scheme used for the level of evidence. Table is adapted from (5).

Drug: Carbamazepine

Carbamazepine is an antiseizure drug used in the treatment of epilepsy. Carbamazepine is also used as an analgesic in trigeminal neuralgia and may be used in the treatment of bipolar disorder (5, 7, 8).

Epilepsy is characterized by spontaneous recurrent epileptic seizures, which may be classified as focal or generalized. Carbamazepine is one of the first-line treatments for focal seizures in adults, adolescents, and children, and it may also be considered for general tonic-clonic seizures.

The symptoms of focal seizures depend upon where the focus of the seizure originates in the brain e.g., jerking of a limb indicates a focus in the contralateral motor cortex. In contrast, generalized seizures appear to originate in all regions of the cortex simultaneously and include absence seizures (sudden impaired consciousness and staring) and general tonic-clonic seizures (loss of consciousness, stiffening of limbs in the tonic phase, and twitching or jerking muscles in the clonic phase).

Carbamazepine is a tricyclic compound that belongs to the class of antiseizure drugs that act by blocking voltage-dependent sodium channels present on neuronal cell membranes. Carbamazepine stabilizes the sodium channel in the inactivated state, leaving fewer of the channels available to open. This prolonged inactivated phase of the channel inhibits the rapid and repetitive generation of action potentials in the epileptic focus (3, 9).

Carbamazepine is metabolized in the liver by the cytochrome P-450 (CYP) system. The major metabolite is carbamazepine-epoxide, which has an anticonvulsant activity of uncertain significance. CYP3A4 is the main enzyme involved in the metabolism of carbamazepine; a lesser role is played by CYP2C8 and possibly CYP3A5. Minor metabolic pathways include multiple CYP enzymes, such as CYP2B6.

Carbamazepine stimulates transcriptional upregulation of CYP3A4 and other genes involved in its own metabolism. In addition, there are many drug-drug interactions with carbamazepine, because numerous drugs have been shown to induce or inhibit CYP3A4, or are metabolized by CYP3A4. Therefore, when carbamazepine is given with drugs that can decrease or increase carbamazepine levels, close monitoring of carbamazepine levels is indicated and dosage adjustment may be required (10, 11).

Carbamazepine-induced Adverse Drug Reactions

In general, there are two categories of adverse drug reactions. Type A reactions account for up to 85–90% of all adverse drug reactions. They are predictable, based on the known properties of the drug, and they can affect any individual if their exposure to the drug is high enough. For carbamazepine, type A adverse effects include sedation, CNS depression, and vestibular symptoms such as nystagmus and ataxia.

Type B reactions account for the remaining 10–15% of adverse drug reactions. These reactions are difficult to predict (idiosyncratic) because they can occur at any dose, and they develop through a mechanism that is unrelated to the mechanism of action of the drug. For carbamazepine, type B adverse reactions include carbamazepine-induced hypersensitivity reactions that typically involve the skin.

Approximately 5–10% of patients taking carbamazepine will experience carbamazepine-induced cutaneous reactions. Most of these are considered to be mild, such as maculopapular exanthema (MPE) and erythema multiforme. However, these cutaneous reactions can cause considerable discomfort to the patient and often lead to the discontinuation of carbamazepine therapy (5, 12, 13). In addition, treatment may be stopped because of the risk of a more severe, cutaneous drug reaction developing.

Stevens-Johnson syndrome (SJS) and the more severe form, toxic epidermal necrolysis (TEN), can be induced by carbamazepine therapy. These are life-threatening conditions that are primarily characterized by lesions of the skin (detachment of the epidermis) and mucous membranes (severe erosions) (11). SJS/TEN occurs in approximately 1–10 per 10,000 patients taking carbamazepine. Onset is delayed and may occur several weeks after the initiation of carbamazepine therapy. The mortality rate is high—up to 10% for SJS, and 50% for TEN (11, 14, 15). Pediatric patients who survive SJS/TEN usually have long-term complications, such as scarring, visual loss and chronic kidney disease (16).

Another severe and potentially life-threatening carbamazepine-induced hypersensitivity reaction is known as drug reaction with eosinophilia and systemic symptoms (DRESS, also known as drug-induced hypersensitivity syndrome, HSS).

The mechanisms underlying this hypersensitivity reaction is poorly understood, but is thought to involve the drug, or a molecule derived from the drug, interacting with the major histocompatibility complex (MHC) expressed on the surface of cells, resulting in a stimulation of the immune system, particularly T cells and eosinophils (5, 15).

Individuals who have specific *HLA* variants are known to be susceptible to carbamazepine-induced hypersensitivity reactions. In 2007, the FDA added a warning to the drug label concerning carbamazepine-induced SJS/TEN, with a recommendation for *HLA-B*15:02* screening in South-East Asian populations (17). Carbamazepine should not be used in patients who have the *HLA-B*15:02* variant (Table 1).

Screening of patients prior to carbamazepine therapy can identify those at higher risk of hypersensitivity reactions, allowing for an alternative drug to be used. Clinical practice guidelines for the treatment of epilepsy, bipolar disorder, and trigeminal neuralgia should be consulted for recommended alternative therapies to carbamazepine. However, caution should be used because of the risk of cross-reactivity between structurally similar antiseizure drugs (oxcarbazepine, lamotrigine, phenytoin, phenobarbital, primidone) (5, 18).

Up to 80% of patients who have an unexpected adverse reaction to carbamazepine will also have an adverse reaction to other antiseizure drugs, thereby restricting treatment options (19). Phenytoin and lamotrigine have both been associated with carbamazepine-induced SJS/TEN, and some evidence also links fosphenytoin, oxcarbazepine, eslicarbazepine acetate with SJS/TEN (20).

HLA gene family

The human leukocyte antigen (*HLA*) genes code for more than 200 different major histocompatibility complex (MHC*)* proteins. The MHC family has been subdivided into three subgroups based on the structure and function of the encoded proteins: Class I, Class II, and Class III.

The class I region contains the proteins encoded by the HLA genes *HLA-A, HLA-B, and HLA-C*. These MHC molecules are expressed on the surfaces of almost all cells and play an important role in processing and presenting antigens. The MHC class I gene region also contains a variety of other genes, many of which are not known to be involved in immune function.

An important role of MHC class I molecules is to present peptide fragments to immune cells (CD8+ T cells). Most of these peptides originate from the breakdown of normal cellular proteins ("self"). However, if foreign peptide fragments are presented, e.g., from a pathogen, CD8+T cells will recognize the peptides as "non-self" and will be activated to release inflammatory cytokines and launch an immune response to dispose of the pathogen (or foreign body).

Because MHC molecules need to present such a wide variety of "self" and "non-self" peptides, the *HLA* genes are both numerous and highly polymorphic. More than 1,500 *HLA-B* alleles have been identified (7). HLA allele nomenclature includes the HLA prefix, followed by the gene, an asterisk and a two digit number that corresponds to antigen specificity, and the assigned allele number (21). For example, the *HLA-B*15:02* allele is composed of:

- HLA: the HLA prefix (the HLA region on chromosome 6)
- B: the B gene (a particular *HLA* gene in this region)
- 15: the allele group (historically determined by serotyping, i.e., a group of alleles that share the same serotype)
- 02: the specific *HLA* allele (a specific protein sequence; determined by genetic analysis).

Additional digits have recently been added to the nomenclature to discriminate alleles that do not differ in the protein amino acid sequence but differ in their genetic sequence (i.e., because of synonymous and noncoding genetic variants).

Variation in *HLA* genes plays an important role in the susceptibility to autoimmune disease and infections. They are also critical in the context of transplant surgery where better outcomes are observed if the donor and recipient are HLA-compatible.

More recently, *HLA* variants have been associated with an increasing number of drug hypersensitivity responses (Type B adverse drug reactions). The strongest HLA-associated drug responses are *HLA-B*15:02* and carbamazepine-induced SJS/TEN in Asian populations, *HLA-B*57:01* and abacavir hypersensitivity syndrome in the Caucasian population, and *HLA-B*58:01* in allopurinol hypersensitivity syndrome and SJS/TEN (22).

Gene: HLA-B, Allele HLA-B*15:02

*HLA-B*15:02* is strongly associated with carbamazepine-induced SJS/TEN in populations where the *HLA-B*15:02* is common (China, Thailand, India, Malaysia, Taiwan). In patients of Asian origin, pharmacogenetic testing for *HLA-B*15:02* is recommended before initiation of carbamazepine therapy (23). The clinical benefits of screening for *HLA-B*15:02* have been confirmed in a Taiwanese study, where genetic testing reduced the incidence of carbamazepine-induced SJS/TEN from ten expected cases to zero (24).

The association between *HLA-B*15:02* and SJS/TEN was first reported in the Han Chinese. In the initial study, every patient who had carbamazepine-induced SJS/TEN was found to have the *HLA-B*15:02* allele (44/44, 100%), whereas the allele was much less common in carbamazepine-tolerant patients (3/101, 3%) (25). The *HLA*15:02* allele has since been associated with carbamazepine-induced SJS/TEN in Taiwanese, Chinese, Indians, Malay, and Chinese-Americans, but not in Caucasians or Japanese individuals (25-32).

The prevalence of carbamazepine-induced SJS/TEN is higher in populations where the *HLA-B*15:02* allele is most common. *HLA-B*15:02* is highly prevalent in Southeast Asia, with an allele frequency of over 15% in Hong Kong, Thailand, Malaysia, Vietnam, and parts of the Philippines. It is slightly less prevalent (10–13%) in Taiwan and Singapore, and in North China (4%). South Asians, including Indians, have intermediate prevalence of *HLA-B*15:02* (2–4%), with higher frequencies in some sub-populations (3, 5, 33-37).

The *HLA-B*15:02* allele is rare (frequency of less than 1%) in East Asia (Japan and Korea) and in individuals who are not of Asian descent. For example, the variant is rare in Europeans, Hispanics, Africans, African Americans, and Native Americans (5, 34). The absence of this variant in these population explains the lack of association of *HLA-B*15:02* with carbamazepine-induced SJS/TEN in Caucasians and Japanese individuals.

Current data suggest that *HLA-B*15:02* is a risk factor only for SJS/TEN because it does not appear to increase the risk of other carbamazepine-induced cutaneous reactions such as MPE and DRESS (5).

Gene: *HLA-A*, Allele *HLA-A*31:01*

The *HLA-A*31:01* allele is important for all types of carbamazepine hypersensitivity reactions. Also, in contrast to *HLA-B*15:02* which is predominantly found in Southeast Asia, the *HLA-A*31:01* is found in many populations worldwide (Table 5) (38-40).

Table 5. Comparison of *HLA-B*15:02* and *HLA-A*31:01* and Carbamazepine Therapy

	*HLA-B*15:02*	*HLA-A*31:01*
Associated phenotype	*HLA-B*15:02* is strongly associated with SJS/TEN	*HLA-A*31:01* is associated with all carbamazepine hypersensitivity phenotypes, including MPE, HSS, and (less strongly) SJS/TEN

Table 5. continued from previous page.

	*HLA-B*15:02*	*HLA-A*31:01*
Allele distribution	Predominantly concentrated in Southeast Asia, e.g., Hong Kong, Thailand, Malaysia, Vietnam, Philippines, Taiwan, Singapore.	Widely distributed across a range of populations including Europeans, Japanese, South Koreans and Han Chinese
Phenotype distribution	The strong association of *HLA-B*15:02* with carbamazepine-induced SJS/TEN is largely confined to individuals from Southeast Asian countries	*HLA-A*31:01* has been associated with carbamazepine-induced hypersensitivity reactions, particularly HSS, across different populations including European and Japanese individuals.
Pharmacogenetic screening recommendations	Screening for *HLA-B*15:02* is mandated in patients from Southeast Asia, prior to initiation of carbamazepine therapy. The FDA states that "patients with ancestry in genetically at-risk populations should be screened for the presence of *HLA-B*15:02* prior to initiating treatment with carbamazepine".	Screening is not currently mandated prior to initiation of carbamazepine therapy. The FDA states that the "risks and benefits of carbamazepine therapy should be weighed before considering carbamazepine in patients known to be positive for *HLA-A*31:01*".
Allele frequencies (reported by the FDA (1))	Greater than 15% of the population is reported positive in Hong Kong, Thailand, Malaysia, and parts of the Philippines, compared to about 10% in Taiwan and 4% in North China. South Asians, including Indians, appear to have intermediate prevalence of *HLA-B*15:02*, averaging 2% to 4%, but higher in some groups. *HLA-B*15:02* is present in less than 1% of the population in Japan and Korea. *HLA-B*1502* is largely absent in individuals not of Asian origin (e.g., Caucasians, African-Americans, Hispanics, and Native Americans).	*HLA-A*31:01* is expected to be positive by more than 15% of patients of Japanese, Native American, South Indian (for example, Tamil Nadu) and some Arabic ancestry; up to about 10% in patients of Han Chinese, Korean, European, Latin American, and other Indian ancestry; and up to about 5% in African- Americans and patients of Thai, Taiwanese, and Chinese (Hong Kong) ancestry.

SJS/TEN: Stevens–Johnson syndrome/ toxic epidermal necrolysis
MPE: maculopapular exanthema
HSS: drug-induced hypersensitivity syndrome

The association between *HLA-A*31:01* and DRESS and MPE has been found in Europeans, Han Chinese, Japanese, and North Americans of mixed ancestries (14, 26, 41-43). *HLA-A*31:01* is also associated with SJS/TEN, but not in Southeast Asians, where the more common HLA-B*15*:02* allele has an extremely strong association with SJS/TEN (5, 38).

The *HLA-A*31:01* variant is common globally with frequencies of at least 3% in many populations (2–5% in Northern Europeans, 2% in Han Chinese, 7–12% in Japanese populations) (5, 14, 38, 42). The highest frequencies have been reported in South American countries, such as Argentina (25%–38.6%) (38).

Gene: *HLA-B, HLA-B*15:11* and other alleles

The *HLA-B*15:11* variant has been found to be a risk factor for SJS/TEN in Japan (44, 45) and Korea (46). In Central China, *HLA-B*15:11* may be a risk factor for some patients with CBZ-induced SJS negative for *HLA-B*15:02* (47), and one study found that *HLA-A*11:01* for CBZ-induced SJS/TEN was a risk factor in the Spanish Caucasian population (39).

The *HLA-B*15:11* variant is found in frequencies above 1% in specific Asian populations only:Han Chinese, Koreans, Thai (34, 48).

Other alleles considered to be high risk, particularly in high-frequency areas such as Indonesia, Malaysia, and Thailand, include *HLA-B*15:08* and *HLA-B*15:21* (49, 50).

Genetic Testing

The NIH Genetic Testing Registry provides examples of the genetic tests that are currently available for the carbamazepine response, and the *HLA-B* and *HLA-A* genes.

The FDA recommends testing for *HLA-B*15:02* prior to initiating carbamazepine therapy in patients with ancestry in populations with increased frequency of *HLA-B*15:02*. In deciding which patients to screen, the FDA states that the prevalence rates of *HLA-B*15:02* (Table 1) may offer a rough guide, keeping in mind the limitations of these figures due to wide variability in rates within ethnic groups, the difficulty in ascertaining ethnic ancestry, and the likelihood of mixed ancestry (1).

The genotype results for an *HLA* allele such as *HLA-B*15:02* can either be "positive" or "negative" (Table 6). There are no intermediate phenotypes because the HLA genes are expressed in a codominant manner. A positive result is either "heterozygous" or "homozygous", depending upon whether the patient has one or two copies of the **15:02* allele, respectively.

For patients who are positive for *HLA-B*15:02*, the FDA states that carbamazepine should not be used unless the benefits clearly outweigh the risks. For patients who are known to be positive for *HLA-A*31:01*, the FDA states that the risks and benefits of carbamazepine therapy should be weighed before considering carbamazepine.

A negative result indicates that the patient does not have the *HLA-B*15:02* allele. However, a negative result does not rule out the possibility of a patient developing carbamazepine hypersensitivity. Therefore, clinicians should carefully monitor all patients according to standard practices.

Table 6. CPIC (2017). Assignment of likely *HLA-B and HLA-A* genotype

Genotype	Definition	Examples of diplotypes
*HLA-B*15:02* negative	Homozygous for an allele other than *HLA-A*15:02*	$*X/*X^a$
*HLA-B*15:02* positive	Heterozygous or homozygous variant	$*15:02/*X^a$, **15:02/*15:02*
*HLA-A*31:01* negative	Homozygous for an allele other than *HLA-A*31:01*	$*Y^b/*Y^b$
*HLA-A*31:01* positive	Heterozygous or homozygous variant	$31:01/*Y^b$, **31:01/*31:01*

[a] Where *X 5 any *HLA-B* allele other than *HLA-B*15:02*.
[b] Where *Y 5 any *HLA-A* allele other than *HLA-A*31:01*.
Table is adapted from Phillips EJ, Sukasem C, Whirl-Carrillo M, Müller DJ, Dunnenberger HM, Chantratita W, Goldspiel B, Chen YT, Carleton BC, George ALJ, Mushiroda T, Klein T, Gammal RS, and Pirmohamed M. Clinical Pharmacogenetics Implementation Consortium Guideline for HLA Genotype and Use of Carbamazepine and Oxcarbazepine: 2017 Update. Clinical pharmacology and therapeutics (4).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

[1] *The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations where necessary, other author insertions are shown in square brackets.*

2018 Statement from the US Food and Drug Administration (FDA)

SJS/TEN and HLA-B*1502 Allele

Retrospective case-control studies have found that in patients of Chinese ancestry there is a strong association between the risk of developing SJS/TEN with carbamazepine treatment and the presence of an inherited variant of the *HLA*-B gene, *HLA-B*15:02*. The occurrence of higher rates of these reactions in countries with higher frequencies of this allele suggests that the risk may be increased in allele-positive individuals of any ethnicity.

Across Asian populations, notable variation exists in the prevalence of *HLA-B*15:02*. Greater than 15% of the population is reported positive in Hong Kong, Thailand, Malaysia, and parts of the Philippines, compared to about 10% in Taiwan and 4% in North China. South Asians, including Indians, appear to have intermediate prevalence of *HLA-B*1502*, averaging 2 to 4%, but higher in some groups. *HLA- B*15:02* is present in <1% of the population in Japan and Korea.

*HLA- B*15:02* is largely absent in individuals not of Asian origin (e.g., Caucasians, African-Americans, Hispanics, and Native Americans).

Prior to initiating carbamazepine therapy, testing for *HLA-B*15:02* should be performed in patients with ancestry in populations in which *HLA-B*15:02* may be present. In deciding which patients to screen, the rates provided above for the prevalence of *HLA- B*15:02* may offer a rough guide, keeping in mind the limitations of these figures due to wide variability in rates even within ethnic groups, the difficulty in ascertaining ethnic ancestry, and the likelihood of mixed ancestry. Carbamazepine should not be used in patients positive for *HLA-B*15:02* unless the benefits clearly outweigh the risks. Tested patients who are found to be negative for the allele are thought to have a low risk of SJS/TEN.

Over 90% of carbamazepine treated patients who will experience SJS/TEN have this reaction within the first few months of treatment. This information may be taken into consideration in determining the need for screening of genetically at-risk patients currently on carbamazepine.

The *HLA-B*15:02* allele has not been found to predict risk of less severe adverse cutaneous reactions from carbamazepine, such as maculopapular eruption (MPE) or to predict Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS).

Limited evidence suggests that *HLA-B*15:02* may be a risk factor for the development of SJS/TEN in patients of Chinese ancestry taking other anti-epileptic drugs associated with SJS/TEN, including phenytoin. Consideration should be given to avoiding use of other drugs associated with SJS/TEN in *HLA-B*15:02* positive patients, when alternative therapies are otherwise equally acceptable.

Hypersensitivity Reactions and *HLA-A*31:01* Allele

Retrospective case-control studies in patients of European, Korean, and Japanese ancestry have found a moderate association between the risk of developing hypersensitivity reactions and the presence of *HLA-A*31:01*, an inherited allelic variant of the *HLA-A* gene, in patients using carbamazepine. These hypersensitivity reactions include SJS/TEN, maculopapular eruptions, and Drug Reaction with Eosinophilia and Systemic Symptoms.

*HLA-A*31:01* is expected to be present in the following approximate frequencies: greater than 15% in patients of Japanese and Native American ancestry; up to about 10% in patients of Han Chinese, Korean, European, and Latin American ancestry; and up to about 5% in African-Americans and patients of Indian, Thai, Taiwanese, and Chinese (Hong Kong) ancestry.

The risks and benefits of carbamazepine therapy should be weighed before considering carbamazepine in patients known to be positive for *HLA-A*31:01*.

General Information on *HLA* Genotyping and Hypersensitivity

Application of *HLA* genotyping as a screening tool has important limitations and must never substitute for appropriate clinical vigilance and patient management. Many *HLA-B*15:02*-positive and *HLA- A*31:01*-positive patients treated with carbamazepine will not develop SJS/TEN or other hypersensitivity reactions, and these reactions can still occur infrequently in *HLA-B*15:02*-negative and *HLA-A*31:01*-negative patients of any ethnicity. The role of other possible factors in the development of, and morbidity from, SJS/TEN and other hypersensitivity reactions, such as antiepileptic drug (AED) dose, compliance, concomitant medications, co-morbidities, and the level of dermatologic monitoring have not been studied.

Please review the complete therapeutic recommendations that are located here: (1).

2015 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

*HLA-B*15:02*: CARBAMAZEPINE

Patients with this genetic variation have a severely increased risk of experiencing the life-threatening cutaneous adverse event Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN). The risk of carbamazepine-induced SJS/TEN in these patients is 1.8-3.4%.

Recommendation:

1 choose an alternative if possible

*HLA-A*31:01*: CARBAMAZEPINE

Patients with this genetic variation have an increased risk of experiencing the life-threatening cutaneous adverse events DRESS and Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN). The risk of carbamazepine-induced DRESS in these patients is 0.89%.

Recommendation:

1. carefully weigh the risk of DRESS and SJS/TEN against the benefits
2. if an alternative is an option, choose an alternative

*HLA-B*15:11*: CARBAMAZEPINE

Patients with this genetic variation have an increased risk of experiencing the life-threatening cutaneous adverse event Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN). The risk of carbamazepine-induced SJS/TEN in patients with the HLA-B*15:02 allele, which carries a 4.6-6.6 times higher risk than the HLA-B*15:11 allele, is 1.8-3.4%. This would equate to a risk of carbamazepine-induced SJS/TEN in these patients of 0.27-0.73%.

Recommendation:

1. carefully weigh the risk of SJS/TEN against the benefits
2. if an alternative is an option, choose an alternative

Please review the complete therapeutic recommendations that are located here: (2).

2017 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC)

The therapeutic recommendations for *HLA-B*15:02* and carbamazepine remain unchanged from the original guideline (3) but in this update they are now also applicable to oxcarbazepine (4). These recommendations hold irrespective of the patient's region of origin or ethnic group. For patients who are *HLA-B*15:02* negative, carbamazepine or oxcarbazepine may be prescribed per standard guidelines. If a patient is carbamazepine-naïve or oxcarbazepine-naïve and *HLA-B*15:02* positive, carbamazepine and oxcarbazepine should be avoided, respectively, due to the greater risk of SJS/TEN. Other aromatic anticonvulsants, including eslicarbazepine, lamotrigine, phenytoin, fosphenytoin, and phenobarbital, have very limited evidence, if any, linking SJS/TEN with the *HLA-B*15:02* allele; however, caution should still be used when choosing an alternative agent. With regular dosing, carbamazepine- or oxcarbazepine-induced SJS/TEN usually develops within the first 4–28 days of therapy; therefore, patients who have been continuously taking carbamazepine or oxcarbazepine for longer than 3 months without developing cutaneous reactions are at extremely low risk (but not zero) of carbamazepine- or oxcarbazepine-induced adverse events in the future, regardless of *HLA-B*15:02* status.

For patients who are *HLA-A*31:01* negative, carbamazepine may be prescribed per standard guidelines (Table 3). If a carbamazepine-naïve patient also received testing for *HLA-B*15:02* and is positive for this allele, carbamazepine should be avoided regardless of the *HLA-A*31:01* genotype result. If a patient is carbamazepine-naïve and *HLA-A*31:01* positive, and if alternative agents are available, carbamazepine should be avoided due to the greater risk of SJS/TEN, DRESS, and MPE. Other aromatic anticonvulsants, including oxcarbazepine, have very limited evidence, if any, linking SJS/TEN, DRESS, and/or MPE with the *HLA-A*31:01* allele, and thus no recommendation can be made with respect to choosing another aromatic anticonvulsant as an alternative agent. If alternative agents are not available, consider the use of carbamazepine with increased frequency of clinical monitoring. Discontinue therapy at the first evidence of a cutaneous adverse reaction. As previously mentioned, since the latency period for cutaneous adverse drug reactions is known, if the patient is *HLA-A*31:01* positive and has previously used carbamazepine for longer than 3 months without incidence of a cutaneous adverse reaction, cautiously consider use of carbamazepine.

Please review the complete therapeutic recommendations that are located here: (4).

2014 Recommendations from the Canadian Pharmacogenomics Network for Drug Safety (CPNDS)

Recommendation 1.1: Genetic testing for *HLA- B*15:02* is recommended for all carbamazepine (CBZ)-naive patients before initiation of carbamazepine therapy (Level A – strong in patients originating from populations where *HLA- B*15:02* is common, its frequency unknown or whose origin is unknown; Level C – optional in patients originating from populations where *HLA-B*15:02* is rare). Genetic testing for *HLA-A*31:01* is recommended for all carbamazepine-naive patients before initiation of carbamazepine therapy (Level B – moderate in all patients; Table 6).

Recommendation 1.2: In patients who have previously taken carbamazepine for > 3 months without any adverse effects, and in whom reinitiation of carbamazepine is considered, genetic testing is NOT recommended (B). In patients who have previously taken carbamazepine for a shorter period, genetic testing should be considered (B).

Recommendation 1.3: In patients who have previously experienced a hypersensitivity reaction (HSR) potentially related to carbamazepine, genetic testing is recommended as part of the differential diagnosis and for the direction of future therapy (B).

Recommendation 1.4: In patients for whom no alternative treatment options are available, genetic testing is recommended to ensure increased alertness to hypersensitivity symptoms in positive patients (B).

Recommendation 2.1: Genetic testing for *HLA- B*15:02* is most beneficial in patients originating from a population where *HLA-B*15:02* is common (e.g., Chinese, Thai, Indian, Malay, Filipino, Indonesian; A). Nevertheless, genotyping for *HLA-B*15:02* should be considered in ALL patients, irrespective of their ancestry, as the safest option (C).

Recommendation 2.2: *HLA-A*31:01* is common in most populations studied so far. Therefore, genetic testing for this variant is recommended in patients of all ancestries (B).

Recommendation 3.1: In patients who are positive for *HLA-B*15:02* or *HLA-A*31:01*, alternative medications should be used as first-line therapy (A). Consideration in the choice of alternative medications should be given to the possibility of cross-reactivity with structurally similar antiepileptic drugs (AED) (oxcarbazepine, lamotrigine, phenytoin, phenobarbital, primidone).

Recommendation 3.2: In patients who are negative for *HLA-B*15:02* and *HLA-A*31:01*, carbamazepine can be used as first-line therapy (A). However, the occurrence of a hypersensitivity reaction cannot be excluded based on a negative genetic test result.

Table 7. Grading scheme used for clinical practice recommendations

Level	Strength	Evidence basis
A	Strong	Based on strong scientific evidence; benefits clearly outweigh risks
B	Moderate	Based on reduced confidence scientific evidence and expert opinion; benefits likely to outweigh risks
C	Optional	Based mainly on expert opinion, for use with evidence development in a research context

Table adapted from: Amstutz, U., N.H. Shear, M.J. Rieder, S. Hwang, et al., Recommendations for *HLA-B*15:02* and *HLA-A*31:01* genetic testing to reduce the risk of carbamazepine-induced hypersensitivity reactions. Epilepsia, 2014. 55(4): p. 496-506 (5).

Please review the complete therapeutic recommendations that are located here: (5).

Nomenclature

Nomenclature for selected HLA alleles

Allele name	dbSNP reference identifier for allele location	HGVS	IPD-IMGT/HLA
*HLA-B*15:02*	rs2844682 and rs3909184	NG_023187.1:c.[5T>G; 11T>C; 44C>G; 45G>A; 103T>G; 106G>A; 142T>G; 204A>G; 205G>A; 206A>T; 209A>C; 213G>C; 222G>A; 272A>C; 277G>A; 280C>A; 282G>C; 283G>A; 292G>T; 353C>T; 355C>A; 363C>G; 369C>T; 409C>T; 419A>C; 463C>A; 477C>G; 539G>T; 559G>C; 560A>T; 603C>G; 605A>C; 610G>C; 618T>G; 636C>T; 693T>C; 756T>C; 900G>A; 916G>A; 985G>A; 1008T>C; 1046G>C]	Allele Report for B*15:02:01 (HLA00165)

Nomenclature for selected continued from previous page.

Allele name	dbSNP reference identifier for allele location	HGVS	IPD-IMGT/HLA
*HLA-A*31:01*	rs1061235 and rs16333021	NM_002116.7:c.[41C>T; 97T>A; 98T>C; 238G>A; 243G>T; 282G>C; 290C>T; 363A>G; 413G>A; 448C>T; 502A>C; 524A>G; 527A>T; 555T>G; 633A>G; 642C>T; 649C>G; 651C>T; 652A>G; 691G>A; 808G>T; 829G>C; 870G>C; 899T>C; 945G>A; 952C>T; 964A>T; 967A>G; 987C>T; 992T>G; 1029T>C; 1033A>T; 1072G>A; 1077C>T]	Allele Report for A*31:01:02:01 (HLA00097)
*HLA-B*15:11*		NG_023187.1:c.[5T>G; 11T>C; 44C>G; 45G>A; 103T>G; 106G>A; 142T>G; 204A>G; 205G>A; 206A>T; 209A>C; 213G>C; 222G>A; 277G>A; 280C>A; 282G>C; 283G>A; 292G>T; 363C>G; 419A>C; 463C>A; 477C>G; 538C>T; 559G>C; 560A>T; 603C>G; 605A>C; 610G>C; 618T>G; 636C>T; 693T>C; 756T>C; 900G>A; 916G>A; 985G>A; 1008T>C; 1046G>C]	Allele Report for B*15:11:01 (HLA00174)

The IPD-IMGT/HLA Database includes the official sequences named by the WHO Nomenclature Committee for Factors of the HLA System. IPD: Immuno Polymorphism Database, IMGT: international ImMunoGeneTics project, HLA: Human Leucocyte Antigen. The sequence variation descriptions used by IMGT/HLA are in line with the HGVS recommendations for sequence variant descriptions.
Note: For the *MHC* region, variations in genes such as *HLA-B* occur across the whole sequence of the gene, not a single locus. Therefore, the *HLA-B*15:02* allele is defined by its sequence rather than single coding or protein variations. If there is strong linkage disequilibrium between one or more SNPs and a specific *HLA* allele, the presence of these SNPs (tag SNPs) may be used for *HLA* typing (51).
Because of the extreme diversity at the HLA locus, different tag SNPs may be associated with different HLA variants in different populations. For *HLA-B*15:02*, rs2844682 and rs3909184 are the tag SNPs (51). For *HLA-A*31:01*, rs1061235 is a tag SNP in Europeans (14) and rs16333021 is a tag SNP in Japanese (41). A study involving North American children of various ancestries showed that rs1061235 is not a suitable tag SNP in non-Caucasian individuals (42).
Guidelines on nomenclature of the HLA system are available from HLA Nomenclature.

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Version History

To view the 2015 version of this summary (created: October 14, 2015) please click here.

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Carisoprodol Therapy and *CYP2C19* Genotype

Laura Dean, MD[1]

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Introduction

Carisoprodol is a centrally acting muscle relaxant used to relieve acute back pain. Due to the risk of dependence and abuse, carisoprodol should only be used for treatment periods of up to two or three weeks. Carisoprodolol is a Schedule IV controlled substance and carisoprodol overdose can lead to CNS respiratory depression, seizures, and death.

Carisoprodol is metabolized by CYP2C19 to meprobamate, a sedative used to treat anxiety disorders. In individuals who have little or no CYP2C19 activity ("CYP2C19 poor metabolizers"), standard doses of carisoprodol can lead to a 4-fold increase in exposure to carisoprodol and a concomitant 50% reduced exposure to meprobamate compared to normal metabolizers. Approximately 3–5% of Caucasians and Africans, and 15–20% of Asians, are CYP2C19 poor metabolizers (1).

The FDA-approved drug label for carisoprodol states that caution should be used when administering carisoprodol to patients with reduced CYP2C19 activity and when co-administering drugs that inhibit or induce CYP2C19 (1). There are no data on the use of carisoprodol in pregnancy, and the efficacy, safety, and pharmacokinetics of carisoprodol have not been established in pediatric patients (less than 16 years of age).

Drug: Carisoprodol

Carisoprodol is a centrally acting muscle relaxant used to treat acute musculoskeletal pain. It is often used to treat acute low back pain, providing pain relief and helping patients mobilize. However, its clinical use is limited by the risk of abuse (it is a Schedule IV controlled substance) and its toxic effects in overdose, which may be fatal.

The mechanism of action of carisoprodol is not well understood, but it is an indirect agonist of the $GABA_A$ receptor associated with altered neuronal communication at the reticular formation in the brainstem and at the spinal cord. In addition to its skeletal muscle relaxing effects, carisoprodol also has weak anticholinergic, antipyretic, and analgesic properties. Adverse effects include sedation, tachycardia, shortness of breath, and dizziness (2, 3).

Carisoprodol is metabolized by CYP2C19 into meprobamate—an active metabolite that has similar potency to carisoprodol. Meprobamate is used to treat anxiety. Again, its mechanism of action is not well understood, but it has barbiturate-like properties and is toxic in overdose (4).

Individuals who have reduced or absent activity of CYP2C19 have higher plasma levels of carisoprodol, and a higher ratio of carisoprodol:meprobamate, compared to individuals who have normal levels of CYP2C19 activity. Carisoprodol's narrow therapeutic index implies there may be increased risk of toxicity in CYP2C19 poor metabolizers. However, data are limited. Small studies have found no evidence to support an association between *CYP2C19* genotype status and the mortality risk of carisoprodol or adverse effects after a single dose of carisoprodol (4-6).

The FDA-approved drug label for carisoprodol states that caution should be used when administering carisoprodol to patients with reduced CYP2C19 activity. The label also states that the co-administration of

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CYP2C19 inhibitors, such as omeprazole or fluvoxamine, could result in increased exposure of carisoprodol and decreased exposure of meprobamate, and the co-administration of CYP2C19 inducers, such as rifampin or St. John's Wort, could result in decreased exposure of carisoprodol and increased exposure of meprobamate. Low dose aspirin also showed induction effect on CYP2C19. The label states that the full pharmacological impact of these potential alterations of exposures in terms of either efficacy or safety of carisoprodol is unknown (1).

Gene: *CYP2C19*

The CYP2C19 enzyme contributes to the metabolism of a range of clinically important drugs, such as several proton pump inhibitors, clopidogrel, benzodiazepines, and several tricyclic antidepressants, including imipramine.

The *CYP2C19* gene is highly polymorphic—35 variant star (*) alleles are catalogued at the Human Cytochrome P450 (CYP) Allele Nomenclature Database: http://www.cypalleles.ki.se/cyp2c19.htm.

The *CYP2C19*1* wild-type allele is associated with normal enzyme activity and the "normal metabolizer" phenotype, whereas the *CYP2C19*17* allele is associated with increased enzyme activity and the "rapid" and "ultrarapid" metabolizer phenotypes (7).

The most common loss-of-function variant is *CYP2C19*2*, which contains a c.681G>A variant in exon 5 that results in an aberrant splice site. This leads to the production of a truncated and non-functioning protein. The *CYP2C19*2* allele frequencies are ~15% in Caucasians and Africans, and ~29–35% in Asians (7, 8).

Another commonly tested loss-of-function variant is *CYP2C19*3*, which contains a c.636G>A variant in exon 4 that causes a premature stop codon. The *CYP2C19*3* allele frequencies are ~2–9% in Asian populations, but rare in other racial groups. Other loss-of-function variants occur in less than 1% of the general population and include *CYP2C19*4-*8* (7, 8).

CYP2C19 intermediate metabolizers carry one copy of an allele that encodes reduced or absent function (e.g. **1/*2*), whereas "poor metabolizers" are homozygous or compound heterozygous for two loss-of-function alleles (e.g., **2/*2*, **2/*3*) (table 1).

Table 1. *CYP2C19* functional status and phenotypes

Phenotype	Genotype	Examples of diplotypes
CYP2C19 Ultrarapid metabolizer (~2–5% of patients)[a]	An individual carrying two increased function alleles.	**17/*17*
CYP2C19 Rapid metabolizer (~2–30% of patients)	An individual carrying one normal function allele and one increased function allele.	**1/*17*
CYP2C19 Normal metabolizer (~35–50% of patients)	An individual carrying two normal function alleles.	**1/*1*
CYP2C19 Intermediate metabolizer (~18–45% of patients)	An individual carrying one normal function allele and one no function allele or one no function allele and one increased function allele.	**1/*2* **1/*3* **2/*17*[b]

Table 1. continued from previous page.

Phenotype	Genotype	Examples of diplotypes
CYP2C19 Poor metabolizer (~2–15% of patients)	An individual carrying two no function alleles.	**2/*2* **2/*3* **3/*3*

[a] CYP2C19 metabolizer status frequencies are based on average multi-ethnic frequencies. See the *CYP2C19* Frequency Tables for population-specific allele and phenotype frequencies (9).
[b] The predicted metabolizer phenotype for the**2/*17* genotype is a provisional classification. The currently available evidence indicates that the *CYP2C19*17* increased function allele is unable to completely compensate for the *CYP2C19*2* no function allele.
Table is adapted from Hicks J.K., Sangkuhl K., Swen J.J., Ellingrod V.L., Müller D.J., Shimoda K., Bishop J.R., Kharasch E.D., Skaar T.C., Gaedigk A., Dunnenberger H.M., Klein T.E., Caudle K.E., and Stingl J.C. Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC®) for CYP2D6 and CYP2C19 Genotypes and Dosing of Tricyclic Antidepressants: 2016 Update. 2016 Dec 20; doi: 10.1002/cpt.597. [Epub ahead of print] (9)
Note: The nomenclature used in this table reflects the standardized nomenclature for pharmacogenetic terms proposed by CPIC in a 2016 paper, "Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC)" (10).

Genetic Testing

Clinical genotyping tests are available for several *CYP2C19* alleles. The NIH's Genetic Testing Registry (GTR) provides examples of the genetic tests that are currently available for carisoprodol response, CYP2C19-related poor drug metabolism, and the CYP2C19 gene.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2016 Statement from the US Food and Drug Administration (FDA):

Carisoprodol Tablets are metabolized in the liver by CYP2C19 to form meprobamate. Co-administration of CYP2C19 inhibitors, such as omeprazole or fluvoxamine, with Carisoprodol Tablets could result in increased exposure of carisoprodol and decreased exposure of meprobamate. Co-administration of CYP2C19 inducers, such as rifampin or St. John's Wort, with Carisoprodol Tablets could result in decreased exposure of carisoprodol and increased exposure of meprobamate. Low dose aspirin also showed induction effect on CYP2C19.

The full pharmacological impact of these potential alterations of exposures in terms of either efficacy or safety of Carisoprodol Tablets is unknown.

[...]

Patients with Reduced CYP2C19 Activity: Carisoprodol Tablets should be used with caution in patients with reduced CYP2C19 activity. Published studies indicate that patients who are poor CYP2C19 metabolizers have a 4-fold increase in exposure to carisoprodol, and concomitant 50% reduced exposure to meprobamate compared to normal CYP2C19 metabolizers. The prevalence of poor metabolizers in Caucasians and African Americans is approximately 3-5% and in Asians is approximately 15-20%.

Please review the complete therapeutic recommendations that are located here: (1).

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

Nomenclature for selected *CYP2C19* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C19*2*	681G>A Pro227Pro	NM_000769.1:c.681G>A	NP_000760.1:p.Pro227=	rs4244285
*CYP2C19*3*	636G>A Trp212Ter	NM_000769.1:c.636G>A	NP_000760.1:p.Trp212Ter	rs4986893
*CYP2C19*17*	-806C>T	NM_000769.2:c.-806C>T	Not applicable—variant occurs in a non-coding region	rs12248560

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: http://www.cypalleles.ki.se/

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Carvedilol Therapy and *CYP2D6* Genotype

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Introduction

Carvedilol (brand name Coreg) is used to treat heart failure and high blood pressure (hypertension). It is also used in patients who developed left ventricular dysfunction after having a heart attack (myocardial infarction, MI). In patients with cardiovascular disease, carvedilol is associated with improvements in quality of life, hospitalization rates, and survival.

Carvedilol is a non-selective beta blocker (beta 1 and beta 2) and an alpha 1 blocker. It reduces the energy demands on the heart by blocking cardiac beta receptors, which decreases the heart rate and the force of heart contractions. Carvedilol lowers blood pressure by blocking alpha receptors on blood vessels, which relaxes and dilates blood vessels.

CYP2D6 is one of the primary enzymes involved in activating and metabolizing carvedilol. Approximately 8% of Caucasians and 2% of most other populations have absent CYP2D6 activity and are predicted to be "CYP2D6 poor metabolizers."

The FDA-approved drug label for carvedilol states that plasma concentrations of carvedilol may be higher in CYP2D6 poor metabolizers compared to normal metabolizers, but does not discuss altering carvedilol dosing based on a patient's *CYP2D6* genotype (1). However, the label does state the dose of carvedilol should be individualized, and the dose should be monitored as it is gradually increased (up-titrated), based on tolerability and clinical response (Table 1).

The Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP) recommend that no action is needed for carvedilol and *CYP2D6* genotype. For CYP2D6 poor metabolizers, DPWG states that the plasma concentration of carvedilol can be elevated, but this does not result in an increase in side effects (Table 2) (2).

Table 1. FDA (2017) Drug Label for Carvedilol. Therapeutic recommendations based on *CYP2D6* Genotype.

Phenotype	Carvedilol
CYP2D6 poor metabolizers	Retrospective analysis of side effects in clinical trials showed that poor CYP2D6 metabolizers had a higher rate of dizziness during up-titration, presumably resulting from vasodilating effects of the higher concentrations of the α-blocking R(+) enantiomer.

Please see Therapeutic Recommendations based on Genotype for more information from FDA. This table is adapted from (1).

Table 2. DPWG (2016) Recommendations for Carvedilol and *CYP2D6*

Phenotype	Recommendations
CYP2D6 poor metabolizers	No action is required for this gene-drug interaction. The plasma concentration of carvedilol can be elevated. This does not, however, result in an increase in side effects.
CYP2D6 intermediate metabolizers	No action is required for this gene-drug interaction. The plasma concentration of carvedilol can be elevated. This does not, however, result in an increase in side effects.

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Table 2. continued from previous page.

Phenotype	Recommendations
CYP2D6 ultrarapid metabolizers	No action is required for this gene-drug interaction. The plasma concentration of carvedilol can be reduced. This does not, however, result in a decrease in the effectiveness.

Please see Therapeutic Recommendations based on Genotype for more information from DPWG. This table is adapted from (2).

Drug: Carvedilol

Carvedilol is widely considered to be the standard of care for patients with heart failure, particularly for patients who also have hypertension. Carvedilol is used to treat mild to severe congestive heart failure, as well as hypertension, and left ventricular dysfunction in patients who recently had an MI, but are otherwise stable.

Carvedilol is a non-selective beta blocker (blocks beta 1 and beta 2 receptors) and an alpha 1 blocker. By blocking beta receptors found in the heart, carvedilol reduces the heart rate and decreases the force of heart contractions. By blocking the alpha 1 receptors found on blood vessels, carvedilol relaxes and dilates the blood vessels, which lowers blood pressure.

In the treatment of heart failure, beta blockers such as carvedilol are thought to protect the heart from increased catecholamine stimulation (catecholamines include adrenaline and noradrenaline). In the short term, adrenergic activation can help the heart maintain cardiac performance, but over time, continued activation can be detrimental. Harmful effects include a persistently increased heart rate, down-regulation and impaired functioning of the beta receptors, and myocyte hypertrophy and death—which leads to adverse re-modelling of heart tissue.

Carvedilol exerts its therapeutic effects by protecting the failing heart from harmful adrenergic stimulation. Carvedilol reduces the heart rate, improves left ventricular function, and reduces vasoconstriction. Several large trials (e.g., MOCHA, PRECISE, COPERNICUS) have reported that carvedilol reduces all-cause mortality and decreases hospitalization in patients with heart failure (3-6).

The dose of carvedilol must be individualized and monitored during up-titration. Gradual up-titration should reduce the risk of syncope (fainting) or excessive hypotension (low blood pressure). The FDA drug label recommends carvedilol to be started at 6.25 mg twice daily, which can be increased after 3 to 10 days, based on tolerability, to 12.5 mg twice daily. The dose may then be increased to the target dose of 25 mg twice daily, although patients should be maintained on a lower dose if higher doses are not tolerated. In addition, a lower starting dose may be used (3.125 mg twice daily), and the rate of up-titration may be slowed if clinically indicated (e.g., due to low blood pressure or heart rate, or fluid retention) (1).

Carvedilol is a mixture of equal amounts of left-handed *S*(-) and right-handed *R*(+) enantiomers (a "racemic mixture"). Enantiomers are molecules that are mirror images of each other, but are not superimposable on one another. The nonselective beta-adrenoreceptor blocking activity of carvedilol is present in the *S*(-) enantiomer; and the α1-adrenergic blocking activity is present in both *R*(+) and *S*(-) enantiomers at equal potency.

Even though carvedilol plasma levels are about 50% higher in the elderly compared with young subjects, no overall differences in the safety or effectiveness were observed between these two populations except for higher incidence of dizziness in hypertensive subjects (incidence 8.8% in the elderly versus 6% in younger subjects) (1).

Carvedilol is contraindicated in patients with severe hepatic (liver) impairment because patients with severe liver impairment (i.e., cirrhosis) exhibit a 4- to 7-fold increase in carvedilol plasma levels when compared with healthy subjects (1).

Gene: *CYP2D6*

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP450 genes are highly polymorphic and can result in no, decreased, normal, or increased enzyme activity.

CYP2D6 and CYP2C9 are the primary enzymes involved in the activation and metabolism of carvedilol. Other enzymes involved to a lesser extent include CYP3A4, CYP2C19, CYP1A2, and CYP2E1. The pharmacokinetics of carvedilol are known to be influenced by genetic variation in *CYP2D6*—data do not exist for *CYP2C9*.

Individuals who have two non-functional copies of the *CYP2D6* gene are predicted to be "CYP2D6 poor metabolizers". Plasma concentrations of *R*(+)- carvedilol are 2–3 times higher in poor metabolizers, and levels of *S*(-)-carvedilol are increased by approximately 20% to 25%, compared to normal metabolizers with normal CYP2D6 activity (1).

Retrospective analysis of side effects in clinical trials showed that individuals who are CYP2D6 poor metabolizers had a higher rate of dizziness during up-titration. This is thought to result from vasodilating effects of the 2–3 times higher concentrations of the α-blocking *R*(+) enantiomer (1).

Variation in *CYP2D6* does not appear to be associated with a change in the response to carvedilol therapy. This may be because other CYP450 enzymes can convert carvedilol to its active metabolite. One small study (n=93) reported that there were no significant differences of carvedilol dose as well as the number of adverse drug reactions among patients with different *CYP2D6* genotypes (7). Another small study (n=110) found that there were significant *CYP2D6* allele-specific differences in carvedilol pharmacokinetics, but *CYP2D6* genotype had no effect on heart rate, blood pressure or adverse effects (8).

The *CYP2D6* genotype may be associated with carvedilol dosage, however. Two small studies reported that higher maintenance doses of carvedilol were tolerated by carriers of non-functional *CYP2D6* alleles (n=65) (9), and by CYP2D6 poor metabolizers (n=93) (10).

Genetic Testing

The NIH's Genetic Testing Registry (GTR) displays genetic tests that are currently available for carvedilol response and the *CYP2D6* gene. According to the FDA, the pharmacokinetics of carvedilol do not appear to be different in patients with decreased or absent CYP2D6 activity. Therefore, genetic testing prior to the use of carvedilol is not recommended.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2017 Statement from the US Food and Drug Administration (FDA)

The primary P450 enzymes responsible for the metabolism of both *R*(+) and *S*(-)-carvedilol in human liver microsomes were CYP2D6 and CYP2C9 and to a lesser extent CYP3A4, 2C19, 1A2, and 2E1. CYP2D6 is

[1] *The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations where necessary, other author insertions are shown in square brackets.*

thought to be the major enzyme in the 4'- and 5'-hydroxylation of carvedilol, with a potential contribution from 3A4. CYP2C9 is thought to be of primary importance in the O-methylation pathway of *S*(-)-carvedilol.

[…]

Carvedilol is subject to the effects of genetic polymorphism with poor metabolizers of debrisoquin (a marker for cytochrome P450 2D6) exhibiting 2- to 3-fold higher plasma concentrations of *R*(+)-carvedilol compared with extensive metabolizers. In contrast, plasma levels of *S*(-)-carvedilol are increased only about 20% to 25% in poor metabolizers, indicating this enantiomer is metabolized to a lesser extent by cytochrome P450 2D6 than *R*(+)-carvedilol. The pharmacokinetics of carvedilol do not appear to be different in poor metabolizers of *S*-mephenytoin (patients deficient in cytochrome P450 2C19).

Please review the complete therapeutic recommendations that are located here: (1).

2016 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

CYP2D6 PM: CARVEDILOL

Pharmacist text

NO action is required for this gene-drug interaction.

The plasma concentration of carvedilol can be elevated. This does not, however, result in an increase in side effects.

Background information

Carvedilol is primarily converted by CYP2D6 to 4'-hydroxycarvedilol and 5'-hydroxycarvedilol. Data from pre-clinical studies suggest that these metabolites are active.

Carvedilol is also converted by other CYP450 enzymes to the active metabolite desmethylcarvedilol.

CYP2D6 IM: CARVEDILOL

Pharmacist text

NO action is required for this gene-drug interaction.

The plasma concentration of carvedilol can be elevated. This does not, however, result in an increase in side effects.

Background information

Carvedilol is primarily converted by CYP2D6 to 4'-hydroxycarvedilol and 5'-hydroxycarvedilol. Data from pre-clinical studies suggest that these metabolites are active.

Carvedilol is also converted by other CYP450 enzymes to the active metabolite desmethylcarvedilol.

CYP2D6 UM: CARVEDILOL

Pharmacist text

NO action is required for this gene-drug interaction.

The plasma concentration of carvedilol can be reduced. This does not, however, result in a decrease in the effectiveness.

Background information

Carvedilol is primarily converted by CYP2D6 to 4'-hydroxycarvedilol and 5'-hydroxycarvedilol. Data from pre-clinical studies suggest that these metabolites are active.

Carvedilol is also converted by other CYP450 enzymes to the active metabolite desmethylcarvedilol.

Please review the complete therapeutic recommendations that are located here: (2)

Acknowledgments

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Celecoxib Therapy and *CYP2C9* Genotype

Laura Dean, MD[1]

Created: August 18, 2016.

Introduction

Celecoxib is a nonsteroidal anti-inflammatory drug (NSAID) that is used in the management of osteoarthritis, rheumatoid arthritis, menstrual symptoms, and acute pain. Most NSAIDs inhibit both types of cyclooxygenase, COX-1 and COX-2. These enzymes catalyze pathways that play a key role in the generation of the inflammatory response; however, celecoxib, selectively inhibits COX-2.

The *CYP2C9* gene encodes an enzyme involved in the metabolism of many drugs, and is one of the main enzymes that metabolizes and inactivates celecoxib. Two common variants, *CYP2C9*2* and *CYP2C9*3*, are associated with significantly reduced CYP2C9 enzyme activity. Individuals who carry two copies of these variants (or other loss-of-function variant *CYP2C9* alleles) are considered CYP2C9 "poor metabolizers" and may be exposed to high drug levels after standard celecoxib doses.

The FDA-approved drug label for celecoxib states: "patients who are known or suspected to be poor CYP2C9 metabolizers based on genotype or previous history/experience with other CYP2C9 substrates (such as warfarin, phenytoin) should be administered celecoxib with caution. Consider starting treatment at half the lowest recommended dose in poor metabolizers (i.e., *CYP2C9*3/*3*). Consider using alternative management in juvenile rheumatoid arthritis (JRA) patients who are poor metabolizers" (1).

Drug: Celecoxib

Celecoxib is a NSAID that is used to treat osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, painful menstruation, and acute pain (1). It is also used to reduce the number of colon and rectum polyps in patients with familial adenomatous polyposis.

Worldwide, it is estimated that more than 30 million people receive NSAIDs daily (2). They are one of the most commonly used classes of medicine. Several NSAIDs (aspirin, ibuprofen, and naproxen) are available over-the-counter, but stronger doses and other types of NSAIDs, such as celecoxib, are only available via prescription. It is thought that approximately 25% of the population has experienced NSAID-related side effects that require medical care (3).

Most NSAIDs are non-selective COX inhibitors that reduce the production of pro-inflammatory prostaglandins by inhibiting both COX-1 and COX-2. COX is the central enzyme in the synthesis of prostaglandins and thromboxane A_2 from arachidonic acid. Prostaglandins can be protective (e.g., protect the gastric mucosal lining and aids platelet aggregation) or inflammatory (e.g., recruiting inflammatory white blood cells).

Celecoxib is a selective COX-2 inhibitor, which promotes the production of the gastric mucosal lining. Although celecoxib may be more gastroprotective than non-selective NSAIDs (4-7), the use of celecoxib still increases the risks of gastrointestinal adverse events. The FDA-approved label for celecoxib includes the warning that:

NSAIDs, including CELECOXIB, cause an increased risk of serious gastrointestinal adverse events including bleeding, ulceration, and perforation of the stomach or intestines, which can be fatal. These events can occur at any time during use and without warning symptoms (1).

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In the US, acute gastrointestinal bleeding associated with the use of NSAIDs may cause more than 30,000 hospitalizations per year (8). Several risk factors for NSAID-related bleeding have been identified, including old age, a history of peptic ulcer disease, high dosages of NSAIDs, concomitant use of different NSAIDs (9), and *CYP2C9* genotype.

Gene: *CYP2C9*

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs in the liver. The CYP450 genes are very polymorphic and can result in reduced, absent, or increased enzyme activity. CYP2C9 metabolizes approximately 15% of clinically used drugs, and atypical metabolic activity caused by genetic variants in the *CYP2C9* gene can play a major role in adverse drug reactions (10, 11).

At least 16 different NSAIDS are metabolized, in part, by CYP2C9 (12). Celecoxib is extensively metabolized by CYP2C9, with minor contributions from CYP3A4, CYP2C8 and CYP2C19 (3).

*CYP2C9*1* is the wild-type allele and is associated with normal enzyme activity and the normal metabolizer phenotype. Two common variants, *CYP2C9*2* (p.Arg144Cys) and *CYP2C9*3* (p.Ile359Leu), are associated with significantly reduced enzyme activity. Carriers of these variants have altered pharmacokinetics of several NSAIDs: celecoxib, flurbiprofen, ibuprofen, and tenoxicam (12, 13). This could potentially lead to dose recommendations based upon *CYP2C9* genotype, and be used to identify individuals who are at increased risk of adverse events. However, pharmacogenetic testing has been limited to retrospective studies to identify the causes of an atypical response to NSAID (11).

Studies have found that *CYP2C9*3* is associated with an increased risk of bleeding associated with NSAID use (9, 14). In contrast, *CYP2C9*3* was found to be beneficial in a trial where celecoxib was given to prevent colorectal adenomas. High dose celecoxib had greater efficacy in preventing new adenomas than low-dose celecoxib, but only among individuals who were carriers of *CYP2C9*3* (15, 16).

The frequencies of variant *CYP2C9* alleles vary between different ethnic groups (17-19). The **2* allele is more common in Caucasian and Middle Eastern populations (10-20%), than in Asian or African populations (0-6%) (19-21). The **3* allele is less common (<10% in most populations) and extremely rare in African populations (19, 22).

The influence of other variant alleles, such as *CYP2C9*8* and *CYP2C9*11*, on celecoxib levels in the plasma has not yet been evaluated.

Genetic Testing

Clinical genotyping tests are available for several *CYP2C9* alleles, and a list of tests is available at the Genetic Testing Registry (GTR) of the National Institutes of Health: http://www.ncbi.nlm.nih.gov/gtr/tests/?term=1559[geneid].

The variants that are most commonly tested for are *CYP2C9*2* and *CYP2C9*3*. Test results are typically reported as a diplotype (e.g., *CYP2C9 *3/*3*), and may also include an interpretation of the patient's predicted metabolizer phenotype: ultrarapid, normal (extensive), intermediate, or poor.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2015 Statement from the US Food and Drug Administration (FDA): Poor Metabolizers of CYP2C9 Substrates: Patients who are known or suspected to be poor CYP2C9 metabolizers based on genotype or previous history/experience with other CYP2C9 substrates (such as warfarin, phenytoin) should be administered celecoxib with caution. Consider starting treatment at half the lowest recommended dose in poor metabolizers (i.e., *CYP2C9*3/*3*). Consider using alternative management in junior rheumatoid arthritis (JRA) patients who are poor metabolizers.

Please review the complete therapeutic recommendations that are located here: (1).

Nomenclature

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C9*2*	430C>T Arg144Cys	NM_000771.3:c.430C>T	NP_000762.2:p.Arg144Cys	rs1799853
*CYP2C9*3*	1075A>C Ile359Leu	NM_000771.3:c.1075A>C	NP_000762.2:p.Ile359Leu	rs1057910

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: http://www.cypalleles.ki.se/

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[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

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Clobazam Therapy and *CYP2C19* Genotype

Laura Dean, MD[1]

Created: September 23, 2019.

Introduction

Clobazam (brand names Onfi, Sympazan) is approved by the FDA to treat seizures associated with Lennox-Gastaut syndrome (LGS) in patients aged 2 years and older (1). The drug is widely used in the chronic treatment of focal and generalized seizures, and has application in the treatment of diverse epilepsy syndromes, including epileptic encephalopathies other than LGS, such as Dravet syndrome (2-6).

Lennox-Gastaut syndrome is characterized by different types of seizures that typically begin in early childhood and may be associated with intellectual disability. Clobazam has been shown in controlled clinical trials to reduce drop (atonic) seizures in children with LGS, but there is evidence that it is effective for other seizure types as well.

Clobazam is a 1,5-benzodiazepine that acts as a positive allosteric modulator of $GABA_A$ receptors. It is often used in combination with other drugs, including stiripentol, cannabidiol, and many others.

Clobazam is extensively metabolized in the liver by cytochrome P450 (CYP) and non-CYP transformations. The major metabolite is N-desmethylclobazam (norclobazam), which has similar activity to clobazam on $GABA_A$ receptors and is an active antiseizure agent. During chronic treatment, levels of norclobazam are 8–20 times higher than those of the parent drug so that seizure protection during chronic therapy is mainly due to this metabolite.

Norclobazam is principally metabolized by CYP2C19. Individuals who lack CYP2C19 activity ("CYP2C19 poor metabolizers") have higher plasma levels of norclobazam and are at an increased risk of adverse effects.

The FDA-approved drug label states that for patients known to be CYP2C19 poor metabolizers, the starting dose of clobazam should be 5 mg/day. Dose titration should proceed slowly according to weight, but to half the standard recommended doses, as tolerated. If necessary and based upon clinical response, an additional titration to the maximum dose (20 mg/day or 40 mg/day, depending on the weight group) may be started on day 21 (Table 1) (1).

Table 1. The FDA (2019) Drug Label for Clobazam: Recommended Total Daily Dosing by Weight Group

	Less than or equal to 30 kg body weight	Greater than 30 kg body weight	CYP2C19 poor metabolizers
Starting dose	5 mg	10 mg	In patients known to be CYP2C19 poor metabolizers, the starting dose should be 5 mg/day and dose titration should proceed slowly according to weight, but to half the recommended total daily doses presented in this table, as tolerated. If necessary, and based upon clinical response, an additional titration to the maximum dose (20 mg/day or 40 mg/day, depending on the weight group) may be started on day 21.
Starting day 7	10 mg	20 mg	
Starting day 14	20 mg	40 mg	

This FDA table is adapted from (1).

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Drug: Clobazam

Clobazam is a 1,5-benzodiazepine that is an adjunct treatment of seizures associated with LGS. Clobazam is used in patients aged 2 years and above, and is dosed according to body weight (1). Clobazam was licensed for use in the United States in 2011.

Lennox-Gastaut syndrome is characterized by severe seizures in childhood. Typically, seizures begin between 3–5 years of age, and there may be different seizure types (e.g., absent, tonic, atonic, myoclonic). In addition, there may be signs of mental retardation.

Over half of all cases of LGS are associated with another condition, such as tuberous sclerosis, meningitis, or head injuries. For approximately 40% of cases, the cause is not known, but increasingly, genetic disorders are being identified, such as de novo mutations or chromosomal syndromes.

The seizures associated with LGS are often difficult to treat. Therapy is influenced by the underlying cause of the syndrome, and certain antiseizure drugs, such as clobazam, have been found to be helpful. Previously, 2 studies reported that clobazam was effective at reducing drop seizures in children with LGS (5, 7). Other treatment options include a ketogenic diet, as well as surgical options (8).

The FDA-approved drug label for clobazam contains a boxed warning regarding the risks of the concomitant use of benzodiazepines (such as clobazam) with opioids. The warning states that this may result in profound sedation, respiratory depression, coma, and death. Other adverse effects of clobazam therapy include sedation, lethargy, drooling, severe dermatological reactions, and dependence (1).

Clobazam may cause fetal harm. There are no adequate studies in pregnant women, but in animal studies, the administration of clobazam during pregnancy resulted in fetal toxicity, including an increased incidence of fetal malformations. Therefore, clobazam should only be used during pregnancy if the potential benefit to the mother justifies the potential risk to the fetus. Infants born to mothers who have taken benzodiazepines in later stages of pregnancy can develop dependence and subsequently undergo withdrawal in the postnatal period.

Clobazam is primarily metabolized by CYP3A4, and to a lesser extent, by CYP2C19 and CYP2B6. The active metabolite, N-desmethylclobazam (norclobazam) is an antiseizure agent that is less potent than clobazam, but during chronic clobazam therapy, the circulating levels of norclobazam are 8–20 times higher than clobazam levels. Therefore, seizure protection during chronic therapy is mainly due to norclobazam.

With long-term exposure, tolerance to clobazam does occur in some patients; however, many patients exhibit continued efficacy. Therefore, the propensity for tolerance maybe less than with some other benzodiazepines (1, 9–11).

In individuals who lack CYP2C19 activity ("CYP2C19 poor metabolizers"), standard doses of clobazam lead to higher levels of norclobazam. Compared with individuals with normal CYP2C19 activity, poor metabolizers have up to 5 fold higher plasma levels of norclobazam, increasing the risk of adverse effects.

The recommended total daily doses of clobazam, by weight, are provided in the drug label (Table 1). The label states that each dose should be individualized within each body weight group based on clinical efficacy and tolerability. Any dose greater than 5 mg should be divided into a twice daily dose, and increases in doses should not be increased more rapidly than weekly, because the serum concentrations of clobazam and its active metabolite requires 5 and 9 days, respectively, to reach steady-state (1). Steady-state levels of clobazam are typically reached in under 3 weeks in normal metabolizers, but may take several months in CYP2C19 poor metabolizers (12).

Determining a patient's CYP2C19 status may be helpful in preventing an overdose when starting clobazam therapy, because levels of norclobazam will be increased in CYP2C19 poor metabolizers (13–15). The drug label

states that in patients known to be CYP2C19 poor metabolizers, the starting dose should be 5 mg/day (1). A 2015 study recommends a lower starting dose of 2.5 mg/day (16).

In CYP2C19 poor metabolizers, the drug label states that dose titration should proceed slowly, as tolerated, and according to weight, but to half the dose presented in Table 1. If necessary and based upon clinical response, an additional titration to the maximum dose (20 mg/day or 40 mg/day, depending on the weight group) may be started on day 21 (1).

Gene: CYP2C19

The CYP superfamily is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP genes are very polymorphic and can result in reduced, absent, or increased drug metabolism.

The CYP2C19 enzyme contributes to the metabolism of a range of clinically important drugs, such as antidepressants, antiplatelet agents, some proton pump inhibitors, and benzodiazepines such as clobazam.

The *CYP2C19* gene is highly polymorphic as there currently are 35 variant star (*) alleles catalogued by the Pharmacogene Variation (PharmVar) Consortium. The *CYP2C19*1* is considered the wild-type allele when no variants are detected and is associated with normal enzyme activity and the "normal metabolizer" phenotype.

The *CYP2C19*17* allele is associated with increased enzyme activity and is found among individuals with "rapid" (**1/*17*) and "ultrarapid" (**17/*17*) metabolizer phenotypes. Heterozygous carriers of non-functional alleles (e.g., **2* and **3*) are classified as "intermediate metabolizers" (e.g., **1/*2*), and individuals who have 2 non-functional alleles are classified as "poor metabolizers" (e.g., **2/*2*, **2/*3*) (Table 2).

Table 2. CPIC (2016). Assignment of *CYP2C19* Phenotypes.

Phenotype	Genotype	Examples of diplotypes
CYP2C19 ultrarapid metabolizer (approximately 2–5% of individuals)[a]	An individual who has 2 increased function alleles	**17/*17*
CYP2C19 rapid metabolizer (approximately 2–30% of individuals)	An individual who has one normal function allele and one increased function allele	**1/*17*
CYP2C19 normal metabolizer (approximately 35–50% of individuals)	An individual who has 2 normal function alleles	**1/*1*
CYP2C19 intermediate metabolizer (approximately 18–45% of individuals)	An individual who has one normal function allele and one no function allele, or one no function allele and one increased function allele	**1/*2* **1/*3* **2/*17*[b]
CYP2C19 poor metabolizer (approximately 2–15% of individuals)	An individual who has 2 no function alleles	**2/*2* **2/*3* **3/*3*

[a] CYP2C19 metabolizer status frequencies are based on average multi-ethnic frequencies. See the *CYP2C19* Frequency Tables for population-specific allele and phenotype frequencies (17).
[b] The predicted metabolizer phenotype for the **2/*17* genotype is a provisional classification. The currently available evidence indicates that the *CYP2C19*17* increased function allele is unable to completely compensate for the *CYP2C19*2* no function allele.
This CPIC table is adapted from (17).

Approximately 2% of Caucasians, 4% of African Americans, and 15–25% in East Asians are *CYP2C19* poor metabolizers; and up to 45% of patients are CYP2C19 intermediate metabolizers (17–19).

The most common no function allele is *CYP2C19*2*, which is defined by a c.681G>A variant in exon 5 that creates an aberrant splice site that translates a truncated and nonfunctioning protein. The *CYP2C19*2* allele frequencies are ~15% in Caucasians and Africans, and ~29–35% in Asians (20).

For CYP2C19, another commonly tested no function variant is *CYP2C19*3*, which contains a c.636G>A variant in exon 4 that causes a premature stop codon. The *CYP2C19*3* allele frequencies are ~2–9% in Asian populations, but rare in other racial groups. Other no function variants occur in less than 1% of the general population, and include *CYP2C19*4–*8* (20).

Genetic Testing

Clinical genotyping tests are available for several *CYP2C19* alleles. The NIH's Genetic Testing Registry (GTR) provides examples of the genetic tests that are currently available for the clobazam response and the *CYP2C19* gene. In addition, variant *CYP2C19* alleles to be included in clinical genotyping assays have been recommended by the Association for Molecular Pathology (21).

Usually an individual's result is reported as a diplotype, such as *CYP2C19 *1/*1*, and may also include an interpretation of the individual's predicted metabolizer phenotype (ultrarapid, normal, intermediate, or poor). Table 2 summarizes common *CYP2C19* phenotypes.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2019 Statement from the US Food and Drug Administration (FDA)

2.5 Dosage Adjustments in CYP2C19 Poor Metabolizers

In CYP2C19 poor metabolizers, levels of N-desmethylclobazam, clobazam's active metabolite, will be increased. Therefore, in patients known to be CYP2C19 poor metabolizers, the starting dose should be 5 mg/day and dose titration should proceed slowly according to weight, but to half the dose presented in Table 1, as tolerated. If necessary and based upon clinical response, an additional titration to the maximum dose (20 mg/day or 40 mg/day, depending on the weight group) may be started on day 21.

[…]

8.6 CYP2C19 Poor Metabolizers

Concentrations of clobazam's active metabolite, N-desmethylclobazam, are higher in CYP2C19 poor metabolizers than in normal metabolizers. For this reason, dosage modification is recommended

[…]

12.5 Pharmacogenomics

The polymorphic CYP2C19 is the main enzyme that metabolizes the pharmacologically active N-desmethylclobazam. Compared with CYP2C19 normal metabolizers, N-desmethylclobazam AUC and Cmax are approximately 3–5 times higher in poor metabolizers (e.g., subjects with *2/*2 genotype) and 2 times higher in

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

intermediate metabolizers (e.g., subjects with *1/*2 genotype). The prevalence of CYP2C19 poor metabolism differs depending on racial/ethnic background. Dosage in patients who are known CYP2C19 poor metabolizers may need to be adjusted.

The systemic exposure of clobazam is similar for both CYP2C19 poor and normal metabolizers.

Please review the complete therapeutic recommendations that are located here: (1).

Nomenclature for Selected *CYP2C19* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C19*2*	681G>A Pro227Pro	NM_000769.1:c.681G>A	NP_000760.1:p.Pro227=	rs4244285
*CYP2C19*3*	636G>A Trp212Ter	NM_000769.1:c.636G>A	NP_000760.1:p.Trp212Ter	rs4986893
*CYP2C19*17*	-806C>T	NM_000769.1:c.-806C>T	Not applicable –– variant occurs in a non-coding region	rs12248560

dbSNP: The Single Nucleotide Polymorphism Database
Note: the normal "wild-type" allele is *CYP2C19*1*.
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (22).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for cytochrome P450 enzymes is available from Pharmacogene Variation (PharmVar) Consortium.

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Clopidogrel Therapy and *CYP2C19* Genotype

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Introduction

Clopidogrel (brand name Plavix) is an antiplatelet agent. Clopidogrel reduces the risk of myocardial infarction (MI) and stroke in patients with acute coronary syndrome (ACS), and in patients with atherosclerotic vascular disease (indicated by a recent MI or stroke, or established peripheral arterial disease) (1). Clopidogrel is also indicated in combination with aspirin in patients undergoing percutaneous coronary interventions (PCI), e.g., the placement of a stent.

The effectiveness of clopidogrel depends on its conversion to an active metabolite by CY2C19. Individuals who carry 2 non-functional copies of the *CYP2C19* gene are classified as CYP2C19 poor metabolizers. They have no enzyme activity and cannot activate clopidogrel via the CYP2C19 pathway, which means the drug will have no effect. Approximately 2% of Caucasians, 4% of African Americans, and 14% of Chinese are CYP2C19 poor metabolizers.

The 2017 FDA-approved drug label for clopidogrel includes a boxed warning concerning the diminished antiplatelet effect of clopidogrel in CYP2C19 poor metabolizers (Table 1). The warning states that tests are available to identify patients who are CYP2C19 poor metabolizers, and to consider the use of another platelet P2Y12 inhibitor in patients identified as CYP2C19 poor metabolizers.

The effectiveness of clopidogrel is also reduced in individuals who are CYP2C19 intermediate metabolizers. These individuals carry one non-functional copy of *CYP2C19*, with either one normal function copy or one increased function copy. For patients with ACS who are undergoing PCI, the 2013 Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for clopidogrel recommends an alternative antiplatelet therapy (e.g., prasugrel, ticagrelor) for CYP2C19 poor or intermediate metabolizers, if there is no contraindication (Table 2) (2).

The Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP) have also made antiplatelet therapy recommendations based on *CYP2C19* genotype. For patients with ACS who receive PCI, they recommend an alternative drug to clopidogrel in poor metabolizers, and for intermediate metabolizers, they recommend choosing an alternative drug, or doubling the dose of clopidogrel to 150 mg daily dose, 600 mg loading dose (Table 3) (3).

Table 1. FDA (2017) Drug Label for Clopidogrel. Warning: Diminished Antiplatelet Effect in Patients with 2 Loss-of-Function Alleles of the *CYP2C19* Gene.

Phenotype	Recommendations
CYP2C19 poor metabolizer	Consider use of another platelet P2Y12 inhibitor in patients identified as CYP2C19 poor metabolizers

Please see Therapeutic Recommendations based on Genotype for more information from the FDA. This table is adapted from (1).

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Table 2. CPIC (2013) Antiplatelet Therapy Recommendations based on *CYP2C19* Status when considering Clopidogrel for ACS/PCI Patients.

Phenotype	Examples of diplotypes	Implications for clopidogrel	Therapeutic recommendations for clopidogrel in ACS/PCI[a]
Ultrarapid metabolizer	**17/*17*	Increased platelet inhibition; decreased residual platelet aggregation[b]	Dose recommended by drugs label
Rapid metabolizer	**1/*17*		
Normal metabolizer	**1/*1*	Normal platelet inhibition; normal residual platelet aggregation	Dose recommended by drug label
Intermediate metabolizer	**1/*2* **1/*3* **2/*17*	Reduced platelet inhibition; increased residual platelet aggregation; increased risk for adverse cardiovascular events	Alternative antiplatelet therapy recommended if no contraindication, e.g., prasugrel, ticagrelor
Poor metabolizer	**2/*2* **2/*3* **3/*3*	Significantly reduced platelet inhibition; increased residual platelet aggregation; increased risk for adverse cardiovascular events	Alternative antiplatelet therapy recommended if no contraindication, e.g., prasugrel, ticagrelor

[a] The strength of therapeutic recommendations is "moderate" for intermediate metabolizers and "strong" for all other metabolizers. See Supplementary Materials and Methods (Strength of Therapeutic Recommendations) online.
[b] The *CYP2C19*17* allele may be associated with increased bleeding risks, see (4).
ACS, acute coronary syndrome
PCI, percutaneous coronary intervention
Please see Therapeutic Recommendations based on Genotype for more information from CPIC. This table is adapted from (2).
The nomenclature used in this table reflects the standardized nomenclature for pharmacogenetic terms proposed by CPIC in a 2017 paper, "Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC)" (5). Note: the 2013 CPIC guideline for clopidogrel therapy includes *1/*17 as an example diplotype for the ultrarapid metabolizer phenotype and does not include the rapid metabolizer phenotype. However, in more recent guidelines using updated nomenclature, *1/*17 is included as an example diplotype for the rapid metabolizer phenotype (Table 4).

Table 3. DPWG (2017) Recommendations for Clopidogrel and *CYP2C19* Phenotype.

Phenotype	Recommendation
Ultrarapid metabolizer	NO action is required for this gene-drug interaction
Intermediate metabolizer	Percutaneous coronary intervention: 1 choose an alternative or double the dose to 150 mg/day (600 mg loading dose) Prasugrel and ticagrelor are not metabolised by CYP2C19 (or to a lesser extent) Other indications: 1 no action required
Poor metabolizer	Percutaneous coronary intervention: 1 choose an alternative Prasugrel and ticagrelor are not metabolised by CYP2C19 (or to a lesser extent) Other indications: 1. determine the level of inhibition of platelet aggregation by clopidogrel 2. consider an alternative in poor responders Prasugrel and ticagrelor are not metabolised by CYP2C19 (or to a lesser extent)

Please see Therapeutic Recommendations based on Genotype for more information from DPWG. This table is adapted from (3).

Drug: Clopidogrel

Clopidogrel is an antiplatelet drug used in the treatment of patients with ACS, managed medically or with PCI. Clopidogrel is also used in the treatment of patients with atherosclerotic vascular disease, as indicated by a recent MI, a recent ischemic stroke, or symptomatic peripheral arterial disease. Clopidogrel has been shown to reduce the rate of subsequent MI and stroke in these patients (1, 6).

Clopidogrel is a P2RY12 inhibitor (purinergic receptor P2Y, G-protein coupled 12). Clopidogrel acts by irreversibly binding to the platelet P2RY12 receptor, and blocking adenosine diphosphate (ADP)-mediated platelet activation and aggregation. Clopidogrel belongs to the second generation of thienopyridine antiplatelet agents.

Clopidogrel is given to treat or to prevent further occurrences of arterial thrombosis, which occurs when a blood clot (thrombus) forms inside an artery. Often, arterial thrombosis is triggered in response to the rupturing of the atherosclerotic plaque lining the arterial wall. If the thrombus occludes the arterial lumen, the blood flow is reduced or stopped, resulting in ischemia. In the brain, thrombosis in the cerebral arteries can cause a transient ischemic attack (TIA) or ischemic stroke. In the peripheral vessels, thrombosis can cause peripheral artery disease, and in the heart, a thrombosis in the coronary arteries is a common cause of ACS. Platelet inhibitors such as clopidogrel interrupt the formation of the thrombus, which involves the rapid recruitment and activation of platelets.

ACS reflects a decreased blood flow in the coronary arteries and comprises unstable angina and MI. Unstable angina occurs suddenly, often at rest or with minimal exertion, and may be new in onset or may occur with less exertion than previously. An MI may be classified as "STEMI" or "NSTEMI" based on EKG findings. EKG findings that include ST segment elevation are termed "ST segment elevation MI" (STEMI). If no ST segment elevation is present but myocardial biomarkers such as troponin I or T are increased, the term "non-ST segment elevation MI" (NSTEMI) is applied.

In patients with ACS (unstable angina, NSTEMI, or STEMI), the addition of 75 mg daily clopidogrel to aspirin and other standard treatments reduces the risk of MI, stroke, and death, compared with the addition of placebo (7, 8).

However, despite the general efficacy of clopidogrel, resistance is common. Resistance to an antiplatelet drug occurs when there is no significant reduction in platelet function after therapy, compared with baseline platelet function. Clopidogrel treatment failure occurs when there is a thrombotic or ischemic event (e.g., stent thrombosis or recurrent ACS) during clopidogrel therapy in patients with "High on-Treatment Platelet Reactivity" (HTPR).

HTPR occurs when the platelet P2Y12 receptors are still responsive despite clopidogrel therapy. It is tested for by adding an ADP agonist to a plasma sample and measuring aggregation or intracellular markers of platelet activation. It has been estimated that between 16–50% of patients treated with clopidogrel have HTPR (9).

Platelet function assays are used to assess platelet response; they measure "Platelet Reactivity Units" (PRU). The PRU cut-off values vary, but generally, the therapeutic window for clopidogrel is around 95-208 PRU. A PRU value higher than 208 indicates clopidogrel resistance, and a value below 95 is associated with a higher risk for major bleeding (10, 11). Most (but not all) studies report an association between clopidogrel resistance (HTPR or high PRU) and an increased risk of thrombotic/ischemic event following PCI, such as stent thrombosis (12).

A poor response to clopidogrel is due to, in part, genetic variations in the *CYP2C19* gene. Other genes that may influence clopidogrel response include *ABCB1* (13-15), *P2Y12*, and *GPIIIA* (16-18). Clopidogrel is a prodrug, and CYP2C19 is the major enzyme involved in the conversion of clopidogrel into an active metabolite.

Several studies have reported an increase in adverse cardiovascular events in patients who carry one or 2 non-functional copies of the *CYP2C19* gene ("intermediate metabolizers" and "poor metabolizers", respectively), compared with patients with 2 normal copies of the *CYP2C19* gene ("normal metabolizers"). These studies focus on patients with ACS undergoing PCI, with carriers of non-functional alleles also being at a higher risk of stent thrombosis (19-21). These patients may require much higher doses of clopidogrel (e.g., 4-fold higher) or an alternative drug (22, 23).

The studies that did not find a significant association between *CYP2C19* and clinical outcome in patients with ACS, often included some data from non-PCI patients (12, 24, 25).

Several studies of patients with TIA have reported that *CYP2C19* status influences the risk of having an ischemic stroke or adverse clinical outcomes following a stroke (26-28). A recent trial (CHANCE - (Clopidogrel in High-risk Patients with Acute Nondisabling Cerebrovascular Events) found that the use of clopidogrel plus aspirin compared with aspirin alone reduced the risk of a new stroke only in the subgroup of patients who were not carriers of the *CYP2C19* non-functional alleles (29).

Alternative antiplatelet drugs to clopidogrel, such as prasugrel (a third generation thienopyridine) and ticagrelor (a cyclopentyl triazolopyrimidine), are not dependent upon CYP2C19 for activation. Although both clopidogrel and prasugrel form active metabolites with similar potency, in the population overall, prasugrel is a more potent antiplatelet agent than clopidogrel due to the more efficient formation of the active metabolite from the prodrug (30).

A large trial, TRITON-TIMI 38, compared prasugrel with clopidogrel in 13,608 patients with ACS who were undergoing PCI. Prasugrel was found to provide more potent platelet inhibition than clopidogrel: and after 15 months, the patients treated with prasugrel had a lower incidence of the combined endpoint of cardiovascular death, nonfatal MI, or nonfatal stroke as compared with patients treated with clopidogrel (9.9% vs. 12.1%) (31, 32). However, prasugrel was associated with a higher risk of bleeding, leading to the FDA warning that the use of prasugrel is contraindicated in patients with active pathological bleeding, or a history of stroke or TIA (33, 34). In addition, prasugrel has also an FDA box warning for patients with a high probability of undergoing coronary artery bypass grafting (prasugrel should not be started, or when possible, discontinue prasugrel at least 7 days prior to any surgery) (35).

In an analysis from the recent PLATO trial, ticagrelor was found to be superior to clopidogrel in a subgroup of patients with STEMI who were treated with PCI. Consistent with the overall results of the trial, ticagrelor was found to have superior efficacy and similar safety compared with clopidogrel (36).

In addition, the latest guideline from the American College of Cardiology/American Heart Association includes a preference for alternative therapy over clopidogrel in patients with ACS/PCI. This is a class IIa recommendation based on moderate quality, from the 2016 focused update on dual antiplatelet therapy (37). In full, this recommendation states "In patients with ACS (NSTE-ACS or STEMI) treated with dual antiplatelet therapy after coronary stent implantation who are not at high risk for bleeding complications and who do not have a history of stroke or TIA, it is reasonable to choose prasugrel over clopidogrel for maintenance P2Y12 inhibitor therapy" (37).

Although prasugrel is more effective than standard-dose clopidogrel, dual antiplatelet therapy with clopidogrel and aspirin remains the standard of care at many institutions for patients with ACS undergoing PCI (38-40). This may be because clopidogrel has a lower bleeding risk and is less expensive (41). However, the availability of *CYP2C19* genetic testing can facilitate personalized antiplatelet therapy, with individuals with impaired CYP2C19 activity being identified early, and offered an alternative antiplatelet agent, such as prasugrel (39, 42-45).

Recent studies have found that *CYP2C19*-genotype guided antiplatelet therapy results in a higher likelihood of achieving a therapeutic level of on-treatment platelet reactivity (10, 46-48), which may also be cost effective among ACS patients undergoing PCI (39, 49-51). However, more data are needed to determine whether routine genotyping and platelet function tests could help reduce future cardiovascular events in ACS patients (52-54).

Gene: CYP2C19

The cytochrome P450 superfamily (CYP) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP genes are very polymorphic and can result in reduced, absent, or increased drug metabolism.

The CYP2C19 enzyme contributes to the metabolism of a range of clinically important drugs, such as antidepressants, benzodiazepines, voriconazole (55), some proton pump inhibitors, and the antiplatelet agent, clopidogrel.

The variability of clopidogrel metabolism and treatment outcomes between individuals is partly determined by variant alleles of the *CYP2C19* gene.

The *CYP2C19* gene is highly polymorphic—35 variant star (*) alleles are catalogued at the Pharmacogene Variation (PharmVar) Consortium. The *CYP2C19*1* is considered the wild type allele when no variants are detected, and is categorized as normal enzyme activity and the "normal metabolizer" phenotype.

The *CYP2C19*17* allele is associated with increased enzyme activity and, depending on the number of alleles present, is associated with the "rapid" (one **17* allele) and "ultrarapid" (2 **17* alleles) metabolizer phenotypes. Non-functional alleles include *CYP2C19*2* and **3*. *CYP2C19* intermediate metabolizers carry one copy of an allele that encodes a non-functional allele (e.g. **1/*2*), whereas "poor metabolizers" carry 2 non-functional alleles (e.g., **2/*2*, **2/*3*) (Table 4).

Table 4. *CYP2C19* Functional Status and Phenotypes, CPIC 2016.

Phenotype	Genotype	Examples of diplotypes
CYP2C19 ultrarapid metabolizer (~2–5% of patients)[a]	An individual carrying 2 increased function alleles	**17/*17*
CYP2C19 rapid metabolizer (~2–30% of patients)	An individual carrying one normal function allele and one increased function allele	**1/*17*
CYP2C19 normal metabolizer (~35–50% of patients)	An individual carrying 2 normal function alleles	**1/*1*
CYP2C19 intermediate metabolizer (~18–45% of patients)	An individual carrying one normal function allele and one no function allele or one no function allele and one increased function allele	**1/*2* **1/*3* **2/*17*[b]
CYP2C19 poor metabolizer (~2–15% of patients)	An individual carrying 2 no function alleles	**2/*2* **2/*3* **3/*3*

[a] CYP2C19 metabolizer status frequencies are based on average multi-ethnic frequencies. See the *CYP2C19* Frequency Tables for population-specific allele and phenotype frequencies (56).
[b] The predicted metabolizer phenotype for the**2/*17* genotype is a provisional classification. The currently available evidence indicates that the *CYP2C19*17* increased function allele is unable to completely compensate for the *CYP2C19*2* non-functional allele.
This table is adapted from (56).

Approximately 2% of Caucasians, 4% of African Americans, and 14% of Chinese are CYP2C19 poor metabolizers (57); and up to 45% of patients are CYP2C19 intermediate metabolizers. As noted above, ACS/PCI patients that are CYP2C19 intermediate or poor metabolizers and who are treated with clopidogrel have

increased risks for major cardiovascular events including stent thrombosis, compared with similarly treated patients without a non-functional allele (57) (19, 21).

The most common non-functional variant is *CYP2C19*2*, which contains the NM_000769.1:c.681G>A variant in exon 5 that results in an aberrant splice site that produces a truncated and non-functioning protein. The *CYP2C19*2* allele frequencies are ~15% in Caucasians and Africans, and ~29–35% in Asians (2). Approximately 6–12% of the observed variability in antiplatelet effect of clopidogrel is thought to be attributed to *CYP2C19*2* allele(s) (58).

For *CYP2C19*, another commonly tested non-functional variant is *CYP2C19*3*, which contains a c.636G>A variant in exon 4 that causes a premature stop codon. The *CYP2C19*3* allele frequencies are ~2–9% in Asian populations, but rare in other racial groups. Other non-functional variants occur in less than 1% of the general population and include *CYP2C19*4–*8* (2).

Genetic Testing

Clinical genotyping tests are available for several *CYP2C19* alleles. The NIH's Genetic Testing Registry (GTR) provides examples of the genetic tests that are currently available for clopidogrel response, CYP2C19-related poor drug metabolism, and the *CYP2C19* gene.

Usually a patient's result is reported as a diplotype, such as *CYP2C19 *1/*1*, and may also include an interpretation of the patient's predicted metabolizer phenotype (ultrarapid, rapid, normal, intermediate, or poor). Table 2 and Table 3 summarize the common *CYP2C19* phenotypes with antiplatelet therapy recommendations developed by CPIC and DPWG, respectively.

The association between *CYP2C19*2* and **3* and clopidogrel response has been extensively studied; however, the less common non-functional alleles (e.g., *CYP2C19*4–*8*) also likely influence clopidogrel response similar to **2* and **3,* but the body of evidence is not as extensive. Therefore, when other non-functional alleles are identified in patients undergoing PCI, these alleles should be considered to reduce the effectiveness of clopidogrel therapy in a similar manner to the more common *CYP2C19**2 allele (2, 59).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2017 Statement from the US Food and Drug Administration (FDA)

WARNING: DIMINISHED ANTIPLATELET EFFECT IN PATIENTS WITH TWO LOSS-OF-FUNCTION ALLELES OF THE CYP2C19 GENE

The effectiveness of clopidogrel tablets results from its antiplatelet activity, which is dependent on its conversion to an active metabolite by the cytochrome P450 (CYP) system, principally CYP2C19. Clopidogrel tablets at recommended doses form less of the active metabolite and so has a reduced effect on platelet activity in patients who are homozygous for nonfunctional alleles of the CYP2C19 gene, (termed "CYP2C19 poor metabolizers"). Tests are available to identify patients who are CYP2C19 poor metabolizers. Consider use of another platelet P2Y12 inhibitor in patients identified as CYP2C19 poor metabolizers.

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

[...]

CYP2C19 is involved in the formation of both the active metabolite and the 2-oxo-clopidogrel intermediate metabolite. Clopidogrel active metabolite pharmacokinetics and antiplatelet effects, as measured by ex vivo platelet aggregation assays, differ according to CYP2C19 genotype.

Patients who are homozygous for nonfunctional alleles of the CYP2C19 gene are termed "CYP2C19 poor metabolizers". Approximately 2% of White and 4% of Black patients are poor metabolizers; the prevalence of poor metabolism is higher in Asian patients (e.g., 14% of Chinese). Tests are available to identify patients who are CYP2C19 poor metabolizers.

A crossover study in 40 healthy subjects, 10 each in the four CYP2C19 metabolizer groups, evaluated pharmacokinetic and antiplatelet responses using 300 mg followed by 75 mg per day and 600 mg followed by 150 mg per day, each for a total of 5 days. Decreased active metabolite exposure and diminished inhibition of platelet aggregation were observed in the poor metabolizers as compared to the other groups.
Please review the complete therapeutic recommendations that are located here: (1).

2017 Summary of Recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

CYP2C19 PM: CLOPIDOGREL

Genetic variation reduces activation of clopidogrel. This increases the risk of serious cardiovascular events in patients undergoing balloon angioplasty or stent placement (percutaneous coronary intervention). No negative clinical consequences have been proved in other patients.

Recommendation:

- PERCUTANEOUS CORONARY INTERVENTION:
  1 choose an alternative

Prasugrel and ticagrelor are not metabolised by CYP2C19 (or to a lesser extent).

- OTHER INDICATIONS:
  1. determine the level of inhibition of platelet aggregation by clopidogrel
  2. consider an alternative in poor responders

Prasugrel and ticagrelor are not metabolised by CYP2C19 (or to a lesser extent).

CYP2C19 IM: CLOPIDOGREL

Genetic variation reduces activation of clopidogrel. This increases the risk of serious cardiovascular events in patients undergoing balloon angioplasty or stent placement (percutaneous coronary intervention). No negative clinical consequences have been observed in other patients.

Recommendation:

- PERCUTANEOUS CORONARY INTERVENTION:
  1 choose an alternative or double the dose to 150 mg/day (600 mg loading dose)

Prasugrel and ticagrelor are not metabolised by CYP2C19 (or to a lesser extent).

- OTHER INDICATIONS:
  1 no action required

CYP2C19 UM: CLOPIDOGREL

NO action is required for this gene-drug interaction.

The genetic variation results in increased conversion of clopidogrel to the active metabolite. However, this can result in both positive effects (reduction in the risk of serious cardiovascular events) and negative effects (increase in the risk of bleeding).

Please review the complete therapeutic recommendations that are located here: (3).

2013 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC)

Standard dosing of clopidogrel, as recommended in the product insert, is warranted among ACS/PCI patients with a predicted *CYP2C19* extensive metabolizer or ultrarapid metabolizer phenotype (i.e., **1/*1*, **1/*17*, and **17/*17*). If genotyping from a Clinical Laboratory Improvement Amendments–certified laboratory identifies a patient as a *CYP2C19* PM (i.e., **2/*2*), current literature supports the use of an alternative antiplatelet agent (e.g., prasugrel or ticagrelor) when not contraindicated clinically.

The most challenging patient population to address is the *CYP2C19* IM [intermediate metabolizer] phenotype (e.g., **1/*2*, **1/*3*, and **2/*17*). IMs have higher on-treatment residual platelet activity on average as compared with extensive metabolizers, and ACS/PCI *CYP2C19*2* heterozygotes treated with clopidogrel have increased risks for serious adverse CV [cardiovascular] outcomes, including stent thrombosis. Consequently, these data support switching to an alternative antiplatelet agent for IMs when not contraindicated. However, given the wide interindividual variability in residual platelet activity observed among clopidogrel-treated IMs, clinical judgment also taking into account other factors that may place an IM at increased risk of a CV event (or adverse bleeding event) must be considered to most effectively individualize therapy.
Please review the complete therapeutic recommendations that are located here: (2).

Nomenclature of Selected *CYP2C19* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C19*2*	681G>A Pro227Pro	NM_000769.1:c.681G>A	NP_000760.1:p.Pro227=	rs4244285
*CYP2C19*3*	636G>A Trp212Ter	NM_000769.1:c.636G>A	NP_000760.1:p.Trp212Ter	rs4986893
*CYP2C19*17*	-806C>T	NM_000769.1:c.-806C>T	Not applicable - variant occurs in a non-coding region	rs12248560

Note: the normal "wild type" allele is *CYP2C19*1* and is reported when no variant is detected.
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (60).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for cytochrome P450 enzymes is available from Pharmacogene Variation (PharmVar) Consortium.

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Version History

To view the 2015 version of this summary (Created: November 19, 2015) please click here.

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Clozapine Therapy and *CYP2D6, CYP1A2,* and *CYP3A4* Genotypes

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Introduction

Clozapine is one of the most effective antipsychotics available in the treatment of schizophrenia and the only antipsychotic found to be effective in treatment-resistant schizophrenia. Clozapine is also used to reduce the risk of recurrent suicidal behavior in patients with schizophrenia or schizoaffective disorder (1, 2).

Compared to typical antipsychotics, clozapine is far less likely to cause movement disorders, known as extrapyramidal side effects, which include dystonia, akathisia, parkinsonism, and tardive dyskinesia. However, there are significant risks associated with clozapine therapy that limits its use to only the most severely ill patients who have not responded adequately to standard drug therapy. Most notably, because of the risk of clozapine-induced agranulocytosis, clozapine treatment requires monitoring of white blood counts and absolute neutrophil counts, and in the US, the FDA requires that patients receiving clozapine be enrolled in a computer-based registry (3).

Clozapine is metabolized in the liver by the cytochrome P450 (CYP) system of enzymes. CYP1A2 is the main CYP isoform in clozapine metabolism and CYP1A2 activity is an important determinant of clozapine dose (4). Other CYP enzymes involved in clozapine metabolism include CYP2D6 and CYP3A4.

Approximately 6-10% of Caucasians have reduced activity of CYP2D6 ("poor metabolizers"). These individuals may develop higher than expected plasma concentrations of clozapine with usual doses. The FDA-approved drug label for clozapine states that a dose reduction may be necessary in patients who are CYP2D6 poor metabolizers (1).

Drug: Clozapine

Clozapine is an antipsychotic used in the treatment of schizophrenia. Schizophrenia is a severe neurodevelopmental disorder with a worldwide prevalence of around 1%. The etiology of schizophrenia is unknown, but it is thought to result from a combination of complex genetic and environmental factors. Before the discovery of the first antipsychotics in the 1950s, the management of schizophrenia relied heavily upon sedation, electroconvulsive therapy, and institutionalization.

The symptoms of schizophrenia fall in to three main categories: positive, negative, and cognitive. Positive symptoms are generally not found in healthy individuals, but may come and go or persist in individuals with schizophrenia. Positive symptoms include reality distortion (e.g., delusions, hallucinations), and thought disorders. These symptoms often respond well to treatment.

Negative symptoms are deficits in normal emotions and behavior, and may be mistaken for depression. Symptoms divide into reduced expression of emotion (e.g., speaking without moving or with a monotonous voice), and avolition (a lack of motivation to start or continue with a task). No treatment has established efficacy for these pathologies.

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Cognitive symptoms may also be difficult to recognize. They include poor executive functioning (understanding information and using it to make decisions) and trouble focusing or paying attention. And again, no treatment has established efficacy.

Clozapine is unique among the antipsychotics because it effectively treats positive symptoms, and appears to be more effective in treating negative symptoms, and some cognitive symptoms when compared with other antipsychotics that cause negative symptoms or impair cognition (5-7).

Clozapine has also been shown to reduce aggression and reduce the risk of suicide, and is the only antipsychotic found to be effective in treatment-resistant schizophrenia (2, 8-10). More than one third of patients are thought to have schizophrenia that only partially responds or is resistant to standard drugs; these patients may then be treated with clozapine (2, 10, 11).

The first antipsychotics to be discovered in the 1950s were haloperidol and chlorpromazine. Known as "first generation" or "typical" antipsychotics, these drugs are used to treat psychosis (regardless of the cause), chronic psychotic disorders (e.g., schizophrenia), and other psychiatric conditions. However, prominent adverse effects included extrapyramidal side effects such as tardive dyskinesia, muscle rigidity, tremors, and Parkinsonian-like symptoms.

Newer antipsychotics, known as "second generation" or "atypical" antipsychotics, have a lower risk of extrapyramidal side effects such as tardive dyskinesia. However, many have serious metabolic effects. These antipsychotics include aripiprazole, clozapine, iloperidone, olanzapine, and risperidone.

Clozapine was introduced in 1971 as the first atypical antipsychotic, but the manufacturer (Novartis, formerly Sandoz) voluntarily withdrew the drug in 1975 because of safety concerns (7). One of the most dangerous risks reported was that of clozapine-induced neutropenia—a severely low level of neutrophils (a type of white blood cell), which places patients at high risk of infection. However, because it was later shown that clozapine was the most effective antipsychotic in the management of treatment-resistant schizophrenia, in 1989 the FDA reapproved clozapine for that use (5, 7, 9).

The main action of both first-generation and second-generation antipsychotics appears to be the post-synaptic blockade of D2 dopamine receptors in the brain. (An exception is aripiprazole, which is a D2 partial agonist.) Blockade of the D2 receptor in the brain's limbic system are thought to improve the "positive" symptoms of schizophrenia (12).

However, because the first-generation antipsychotics also block dopamine receptors in the nigrostriatal pathway, they cause movement disorders known as extrapyramidal side effects. These disorders include akathisia (motor restlessness), dystonia (abnormal muscle tone), and tardive dyskinesia (involuntary and repetitive movements).

Clozapine only transiently occupies D2 receptors and then rapidly dissociates to allow normal dopamine neurotransmission. It is thought that because clozapine has a relatively low affinity for the D2 receptor and binds "loosely," extrapyramidal side effects are less likely (11, 13).

In addition to binding the D2 receptor, clozapine has a high affinity for the serotonin 5-HT_{2A} receptors. Blockade of 5-HT_{2A} in the mesocortical tract may also provide some protection against extrapyramidal side effects by increasing amounts of dopamine. Clozapine and its major metabolite (N-desmethylclozapine) have been shown to indirectly activate NMDA receptors, and may also modulate GABA and cholinergic pathways. However, despite these findings, it remains unclear what gives clozapine its superior efficacy to other antipsychotics (7).

One of the most prominent side effects of clozapine therapy is weight gain. The most severe side effects are included in five boxed warnings on the drug label: 1) severe neutropenia, 2) seizures (more likely at higher doses), 3) myocarditis (inflammation of the heart muscle induced by clozapine, that can be fatal), 4) increased

mortality in elderly patients with dementia-related psychosis, and 5) an increased risk of orthostatic hypotension, bradycardia, and syncope (1).

Because of the risk of neutropenia, clozapine can only be prescribed according to a schedule that monitors the patient's white blood cell count (WBC) and absolute neutrophil count (ANC). Neutropenia, defined as an ANC of less than 500/mm^3, is estimated to occur in around 1% of patients, and could prove fatal if not detected early by regular monitoring (14).

Genetic risk factors for clozapine-induced neutropenia have been identified, consisting of two independent amino acid changes in *HLA-DQB1* (126Q) and *HLA-B* (158T). *HLA-DQB1* is associated with autoimmune disease and *HLA-B* is an important component of severe drug reactions, including carbamazepine-induced Stevens-Johnson syndrome and abacavir hypersensitivity. Despite this genetic insight, a genetic test based solely on *HLA-DQB1* and *HLA-B* would not be able to adequately identify if all the patients are truly at low risk of clozapine-induced neutropenia (15).

The Cytochrome P450 Superfamily

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The *CYP450* genes are very polymorphic and can result in reduced, absent, or increased enzyme activity.

Clozapine is extensively metabolized in the liver by CYP450 enzymes, especially by CYP1A2, CYP3A4, and CYP2D6. Most of the metabolites are inactive, but N-desmethylclozapine has been found to have limited activity (7, 16).

The dose of clozapine may need to be adjusted when clozapine is given with medications that inhibit or induce the enzymes responsible for metabolizing clozapine. Inhibitors of CYP enzymes include the antibiotic ciprofloxacin (CYP1A2 inhibitor) and the antidepressant fluvoxamine (CYP3A4 and CYP2D6 inhibitor). Inducers include the antiseizure drug carbamazepine (strong CYP3A4 inducer). In addition, other agents can influence CYP enzymes—caffeine and oral contraceptives are weak or moderate CYP1A2 inhibitors, and tobacco smoke is a moderate inducer of CYP1A2 (and smoking is common among patients with schizophrenia).

Gene: CYP2D6

CYP2D6 is highly polymorphic, with more than 100 star (*) alleles described (17). *CYP2D6*1* is the wild-type allele and is associated with normal enzyme activity and the "extensive metabolizer" phenotype. The *CYP2D6* alleles **2*, **33*, and **35* are also considered to have near-normal activity (Table 1).

Table 1. Activity status of *CYP2D6* alleles

Allele type	*CYP2D6* Alleles
Active	**1, *2, *33, *35*
Decreased activity	**9, *10, *17, *29, *36, *41*
Inactive	**3-*8, *11-*16, *19-*21, *38, *40, *42*

For a detailed list of *CYP2D6* alleles, please see (18).

Individuals who have multiple functional copies of the *CYP2D6* gene are known as "ultrarapid metabolizers," whereas individuals who carry one or two copies of reduced-activity or non-functioning *CYP2D6* alleles are known as "intermediate" or "poor metabolizers."

The most common non-functional alleles include **3*, **4*, **5*, and **6* (19-22), and the most common reduced activity alleles include **10*, **17*, and **41* (23-25). There are large inter-ethnic differences in the frequency of these

alleles, with **3*, **4*, **5*, **6*, and **41* being more common in Caucasians, **17* more common in Africans, and **10* more common in Asians (26-29).

Approximately 6-10% of European Caucasians and their descendants are poor metabolizers, mainly due to the more prevalent nonfunctional **4* and **5* alleles (26, 30). These individuals may develop higher than expected plasma concentrations of clozapine when given in usual doses. Therefore, the FDA-approved drug label for clozapine states that in poor metabolizers, a lower dose of clozapine may be necessary (1).

However, although in theory poor metabolizers may require lower doses of clozapine to achieve the desired therapeutic effects, evidence for this is lacking. Several studies investigating the association between *CYP2D6* genotypes and response to antipsychotic therapy did not report significant findings (31, 32).

Gene: CYP1A2

CYP1A2 alleles influence the treatment response of several antipsychotics (4). However, understanding the pharmacogenomic effects of *CYP1A2* variation is still at an early stage compared with that of other CYP2D6 and other CYP enzymes (33).

CYP1A2 comprises around 13% of all CYP protein in the liver, whereas CYP2D6 comprises around 2%. Approximately 25 variant alleles of *CYP1A2* have been reported, some of which have been shown to alter the activity of CYP1A2. For example, the **1C* allele is associated with decreased enzyme activity (by altering the binding site of an unknown transcription factor in the gene promoter), and the **1F* allele is associated with increased enzyme activity (by increasing the induction of expression) (33, 34).

CYP1A2 is the main CYP isoform in clozapine metabolism (35). Case studies have found that patients with one or more copies of *CYP1A2*1F* (ultrarapid metabolizers) respond poorly to clozapine therapy. However, the treatment response is improved by increasing the dose of clozapine, and also co-administering fluvoxamine, a CYP1A2 inhibitor (36, 37).

The frequency of *CYP1A2*1F* (defined by a C > A polymorphism in intron 1) exists at similar frequencies in all populations (starting at around 0.29) with the highest frequency among Africans (up to 0.51) (38). Environmental factors also strongly influence CYP1A2 activity, such as oral contraceptive use (inhibition) and smoking (induction). Indeed, the sudden cessation of smoking during clozapine therapy may trigger side effects, because of sudden increase in drug levels (39).

Gene: CYP3A4

In contrast to *CYP2D6, CYP1A2,* and other genes that encode drug-metabolizing enzymes, *CYP3A4* shows little genetic variation. Although around 40 variant alleles of *CYP3A4* have been reported, most have not been shown to alter the activity of CYP3A4 (40, 41). To date, only three loss-of-function *CYP3A4* alleles have been identified (*CYP3A4*6, CYP3A4*20* and *CYP3A4*26*) (42, 43).

The *CYP3A4*20* allele contains a premature stop codon which results in a loss-of-function of *CYP3A*. It appears to be the most common *CYP3A4*-defective allele but is still relatively rare, with about 0.2% of European Americans and 0.05% African Americans being carriers. However in Spain, the *CYP3A4*20* allele is present in 1.2% of the population, and up to 3.8% in specific Spanish regions (42).

Genetic Testing

Genetic testing is available for common *CYP2D6, CYP3A4,* and *CYP1A2* alleles. Often a panel of tests is performed. These panels test for variants in multiple genes, which are involved in the metabolism of many drugs,

including clozapine. For examples of the tests available for the clozapine drug response, please see the Genetic Testing Registry.

Results are typically reported as a diplotype, such as *CYP2D6 *1/*1*. A result for copy number, if available, is also important when interpreting *CYP2D6* results (44).

If the test results include an interpretation of the patient's predicted metabolizer phenotype, this should be confirmed by checking the diplotype and assigning an activity score to each allele (e.g., 0 for nonfunctional, 0.5 for reduced function, and 1 for each copy of a functional allele). The phenotype is defined by the sum of the two scores:

- An extensive (normal) metabolizer phenotype has an activity score of 1 to 2
- An intermediate metabolizer has an activity score of 0.5
- A poor metabolizer has an activity score of 0
- An ultrarapid metabolizer has an activity score greater than 2

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2014 Statement from the US Food and Drug Administration (FDA): Dose reduction may be necessary in patients who are CYP2D6 poor metabolizers. Clozapine concentrations may be increased in these patients, because clozapine is almost completely metabolized and then excreted.
Please review the complete therapeutic recommendations that are located here: (1).

Nomenclature

***CYP2D6* Nomenclature**

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*4*	1846G>A	NM_000106.5:c.506-1G>A	Not applicable - variant occurs in a non-coding region	rs3892097
*CYP2D6*5*	Not applicable - variant results in a whole gene deletion			
*CYP2D6*6*	1707 del T Trp152Gly	NM_000106.5:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655
*CYP2D6*10*	100C>T Pro34Ser	NM_000106.5:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
*CYP2D6*17*	Includes at least two functional variants*: 1023C>T (Thr107Ile) 2850C>T (Cys296Arg)	NM_000106.5:c.320C>T NM_000106.5:c.886T>C	NP_000097.3:p.Thr107Ile NP_000097.3:p.Cys296Arg	rs28371706 rs16947
*CYP2D6*41*	2988G>A	NM_000106.5:c.985+39 G>A	Not applicable – variant occurs in a non-coding region	rs28371725

* In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

***CYP1A2* Nomenclature**

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP1A2*1C*	-3860G>A -2964G>A	Unknown	Not applicable—variant occurs in a non-coding region	rs2069514
*CYP1A2*1F*	-	NM_000761.4:c.-9-154C>A	Not applicable—variant occurs in a non-coding region	rs762551

***CYP3A4* Nomenclature**

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP3A4*6*	17661_17662insA 277Frameshift	NM_017460.5:c.830_831insA	NP_059488.2:p.Asp277Glufs	rs4646438
*CYP3A4*20*	1461_1462insA 488Frameshift	NM_017460.5:c.1461_1462insA	NP_001189784.1:p.Pro487Thrfs	rs67666821
*CYP3A4*26*	17633C>T R268Stop			

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: http://www.cypalleles.ki.se/

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Codeine Therapy and *CYP2D6* Genotype

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Introduction

Codeine is used to relieve mild to moderately severe pain, and it belongs to the drug class of opioid analgesics.

The hepatic CYP2D6 enzyme metabolizes a quarter of all prescribed drugs, including codeine. CYP2D6 converts codeine in to its active metabolite, morphine, which provides its analgesic effect. However, pain relief may be inadequate in individuals who carry two inactive copies of *CYP2D6* ("poor metabolizers"), because of reduced morphine levels.

In contrast, individuals who carry more than two normal function copies of the *CYP2D6* gene ("ultrarapid metabolizers") are able to metabolize codeine to morphine more rapidly and more completely. As a result, even with normal doses of codeine, these individuals may experience the symptoms of morphine overdose, which include extreme sleepiness, confusion, and shallow breathing. Nursing mothers may also produce breast milk containing higher than expected levels of morphine that can lead to severe adverse events in their infants (1).

The FDA drug label for codeine states that even at labeled dosage regimens, individuals who are ultra-rapid metabolizers may have life-threatening or fatal respiratory depression or experience signs of overdose. The label also contains a boxed warning, which states that respiratory depression and death have occurred in children who received codeine following tonsillectomy and/or adenoidectomy and had evidence of being ultra-rapid metabolizers of codeine due to a CYP2D6 polymorphism (1).

The Clinical Pharmacogenetics Implementation Consortium (CPIC) recommends that for a patient identified as a CYP2D6 ultrarapid metabolizer, another analgesic should be used to avoid the risk of severe toxicity with a "normal" dose of codeine. CPIC also recommends avoiding codeine in patients identified as CYP2D6 poor metabolizers due to the possibility of lack of effect (see Table 1) (2).

Table 1. 2014 Codeine therapy recommendations based on cytochrome P4502D6 (CYP2D6) phenotype, adapted from CPIC

Phenotype	Activity score	Phenotype details	Genotype	Examples of diplotypes	Recommendations for codeine therapy[1]	Considerations for alternative opioids
Ultrarapid metabolizer (approximately 1–2% of patients)	Greater than 2.0	Increased enzyme activity. Increased formation of morphine following codeine administration, leading to higher risk of toxicity.	More than two copies of normal function alleles	**1/*1xN* **1/*2xN*	Avoid codeine use due to potential for toxicity.	Alternatives that are not affected by this CYP2D6 phenotype include morphine and non-opioid analgesics. Tramadol and, to a lesser extent, hydrocodone and oxycodone are not good alternatives because their metabolism is affected by CYP2D6 activity.

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Table 1. continued from previous page.

Phenotype	Activity score	Phenotype details	Genotype	Examples of diplotypes	Recommendations for codeine therapy[1]	Considerations for alternative opioids
Normal metabolizer (approximately 77–92% of patients)	1.0-2.0*	Normal enzyme activity. Normal morphine formation.	Two normal function alleles, or two decreased function alleles, or one normal function allele and one decreased or no function allele, or combinations of duplicated alleles that result in an activity score of 1.0 to 2.0	**1/*1* **1/*2* **2/*2* **1/*41* **1/*4* **2/*5* **1/*10*	Use label-recommended age- or weight-specific dosing.	
Intermediate metabolizer (approximately 2–11% of patients)	0.5*	Intermediate enzyme activity. Reduced morphine formation.	One decreased function allele and one no function allele	**4/*10* **5/*41*	Use label-recommended age- or weight-specific dosing. If no response, consider alternative analgesics such as morphine or a nonopioid.	Monitor tramadol use for response.
Poor metabolizer (approximately 5–10% of patients)	0	Low or absent enzyme activity. Greatly reduced morphine formation following codeine administration, leading to insufficient pain relief.	Two no function alleles	**4/*4* **4/*5* **5/*5* **4/*6*	Avoid codeine use due to lack of efficacy.	Alternatives that are not affected by this CYP2D6 phenotype include morphine and non-opioid analgesics. Tramadol and, to a lesser extent, hydrocodone and oxycodone are not good alternatives because their metabolism is affected by CYP2D6 activity; these agents should be avoided.

* Activity scores are based on the formation of morphine from codeine. Other investigators may define normal metabolizers with a score of 1.5-2.0, and intermediate metabolizers with a score of 0.5-1.0.

[1] The strength of therapeutic recommendations is "moderate" for intermediate metabolizers, and "strong" for all other metabolizers. Table is adapted from Crews K.R. et al. Clinical Pharmacogenetics Implementation Consortium guidelines for cytochrome P450 2D6 genotype and codeine therapy: 2014 update. Clinical pharmacology and therapeutics. 2014;95(4):376-82 (2). Please note, the nomenclature used in this table reflects the standardized nomenclature for pharmacogenetic terms proposed by CPIC in a 2016 paper, "Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC)" (3).

Drug: Codeine

Codeine is an opioid analgesic. It exerts its effects via the opioid receptors found throughout the body including the central nervous system and the gastrointestinal system. Codeine is a prodrug that only weakly binds the mu opioid receptor. Its analgesic properties depend upon its conversion to morphine that binds to the mu opioid receptor with 200-fold greater affinity than codeine.

Codeine is indicated for the relief of mild to moderately severe pain, where the use of an opioid analgesic is appropriate. Codeine is a Schedule II controlled substance, and there is a risk of misuse and abuse. As with any opioid drug, the dosing regimen should be adjusted for each individual patient. When the patient no longer requires codeine, the doses should be tapered gradually to prevent withdrawal symptoms in patients who have become physically dependent (1).

For codeine to exert its opioid activity, it must first undergo o-demethylation by CYP2D6 to morphine. Only about 5-10% of codeine is metabolized in this pathway, with about 80% of an administered dose of codeine being converted to inactive metabolites and excreted. However, the percentage of codeine converted to morphine can be much higher in individuals who have 3 or more active copies of *CYP2D6* ("ultrarapid metabolizers") (2). In contrast, individuals who lack active copies of *CYP2D6* ("poor metabolizers") have lower levels of morphine.

Morphine is further metabolized to morphine-6-glucuronide, which also has analgesic properties. Other metabolites are thought to be mostly inactive; they include codeine-6-glucuronide (~60%) and norcodeine (~5–10%), both of which share with codeine a similarly weak affinity for the mu opioid receptor (4).

To avoid treatment complications in patients who are either ultrarapid or poor metabolizers, opioids that are not metabolized by CYP2D6 may be used (e.g., morphine, oxymorphone, buprenorphine, fentanyl, methadone, hydromorphone), alongside non-opioids, depending upon the type of pain being treated (2, 5-7).

The most common adverse reactions to codeine include drowsiness, lightheadedness, dizziness, sedation, shortness of breath, nausea, vomiting, and sweating. One of the main serious adverse reactions associated with codeine is respiratory depression. The FDA-drug label for codeine now includes a boxed warning that states "Warning: Death related to ultra-rapid metabolism of codeine to morphine. Respiratory depression and death have occurred in children who received codeine following tonsillectomy and/or adenoidectomy and had evidence of being ultra-rapid metabolizers of codeine due to a CYP2D6 polymorphism" (1, 8, 9).

Gene: *CYP2D6*

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs in the liver. The *CYP450* genes are very polymorphic and can result in decreased, absent, or increased enzyme activity.

CYP2D6 is responsible for the metabolism of many commonly prescribed drugs, including antidepressants, antipsychotics, analgesics, and beta-blockers. The *CYP2D6* gene is highly polymorphic, with more than 100 star (*) alleles described (10).

*CYP2D6*1* is the wild-type allele and is associated with normal enzyme activity and the "normal metabolizer" phenotype. The *CYP2D6* alleles **2*, **33*, and **35* are also considered to have near-normal activity.

About 77–92% of individuals have at least one copy of a normal function allele (**1* or **2*), or two partially functioning alleles. These individuals are also "normal metabolizers" and are most likely to have a phenotypically normal response to codeine. However, there is a large amount of variability in codeine response within patients

genotyped as normal metabolizers, and the causes of this variation, among individuals with the same diplotype, are unknown (2).

Other *CYP2D6* alleles include variants that produce a non-functioning enzyme (e.g., **3*, **4*, **5*, and **6*) (4, 11-13) or an enzyme with decreased activity (e.g., **10*, **17*, and **41*) (14-16) (see Table 2). There are large inter-ethnic differences in the frequency of these alleles, with **3*, **4*, **5*, **6*, and **41* being more common in Caucasians, **17* more common in Africans, and **10* more common in Asians (17).

About 2–11% of patients are intermediate metabolizers—they carry either two decreased function alleles or one decreased function and one no function allele (18). These individuals may not respond as well to codeine because the metabolism of codeine to morphine is reduced.

In Asians and in individuals of Asian descent, only about 50% of *CYPD6* alleles are normal function, and the frequency of *CYP2D6* allele duplications is as high as 45% (19). Common no function variants are *CYP2D6*36* (the most commonly duplicated *CYP2D6* allele in the Asian population) and *CYP2D6*10*. Both these variants contain the SNP "100C>T" (see Nomenclature table) (17, 19-21). In Africans and African Americans, again, only about 50% of *CYPD6* alleles are normal function (11, 16, 17, 22).

About 5–10% of patients are poor metabolizers—they carry two no function alleles (18). In these individuals, codeine will provide little or no pain relief. Poor metabolizers are more commonly found in European Caucasians and their descendants. The majority allele in this population is the normal function *CYP2D6*1* (70%), but the remaining alleles include the no function *CYP2D6*4* and *CYP2D6*5* variants that largely account for the poor metabolizer phenotype in these populations (12, 15, 23).

Patients who are ultrarapid metabolizers carry at least 3 copies of the *CYP2D6* gene. The ultrarapid metabolizer phenotype has been estimated to be present in 1–2% of patients, but the prevalence varies widely in different populations. It is estimated to be present in up to 28% of North Africans, Ethiopians, and Arabs; up to 10% in Caucasians; 3% in African Americans, and up to 1% in Hispanics, Chinese, and Japanese (1, 18).

Each normal function *CYP2D6* allele increases the rate of codeine metabolism, increasing the risk of an initial morphine "overdose", with more side effects and a shorter duration of pain control (24). Even low codeine doses can result in toxic levels of morphine in patients with more than 2 normal function alleles (2). Several case reports have recorded the severe or life-threatening adverse effects that have occurred in patients who were ultrarapid metabolizers and were treated with standard doses of codeine (25, 26).

Genetic Testing

Genetic testing is available for many (~30) of the variant *CYP2D6* alleles. Usually a patient's result is reported as a diplotype, which includes one maternal and one paternal allele, e.g., *CYP2D6 *1/*2*. When patients have more than two copies of the *CYP2D6*, the copies of the allele are denoted by an "xN", e.g., *CYP2D6*2x2*.

If the test results include an interpretation of the patient's predicted metabolizer phenotype, this should be confirmed by checking the diplotype and calculating the CYP2D6 activity score. Each allele is assigned an activity value: 0 for no function, 0.5 for decreased function, and 1 for each copy of a normal function allele. The total CYP2D6 activity score is the sum of the values assigned to each allele—patients with a score of 1.0, 1.5, or 2.0 represent a range of normal metabolizers with normal enzyme activity. Poor metabolizers have an activity score of 0, patients with a score of 0.5 are intermediate metabolizers, and patients with a score of greater than 2.0 are ultrarapid metabolizers (see Table 1) (2).

Variants in other genes, such as *COMT*, *ABCB1*, *UGT2B7* and *OPRM1*, may also influence an individual's response to codeine. However, evidence is lacking on whether genetic testing for these variants will aid optimum codeine dosing (7, 27-29).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1,2] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2015 Statement from the US Food and Drug Administration (FDA): Respiratory depression and death have occurred in children who received codeine in the post-operative period following tonsillectomy and/or adenoidectomy and had evidence of being ultra-rapid metabolizers of codeine (i.e., multiple copies of the gene for cytochrome P450 isoenzyme 2D6 [CYP2D6] or high morphine concentrations). Deaths have also occurred in nursing infants who were exposed to high levels of morphine in breast milk because their mothers were ultra-rapid metabolizers of codeine.

Some individuals may be ultra-rapid metabolizers because of a specific *CYP2D6* genotype (gene duplications denoted as **1/*1xN* or **1/*2xN*). The prevalence of this CYP2D6 phenotype varies widely and has been estimated at 0.5 to 1% in Chinese and Japanese, 0.5 to 1% in Hispanics, 1 to 10% in Caucasians, 3% in African Americans, and 16 to 28% in North Africans, Ethiopians, and Arabs. Data are not available for other ethnic groups. These individuals convert codeine into its active metabolite, morphine, more rapidly and completely than other people. This rapid conversion results in higher than expected serum morphine levels. Even at labeled dosage regimens, individuals who are ultra-rapid metabolizers may have life-threatening or fatal respiratory depression or experience signs of overdose (such as extreme sleepiness, confusion, or shallow breathing).

Please review the complete therapeutic recommendations that are located here: (1)

2014 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC): A standard starting dose of codeine, as recommended in the product label, is warranted in patients with an extensive metabolizer phenotype (i.e., a CYP2D6 activity score of 1.0–2.0). Likewise, a standard starting dose of codeine is warranted in patients with an intermediate metabolizer phenotype (i.e., activity score of 0.5); these patients should be monitored closely for less-than-optimal response and should be offered an alternative analgesic if warranted. If the CYP2D6 substrate tramadol is selected as alternative therapy in intermediate metabolizers, therapy should be monitored closely due to the possibility of poor response.

If clinical genotyping identifies a patient as a CYP2D6 poor metabolizer (i.e., activity score of 0), current evidence supports the avoidance of codeine and the use of an alternative analgesic due to the possibility of lack of effect. Use of an analgesic other than the CYP2D6 substrates tramadol, hydrocodone, or oxycodone in poor metabolizers may be preferable. There is insufficient evidence in the literature to recommend a higher dose of codeine in poor metabolizers, especially considering the evidence that select adverse effects do not differ between poor and extensive metabolizers. In a patient identified as a CYP2D6 ultrarapid metabolizer (i.e., activity score of >2.0), the choice of another analgesic should be made to avoid the risk of severe toxicity with a "normal" dose of codeine.

Please review the complete therapeutic recommendations that are located here: (2).

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labelled all formulations containing the generic drug.

[2] Please note, the term "extensive metabolizer" has been replaced by the term "normal metabolizer" in the standardized nomenclature for pharmacogenetic terms proposed by CPIC in a 2016 paper, "Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC)" 3. Caudle, K.E., H.M. Dunnenberger, R.R. Freimuth, J.F. Peterson, et al., *Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC).* Genet Med, 2017. **19**(2): p. 215-223..

2013 Clinical practice Guideline from the "Canadian Pharmacogenomics Network for Drug Safety (CPNDS) Clinical Recommendations Group: *CYP2D6* genotyping for safe and efficacious codeine therapy":

1. Who should be tested and when?
    - Young children about to receive codeine for pain management and women about to receive codeine for postpartum pain while breastfeeding should be tested for CYP2D6 (Grade A – strong recommendation).
    - Children and adults who continue to have pain despite high doses of codeine should be tested for CYP2D6 (Grade B – moderate recommendation).
    - Genetic testing for CYP2D6 should be considered before administering codeine for the first time in all children and adults in order to rule out non-responders and to identify individuals who may be susceptible to adverse effects from codeine (Grade C – optional recommendation).
2. What gene variants should be tested?

Given the numerous polymorphisms in CYP2D6 and the diversity of the Canadian population, a full-scale analysis of both common and rare CYP2D6 variants is advised (Grade B- moderate recommendation)

- *CYP2D6* alleles with decreased or no function: *CYP2D6 *3- 12, 14-15, 17, 19-20, 29, 40-42, 44, 49, 50, 54-56, 59; *4XN, *10XN*
- *CYP2D6* alleles with normal or increased function: *CYP2D6 *2* (normal), **1XN* (increased), **2XN* (increased), **17XN*, **35XN* (increased), **41XN*, in addition to *CYP2D6* copy number determination.

Recommendations: Genotype-Specific Treatment Options

- Poor metabolizers of CYP2D6 should not receive codeine for pain relief (Grade A- strong recommendation).
- Ultrarapid metabolizers of CYP2D6 should avoid codeine for pain relief and receive alternative analgesics that do not have potent CYP2D6 metabolites (Grade B- moderate recommendation).
- Certain populations, especially opioid naïve breastfed neonates of mothers with functional *CYP2D6* gene duplications taking codeine and young children may be particularly susceptible to codeine-induced central nervous system depression. Breastfeeding mothers and young children who are ultrarapid metabolizers of CYP2D6 should avoid codeine (Grade A – strong recommendation).
- In individuals with IM or EM *CYP2D6* genotypes, codeine can be used as per standard of care. Existing evidence suggests that caution is still warranted in *CYP2D6* EMs receiving codeine if they are receiving maximal therapeutic doses of codeine and have additional risk factors for toxicity.

Please review the complete therapeutic recommendations that are located here: (30)

Nomenclature

Nomenclature of selected *CYP2D6* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*4*	1846G>A	NM_000106.5:c.506-1G>A	Variant occurs in a non-coding region (splice variant causes a frameshift)	rs3892097
*CYP2D6*5*	Variant results in a whole gene deletion			
*CYP2D6*6*	1707 del T Trp152Gly CYP2D6T	NM_000106.5:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655
*CYP2D6*10*	100C>T (Pro34Ser)	NM_000106.5:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852

Nomenclature of selected continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*17*	1023C>T[1] (Thr107Ile)	NM_000106.5:c.320C>T	NP_000097.3:p.Thr107Ile	rs28371706
	2850C>T[2] (Cys296Arg)	NM_000106.5:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
*CYP2D6*41*	2850C>T[2] (Cys296Arg)	NM_000106.5:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
	2988G>A	NM_000106.5:c.985+39 G>A	Variant occurs in a non-coding region (impacts slicing).	rs28371725

[1] In the literature, 1023C>T is also referred to as 1111C>T
[2] In the literature, 2850C>T is also referred to as 2938C>T

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: http://www.cypalleles.ki.se/

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The Clinical Pharmacogenetics Implementation Consortium: http://www.pharmgkb.org/page/cpic

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Dabrafenib Therapy and *BRAF* and *G6PD* Genotype

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Introduction

Dabrafenib is a kinase inhibitor used in the treatment of patients with unresectable or metastatic melanoma with specific *BRAF* variants. Dabrafenib can be used as a single agent to treat melanoma with the *BRAF* V600E variant, or in combination with the MEK inhibitor trametinib to treat melanoma with *BRAF* V600E or V600K variants.

BRAF is an intracellular kinase in the mitogen-activated protein kinases (MAPK) pathway. BRAF is involved in regulating important cell functions such as cell growth, division, differentiation, and apoptosis. BRAF is also a proto-oncogene—when mutated it has the ability to transform normal cells into cancerous cells.

Variation in the kinase domain of BRAF have been associated with various cancers. The most common *BRAF* variant, V600E, constitutively activates the kinase, and causes cell proliferation in the absence of growth factors that would normally be required. The V600E variant is detected in approximately 50% of melanomas (1, 2).

The FDA-approved label for dabrafenib states that the presence of *BRAF* mutation in tumor specimens (V600E for dabrafenib monotherapy; V600E or V600K for dabrafenib plus trametinib) should be confirmed, using an FDA-approved test, before starting treatment with dabrafenib. Dabrafenib is not indicated for treatment of patients with wild-type *BRAF* melanoma.

The label also states that patients who have glucose-6-phosphate dehydrogenase (G6PD) deficiency should be monitored for signs of hemolytic anemia while taking dabrafenib (3).

Drug: Dabrafenib

Dabrafenib is a BRAF kinase inhibitor indicated for the treatment of patients with unresectable or metastatic melanoma. It acts by decreasing signaling through the MAPK pathway, leading to the reduced transcription of genes involved in various cellular responses.

Dabrafenib can be used as a single agent to treat melanoma with *BRAF* V600E variant, or in combination with trametinib to treat melanoma with *BRAF* V600E or V600K variants (3). Dabrafenib and other BRAF inhibitors have also demonstrated responses in patients with rare *BRAF* V600 variants (V600R, V600D) (4). These agents appear to be less active in pre-clinical studies of melanomas with atypical (non-V600) variants (e.g. L597, K601) (5).

Skin cancer is the most common of all cancers. Although melanoma is the least common type of skin cancer, accounting for approximately 1% of cases, it is responsible for the majority of deaths from skin cancer. In the US, the lifetime risk of melanoma is approximately 2.5% for whites, 0.5% for Hispanics, and 0.1% for blacks (6).

Most cases of malignant melanoma are diagnosed at an early stage, when the tumor is localized and surgical excision can be curative. However, the 5-year survival rate drops from 98% for localized disease, to only 16% for patients with metastatic disease.

For patients with advanced metastatic or unresectable malignant melanoma, treatment options typically include immunotherapy and targeted therapy. Although chemotherapy was once widely used, it does not increase

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survival and therefore its use is now limited to patients who are not candidates for further treatment with either immunotherapy or targeted therapy, and for whom there is no appropriate clinical trial.

High-dose interleukin 2 (IL2) therapy may be successful in a minority of cases, but can only be used in select patients with good organ function because of the risk of severe toxicity. Immunotherapy drugs include antibodies that target programmed cell death protein 1 (PD-1), e.g., nivolumab and pembrolizumab (7); and ipilimumab, a monoclonal antibody that targets cytotoxic T-lymphocyte-associated protein 4 (CTLA4). Oncolytic virus therapy with T-VEC (talimogene laherparepvec) is one of the newer immunotherapy drugs approved for melanoma.

Targeted therapies are designed to inhibit components of the MAPK signaling pathway, primarily when it is constitutively activated in melanomas with the activating *BRAF* variant, V600E. Drugs in this category include vemurafenib and dabrafenib, which inhibit BRAF, and trametinib and cobimetinib, which target downstream kinases MEK1 and MEK2, respectively.

Dabrafenib is a potent inhibitor of the kinase domain of the variant *BRAF* V600E. It acts by decreasing signaling through the MAPK pathway, leading to the reduced transcription of genes involved in various cellular responses. Combining dabrafenib with MEK inhibitors has been shown to extend survival (8, 9), and dabrafenib is often used in combination with a MEK inhibitor, e.g., trametinib.

Dabrafenib increased progression-free survival, compared to cytoxic chemotherapy (e.g., dacarbazine), in patients with advanced melanoma with the *BRAF* V600E variant (10, 11). However, at this time there are no randomized trials that compare targeted therapies such as dabrafenib, with immunotherapy, and there are no data regarding the appropriate combinations and sequencing of these therapies for patients with a V600E variant.

A recent phase 3 trial for patients with melanoma with a V600E variant was stopped early because of positive results. The study found that the combination of dabrafenib plus trametinib led to a higher 3-year overall survival rate, compared to vemurafenib monotherapy (25% versus 11%). In addition, the incidence of cutaneous squamous cell carcinoma was decreased in patients taking the combination of dabrafenib plus trametinib (12).

The drug label advises that a dermatological evaluation should be carried out prior to initiating dabrafenib therapy, and every 2 months during therapy. The most common adverse events associated with dabrafenib are skin lesions (benign and malignant). Other side effects include fever, arthralgia, fatigue, alopecia, and palmar-plantar erythrodysesthesia syndrome ("hand-foot syndrome").

In vitro experiments with BRAF inhibitors, such as dabrafenib, have been found to cause a paradoxical activation of signaling pathways and proliferation in *BRAF* wild-type cells. Therefore, dabrafenib should only be used after the presence of *BRAF* V600E variant in tumor specimens has been confirmed using an FDA-approved test (3). The FDA also recommends to permanently discontinue dabrafenib use in patients who develop RAS mutation-positive non-cutaneous malignancies.

Gene: *BRAF*

RAF is a family of intracellular kinases within the MAPK signaling pathway. The RAF family has three members, ARAF, BRAF, and CRAF (13). RAF, along with RAS (see below), are proto-oncogenes.

Proto-oncogenes are genes that, when mutated or expressed at abnormally high levels, can transform normal cells into cancerous cells. Proto-oncogenes typically encode proteins that stimulate cell division, inhibit cell differentiation, and halt cell death. The increased production of oncogenic proteins can lead to the proliferation of poorly differentiated cancer cells (14).

Germline mutations in *BRAF*, as well as other components of the MAPK signaling pathway, are associated with birth defects, such as cardiofaciocutaneous syndrome, characterized by heart defects, mental retardation, and a distinctive facial dysmorphology. Somatic *BRAF* mutations are also associated with several malignancies, including lung adenocarcinoma, mucinous adenoma, ductal carcinoma of the pancreas, colorectal carcinoma, and malignant melanoma.

Variations in *BRAF* are detectable in approximately 50% of malignant melanomas, and drive progression of the disease (1, 2). The *BRAF* variant V600E accounts for approximately 90% of variants. This variant is a substitution of adenine for thymine at position 1799 and results in the substitution of valine for glutamate at codon 600. The variant BRAF protein kinase is constitutively active and a highly potent oncogene, with an increase in kinase activity by as much as 500-fold compared to the wild-type (15). The second most common *BRAF* variant is V600K. Substitutions at other sites are rarer (16, 17).

Several drugs are being developed to target *BRAF* variants, and so far, two drugs have been FDA- approved: vemurafenib and dabrafenib. Unfortunately, less progress has been made in developing targeted therapies for melanoma with wild-type *BRAF*. There are fewer treatment options available, but these include immunotherapy and MEK inhibitors (7, 18).

Gene: *G6PD*

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an inherited X-linked recessive disorder that results from genetic variation in the *G6PD* gene. The *G6PD* gene is located on the X chromosome and G6PD deficiency occurs almost exclusively in males, who have only one X chromosome. G6PD deficiency mainly affects red blood cells, which carry oxygen from the lungs to tissues throughout the body.

G6PD deficiency affects 400 million people worldwide (19), and is common among African Americans, affecting approximately 12% (20). G6PD deficiency appears to be protective against malaria infection (21).

G6PD catalyzes the initial step in the hexose monophosphate (HMP) pathway. In mature red blood cells, the HMP pathway is the only source of NADPH, a coenzyme essential for protection against oxidative stress and repair of oxidative damage.

Red blood cells that are G6PD deficient are more susceptible to oxidative stress caused by exposure to drugs (e.g. sulfamethoxazole, primaquine, and dabrafenib), infections, diabetic ketoacidosis, or following ingestion of fresh fava beans (favism). Because of the oxidative stress, the red blood cells become rigid, become trapped, and are subsequently destroyed by macrophages in the spleen, bone marrow, and liver. Premature and/or fast destruction of red blood cells is called hemolysis and can result in hemolytic anemia.

Most affected individuals are asymptomatic; however, those with symptoms may suffer from episodes of acute hemolytic anemia or chronic hemolytic anemia. The management of hemolytic episodes depends on the severity of hemolysis. More severe cases may require a transfusion of packed red blood cells. Folic acid may be given to prevent the worsening of anemia in individuals with folate deficiency.

The normal (wild-type) copy of the *G6PD* gene is known as G6PD A+ (p.Asn126Asp), and is found in up to 30% of blacks from Africa (22). More than 400 genetic variants of the *G6PD* gene have been identified so far, and most are missense point mutations (23). Common variants include:

- G6PD A- (p.Asn126Asp and p.Val68Met) which is associated with mild to moderate hemolysis, and is found in up to 15% of African-Americans (24)
- G6PD Mediterranean (p.Ser218Phe) which can cause severe hemolysis, and is the most common variant in Caucasians (25)
- G6PD Canton (p.Arg489Leu) which can cause severe hemolysis, and is found in Asians (26)

All individuals with G6PD deficiency should avoid oxidizing agents when possible, including specific drugs and chemicals. Dabrafenib can cause hemolytic anemia. The FDA-approved drug label for dabrafenib warns that "dabrafenib, which contains a sulfonamide moiety, confers a potential risk of hemolytic anemia in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. Monitor patients with G6PD deficiency for signs of hemolytic anemia while taking dabrafenib" (3).

No cases of hemolytic anemia associated with dabrafenib have been published, although it is unclear whether individuals with G6PD deficiency have received dabrafenib.

Genetic Testing

The NIH Genetic Testing Registry, GTR, displays genetic tests that are currently available for the genes *BRAF* and *G6PD*.

The FDA-approved label for dabrafenib states that the presence of *BRAF* mutation in tumor specimens (V600E for dabrafenib monotherapy; V600E or V600K for dabrafenib plus trametinib) should be confirmed, using an FDA-approved test, before starting treatment with dabrafenib. The label also states that dabrafenib is not indicated for treatment of patients with wild-type *BRAF* melanoma.

G6PD deficiency is typically diagnosed by screening tests that measure the activity of G6PD in red blood cells. A false positive may result immediately after an episode of hemolysis, so the test should be repeated at a later date. Molecular genetic testing can be used to confirm the diagnosis of G6PD, and may also be used to screen females with a family history of G6PD to see if they are carriers (27).

Screening for G6PD deficiency is recommended so that affected individuals can avoid agents that can cause oxidative stress and trigger hemolysis. G6PD deficiency is inherited in an X-linked recessive pattern. A heterozygous mother has a 50% chance of passing G6PD deficiency to a son and a 50% chance of passing the carrier trait to a daughter. Affected fathers pass the variant *G6PD* to their daughters, but not to their sons.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2016 Statement from the US Food and Drug Administration (FDA):

BRAF V600E Mutation-Positive Unresectable or Metastatic Melanoma: Dabrafenib is indicated as a single agent for the treatment of patients with unresectable or metastatic melanoma with BRAF V600E mutation as detected by an FDA-approved test.

BRAF V600E or V600K Mutation-Positive Unresectable or Metastatic Melanoma: Dabrafenib is indicated, in combination with trametinib, for the treatment of patients with unresectable or metastatic melanoma with BRAF V600E or V600K mutations, as detected by an FDA-approved test.

Limitation of Use: Dabrafenib is not indicated for treatment of patients with wild-type BRAF melanoma.

Patient Selection: Confirm the presence of BRAF V600E mutation in tumor specimens prior to initiation of treatment with dabrafenib as a single agent. Confirm the presence of BRAF V600E or V600K mutation in tumor specimens prior to initiation of treatment with dabrafenib and trametinib. Information on FDA-approved tests for the detection of BRAF V600 mutations in melanoma is available at: http://www.fda.gov/CompanionDiagnostics.

[…]

Dabrafenib, which contains a sulfonamide moiety, confers a potential risk of hemolytic anemia in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. Monitor patients with G6PD deficiency for signs of hemolytic anemia while taking dabrafenib.

Please review the complete therapeutic recommendations that are located here: (3).

Nomenclature

Selected *BRAF* variants

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
V600E	p.Val600Glu	NM_004333.4:c.1799T>A	NP_004324.2:p.Val600Glu	rs113488022
V600K	p.Val600Lys	NM_004333.4:c.1798_1799delGTinsAA	NP_004324.2:p.Val600Lys	rs121913227
V600R	p.Val600Arg	NM_004333.4:c.1798_1799delGTinsAG	NP_004324.2:p.Val600Arg	rs121913227
V600D	p.Val600Asp	NM_004333.4:c.1799_1800delTGinsAT	NP_004324.2:p.Val600Asp	rs121913377

Selected *G6PD* variants

Common allele name / condition	Alternative names / condition	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
G6PD A-	p.Asn126Asp and p.Val68Met	NM_000402.4:c.466A>G NM_000402.4:c.292G>A	NP_001035810.1:p.Asn126Asp NP_001035810.1:p.Val68Met	rs1050828
G6PD Mediterranean	p.Ser218Phe	NM_000402.4(G6PD):c.653C>T	NP_000393.4:p.Ser218Phe	rs5030868
GP6D Canton	p.Arg489Leu	NM_000402.4(G6PD):c.1466G>T	NP_000393.4:p.Arg489Leu	rs72554665

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

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Deutetrabenazine Therapy and *CYP2D6* Genotype

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Introduction

Deutetrabenazine (brand name Austedo) is used to treat chorea associated with Huntington disease (HD) and tardive dyskinesia (TD). Both HD and TD are types of involuntary movement disorders.

The recommended starting dose is 6 mg once daily for individuals with HD and 12 mg per day (6 mg twice daily) for individuals with TD. The maximum recommended daily dosage for both conditions is 48 mg (24 mg, twice daily).

The active metabolites of deutetrabenazine are reversible inhibitors of vesicular monoamine transporter 2 (VMAT2). The VMAT2 protein transports the uptake of monoamines, such as dopamine, into the nerve terminal. The inhibition of VMAT2 leads to a depletion of pre-synaptic dopamine and reduces the amount of dopamine realized when that neuron fires. This is thought to lead to fewer abnormal, involuntary movements.

The CYP2D6 enzyme converts the active metabolites of deutetrabenazine to minor, reduced activity metabolites. Individuals who have no CYP2D6 activity ("CYP2D6 poor metabolizers") are likely to have a 3- to 4-fold increased exposure to active metabolites, compared with normal metabolizers, following the recommended standard doses of deutetrabenazine.

The 2018 FDA-approved drug label for deutetrabenazine states that the daily dose of deutetrabenazine should not exceed 36 mg (maximum single dose of 18 mg) for individuals who are CYP2D6 poor metabolizers or concurrently taking a strong CYP2D6 inhibitor (e.g., quinidine, antidepressants such as paroxetine, fluoxetine, and bupropion) (Table 1).

In addition, the drug label cautions that tetrabenazine, a closely related VMAT2 inhibitor, causes QT prolongation. Therefore, a clinically relevant QT prolongation may occur in some individuals treated with deutetrabenazine who are CYP2D6 poor metabolizers or are co-administered a strong CYP2D6 inhibitor (1).

Table 1. The FDA (2017) Deutetrabenazine Dosage and Administration.

Disorder	Maximum dose of deutetrabenazine	
	Standard recommendation	Recommendation for CYP2D6 poor metabolizers
Chorea association with Huntington disease	48 mg (24 mg twice daily)	36 mg per day (18 mg twice daily)
Tardive dyskinesia	48 mg (24 mg twice daily)	36 mg per day (18 mg twice daily)

Please see Therapeutic Recommendations based on Genotype for more information from the FDA.
This FDA table is adapted from (1).

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Drug: Deutetrabenazine

Deutetrabenazine (brand name Austedo) is used in the management of involuntary movement disorders: chorea associated with HD, and TD in adults. The use of deutetrabenazine is also being investigated for the management of tics associated with Tourette syndrome (2, 3).

Deutetrabenazine belongs to the drug class of VMAT2 inhibitors. These agents act centrally by depleting dopamine storage in presynaptic vesicles in the central nervous system. This reduces the amount of dopamine released when neurons fire, which may result in fewer abnormal, involuntary movements. Other drugs in this class include valbenazine (brand name Ingrezza) and tetrabenazine (brand name Xenozine).

The recommended initial dose of deutetrabenazine is 6 mg/day for chorea associated with HD, and 12 mg/day for TD. The dose should be titrated up by weekly increments of 6 mg/day, based on tolerability and the reduction of chorea or TD, until the individual optimal and tolerated dose is established in both conditions. The standard maximum dose is 48 mg/day (24 mg, twice daily) (1). However, in the Aim to Reduce Movements in Tardive Dyskinesia study, the maximum allowable dose was 72 mg/day (4).

Tetrabenazine was the first drug to be licensed for the treatment of chorea associated with HD, but its use was limited by frequent dosing (at least 3 times daily) and dose-related adverse events (e.g., somnolence, anxiety, and depression).

Deutetrabenazine is a modified (deuterated) form of tetrabenazine. In deuterated drugs, key hydrogen atoms have been replaced with the heavier hydrogen isotope, deuterium, while preserving pharmacological activity. Because deuterium-carbon bonds are stronger than hydrogen-carbon bonds, these drugs tend to be more resistant than non-deuterated drugs to metabolizing enzymes (e.g., CYP2D6), resulting in a longer half-life that allows less frequent dosing. In addition, peak drug concentrations are reduced, potentially reducing any side effects that are associated with peak concentrations. The maximum daily dose of tetrabenazine is 100 mg, compared with 48 mg for deutetrabenazine; and deutetrabenazine is dosed less frequently (twice daily, compared with at least 3 times daily for tetrabenazine) (5-11).

The exact mechanism of action of deutetrabenazine is unknown, but it is thought to involve the reversible depletion of monoamines (such as dopamine, serotonin, norepinephrine, and histamine) from nerve terminals. Deutetrabenazine's active alpha and beta metabolites (α-HTBZ and β-HTBZ) are reversible inhibitors of VMAT2, a transporter protein that is localized in the presynaptic neurons in the central nervous system.

Dopamine and other monoamine transmitters are transported by VMAT2 from the neuronal cell cytoplasm into the neuronal synaptic vesicle. Dopamine that is not taken up into the presynaptic vesicle as a result of VMAT2 blockade by deutetrabenazine is rapidly degraded by monoamine oxidase, resulting in presynaptic depletion of dopamine (7, 11-14).

Huntington Disease

Huntington disease is primarily an adult-onset hereditary autosomal dominant progressive neurodegenerative disorder, which is characterized by involuntary movements ("chorea"), psychiatric symptoms, and cognitive dysfunction that can lead to dementia. More than 35,000 people in the US have HD. There is a juvenile form of HD that is characterized by onset of signs and symptoms before 20 years of age.

The prevalence of HD varies across regions of the world. For individuals of European ancestry, the prevalence of HD is estimated to be 3–10 per 100,000. Individuals from the US, Europe, and Australia generally fall within this range. Huntington disease is less common in Japan, China, Korea, Finland, Africa, and South Africa, with estimated prevalence values ranging from 0.1–2 per 100,000 (15, 16).

In the US, more than 35,000 people have HD, and, in Caucasians, the prevalence of HD is estimated to be 4.8 per 100,000. Interestingly, the prevalence of HD is higher for Black Americans (6.4 per 100,000). This suggests that HD is far more common in Blacks living in the US compared with Blacks living in Africa. For example, for Blacks living in South Africa the prevalence of HD is 0.02 per 100,000, and for those living in Zimbabwe it is 1.00 per 100,000 (15).

Huntington disease is caused by an unstable expanded repeat of the cytosine-adenine-guanine (CAG) trinucleotide coding for polyglutamine in the *huntingtin* (*HTT*) gene on chromosome 4. A repeat of 39 or more repeats invariably causes HD. The pathogenesis of HD is not understood, but the mutated huntingtin protein is thought to become toxic, which is accompanied by selective loss of neurons in the caudate and putamen (striatum).

Electron microscopy reveals aggregates of mutated huntingtin protein, which may form because the mutated protein is less soluble, or because it is likelier to form bonds with other proteins, or both of these mechanisms may contribute. The MRI scans of the brain reveal early progressive atrophy of the striatum, with the caudate often more severely affected than the putamen.

Chorea is a defining motor symptom, occurring in approximately 90% of individuals with HD, and is characterized by sudden, random, jerky, involuntary movements that can affect any part of the body. Initially, these movements may be mild and misinterpreted as restlessness, but as HD progresses, the movements increasingly interfere with daily functioning, causing social isolation, and increasing the risk of injury from instability and falls. Chorea tends to stabilize and dissipate during the later stages of HD when other movement impairments such as rigidity and dystonia (involuntary twisting movements) become more prominent (11, 13, 17).

Currently, the mainstay of treatment for HD is symptomatic and supportive care –– no drugs are available to stop or prevent the progression of HD. Deutetrabenazine therapy has been shown to effectively control chorea symptoms compared with placebo and is generally well tolerated. However, comparison data with other VMAT2 inhibitors is limited due to a lack of reported head-to-head trials (6, 9-11, 13, 14, 17, 18).

Tardive Dyskinesia

Tardive dyskinesia (TD) is a medication-induced movement disorder. These movements are involuntary and repetitive, and most commonly affect the tongue, mouth, jaw, and face, but can also affect limbs and trunk. Severe cases are associated with difficulty speaking and swallowing. The condition can be disfiguring and stigmatizing, severely negatively impacting the individual's quality of life (19).

Tardive dyskinesia is caused by medicines that block dopamine receptors –– these include antipsychotic medications (e.g., aripiprazole, clozapine, risperidone, thioridazine) and antiemetic drugs used to treat nausea and vomiting (e.g., metoclopramide and prochlorperazine). Tardive dyskinesia is irreversible and life long, persisting after the causative medicine has been stopped.

Approximately one-third of individuals with schizophrenia treated with antipsychotics have TD (20). Initially, it was thought that the prevalence of TD would decrease as newer antipsychotics were developed that are less likely to cause TD; however, TD remains prevalent. This is partly because the newer antipsychotics are indicated to treat conditions other than schizophrenia, such as depression, bipolar disorder, personality disorder, irritability in autism spectrum disorder, as well as off-label uses including insomnia and anxiety. Therefore, the population exposed to the risk of TD has increased (21-24).

Because TD is irreversible, prevention is crucial –– requiring both the limited use of drugs that cause TD and early diagnosis (25, 26). While not a cure, deutetrabenazine has been shown to reduce the abnormal movements associated with TD and is generally well tolerated. However, there are no head-to-head comparisons between

VMAT2 inhibitors, and TD returns approximately 4 weeks after treatment is discontinued (4, 12, 20, 22, 25, 27-29).

Gene: *CYP2D6*

The cytochrome P450 superfamily (CYP450) is a large and diverse superfamily of enzymes that form the major system for metabolizing or detoxifying lipids, hormones, toxins, and drugs. The *CYP450* genes are often very polymorphic and can result in reduced, absent, or increased enzyme activity.

The CYP2D6 enzyme is responsible for the metabolism of many commonly prescribed drugs, including antidepressants, antipsychotics, analgesics, and beta-blockers. Importantly, CYP2D6 is also the main enzyme that metabolizes the active metabolites of deutetrabenazine (1).

CYP2D6 Alleles

The *CYP2D6* gene is highly polymorphic, as over 100 star (*) alleles have been described and cataloged at the Pharmacogene Variation (PharmVar) Consortium, and each allele is associated with either normal, decreased, or absent enzyme function (Table 2).

The combination of *CYP2D6* alleles that a person has is used to determine their diplotype (e.g., CYP2D6 *4/*4). Based on function, each allele can be assigned an activity score from 0 to 1, which in turn is often used to assign a phenotype (e.g., CYP2D6 poor metabolizer). However, the activity score system is not standardized across clinical laboratories or *CYP2D6* genotyping platforms.

Table 2. Activity Status of Selected *CYP2D6* Alleles

Allele type	*CYP2D6* alleles
Normal function	**1, *2, *33, *35*
Decreased function	**9, *10, *17, *29, *36, *41*
No function	**3, *4, *5, *6, *7, *8, *11, *12, *13, *14, *15, *16, *19, *20, *21, *38, *40, *42*

For a comprehensive list of *CYP2D6* alleles, please see PharmVar.

*CYP2D6*1* is assigned when no variant is detected and is assumed to have normal enzyme activity (CYP2D6 normal metabolizer phenotype). The *CYP2D6* alleles **2*, **33*, and **35* are also considered to have near-normal activity.

Alleles that encode an enzyme with decreased activity include **10*, **17*, and **41*, and alleles that encode a nonfunctional enzyme include **3*, **4*, **5*, and **6*. There are large inter-ethnic differences in the frequency of these alleles, with **3*, **4*, **5*, **6*, and **41* being more common in Caucasians, **10* more common in Asians, and **17* more common in Africans (30).

Additional variant alleles and their multi-ethnic population frequencies have previously been reported (31). Moreover, given the structural variability of the *CYP2D6* region at chromosome 22q13.2, full gene deletion and duplication alleles, as well as complex tandem alleles with *CYP2D6*'s pseudogene, *CYP2D7*, also occur in some individuals, and populations (32).

CYP2D6 Phenotypes

In the US and globally, most individuals, around 70-80%, are classified as "normal metabolizers" (also referred to as "extensive metabolizers"). They either have 2 normal function alleles (e.g., *1/*1) or one normal and one decreased function allele (e.g., *1/*41).

Individuals who have one normal function and one no function allele (e.g., *1/*4) or 2 decreased function alleles (e.g., *41/*41) are also categorized as "normal metabolizers" by recent nomenclature guidelines (33), but have also been categorized as "intermediate metabolizers" (34).

Individuals who have more than 2 normal function copies of the *CYP2D6* gene are classified as "ultrarapid metabolizers," which accounts for 1–10% of Caucasian individuals. For individuals of North African, Ethiopian and Saudi ancestry, the frequency is 16–28% (Table 3) (35).

Individuals who do not have any fully functional alleles are either intermediate metabolizers (one decreased function and one no function allele, e.g., *4/*41) or poor metabolizers (2 no function alleles, e.g., *4/*4).

Approximately 6–10% of European Caucasians and their descendants are poor metabolizers, mainly due to the prevalent nonfunctional **4* and **5* alleles. Compared with Europeans, individuals of Asian descent are likelier to be intermediate metabolizers because of increased prevalence of decreased function alleles, such as *10. Approximately 30% of Asians and individuals of Asian descent are intermediate metabolizers. Similarly, Africans and African Americans are likelier to be intermediate metabolizers than Europeans because of the prevalence of a wide range of decreased function variants (30, 36-38).

Table 3. CPIC (2017). Assignment of likely CYP2D6 Phenotype based on Genotype

Phenotype[a]		Genotype	Examples of *CYP2D6* diplotypes[b]
Metabolizer status	Activity score		
CYP2D6 ultrarapid metabolizer	>2.0	An individual with duplications of functional alleles	*1/*1xN, *1/*2xN, *2/*2xN[c]
CYP2D6 normal metabolizer	1.5–2.0	An individual with 2 normal function alleles or one normal function and one decreased function allele	*1/*1, *1/*2, *1/*9, *1/*41, *2/*2
CYP2D6 normal metabolizer or intermediate metabolizer (controversy remains)[b]	1.0	An individual with 2 decreased function alleles or one normal function and one no function allele	*1/*4, *1/*5, *41/*41
CYP2D6 intermediate metabolizer	0.5	An individual with one decreased function and one no function allele	*4/*10, *4/*41, *5/*9
CYP2D6 poor metabolizer	0	An individual with only no functional alleles	*3/*4, *4/*4, *5/*5, *5/*6

[a] See the *CYP2D6* frequency table in (35) for race-specific allele and phenotype frequencies.
[b] For a complete list of *CYP2D6* diplotypes and resulting phenotypes, see the *CYP2D6* genotype to phenotype table in (35). Note that genotypes with an activity score of 1 are classified as normal metabolizers in the *CYP2D6* genotype to phenotype table on the CPIC website (35).
[c] Where xN represents the number of *CYP2D6* gene copies. For individuals with *CYP2D6* duplications or multiplications, see supplemental data for additional information on how to translate diplotypes into phenotypes.
[d] Individuals with an activity score of 1.0 may be classified as intermediate metabolizers by some reference laboratories. A group of *CYP2D6* experts are currently working to standardize the *CYP2D6* genotype to phenotype translation system.
This Clinical Pharmacogenetics Implementation Consortium (CPIC) table is adapted from (35)

Linking Gene Variation with Treatment Response

The CYP2D6 enzyme is responsible for converting deutetrabenazine active metabolites to minor, reduced activity metabolites. Individuals who are taking a strong CYP2D6 inhibitor (e.g., quinidine, antidepressants such as paroxetine, fluoxetine, and bupropion) have an approximately 3–4 fold higher exposure to active deutetrabenazine metabolites after standard dosing. Therefore, it is likely that individuals who are CYP2D6 poor metabolizers will have a similarly increased exposure to deutetrabenazine.

The FDA-approved drug label for deutetrabenazine cautions that a clinically relevant QT prolongation may occur in CYP2D6 poor metabolizers or individuals who are taking a strong CYP2D6 inhibitor. The drug label also states that a closely related VMAT2 inhibitor, tetrabenazine, has been shown to prolong the QT interval (the time taken for the heart ventricles to depolarize and repolarize). Other drugs with this potential have been associated with life-threatening ventricular tachycardia.

The FDA states that the total daily dosage of deutetrabenazine should be reduced in CYP2D6 poor metabolizers or individuals who are taking a strong CYP2D6 inhibitor. The total daily dose of deutetrabenazine should not exceed 36 mg, with a maximum single dose of 18 mg taken twice daily (the standard recommended total daily dose is 48 mg) (1).

Genetic Testing

The NIH Genetic Testing Registry provides examples of the genetic tests that are currently available for the *CYP2D6* gene.

The *CYP2D6* gene is a particularly complex gene that is difficult to genotype because of the large number of variants and the presence of gene deletions, duplications, multiplications, and pseudogenes. The complexity of genetic variation complicates the correct determination of *CYP2D6* genotype.

Targeted genotyping typically includes up to 30 variant *CYP2D6* alleles (of the more than 100 alleles that have been identified so far). Test results are reported as a diplotype, such as *CYP2D6 *1/*1*. However, it is important to note that the number of variants tested can vary between laboratories, which can result in diplotype result discrepancies between testing platforms and laboratories (35).

A result for copy number, if available, is also important when interpreting *CYP2D6* genotyping results. Gene duplications and multiplications are denoted by "xN"; e.g., *CYP2D6*1xN with xN representing the number of *CYP2D6* gene copies.

If the test results include an interpretation of the individual's predicted metabolizer phenotype, such as "CYP2D6 *1/*1, normal metabolizer", this can be confirmed by checking the diplotype and assigning an activity score assigned to each allele (e.g., 0 for no function, 0.5 for decreased function, and 1.0 for each copy of a normal function allele, Table 3).

The *CYP2D6* phenotype is defined by the sum of the 2 activity scores, which is usually in the range of 0–3.0:

- An ultrarapid metabolizer has an activity score greater than 2
- A normal metabolizer phenotype has an activity score of 1.5–2.0
- A normal metabolizer or intermediate metabolizer has a score of 1.0
- An intermediate metabolizer has an activity score of 0.5
- A poor metabolizer has an activity score of 0 (35)

A standardized *CYP2D6* genotype to phenotype assignment logic is currently being developed by an international working group of *CYP2D6* experts and both the CPIC and the Dutch Pharmacogenetics Working Group (DPWG).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants

2017 Statement from the US Food and Drug Administration (FDA)

2.4 Dosage Adjustment in Poor CYP2D6 Metabolizers

In patients who are poor CYP2D6 metabolizers, the total daily dosage of deutetrabenazine should not exceed 36 mg (maximum single dose of 18 mg).

[...]

5.3 QTc Prolongation

Tetrabenazine, a closely related VMAT2 inhibitor, causes an increase (about 8 msec) in the corrected QT (QTc) interval. A clinically relevant QT prolongation may occur in some patients treated with deutetrabenazine who are CYP2D6 poor metabolizers or are co-administered a strong CYP2D6 inhibitor.

For patients who are CYP2D6 poor metabolizers or are taking a strong CYP2D6 inhibitor, dose reduction may be necessary. The use of deutetrabenazine in combination with other drugs that are known to prolong QTc may result in clinically significant QT prolongations.

[...]

8.7 Poor CYP2D6 Metabolizers

Although the pharmacokinetics of deutetrabenazine and its metabolites have not been systematically evaluated in patients who do not express the drug metabolizing enzyme, it is likely that the exposure to α-HTBZ and β-HTBZ would be increased similarly to taking a strong CYP2D6 inhibitor (approximately 3-fold).

Please review the complete therapeutic recommendations that are located here: (1).

Nomenclature

Nomenclature for Selected *CYP2D6* Alleles

Common allele name	Alternative names / major SNP	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*4*	1846G>A	NM_000106.5:c.506-1G>A	Not applicable - variant occurs in a non-coding region	rs3892097
*CYP2D6*5*	Not applicable - variant results in a whole gene deletion			
*CYP2D6*6*	1707 del T Trp152Gly	NM_000106.5:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655
*CYP2D6*10*	100C>T Pro34Ser	NM_000106.5:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
*CYP2D6*17*	Includes at least two functional variants*: 1023C>T (Thr107Ile) 2850C>T (Cys296Arg)	NM_000106.5:c.320C>T NM_000106.5:c.886T>C	NP_000097.3:p.Thr107Ile NP_000097.3:p.Cys296Arg	rs28371706 rs16947

may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

Nomenclature for Selected continued from previous page.

Common allele name	Alternative names / major SNP	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*41*	2988G>A	NM_000106.5:c.985+39 G>A	Not applicable – variant occurs in a non-coding region	rs28371725

SNP= Single Nucleotide Polymorphism
Note: In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.
Note: The variant 1846G>A often occurs with both 4180G>C and 100C>T; and the variant 988G>A occurs with 2850C>T (Cys296Arg).
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (39).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for Cytochrome P450 enzymes is available from Pharmacogene Variation (PharmVar) Consortium.

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Diazepam Therapy and *CYP2C19* Genotype

Laura Dean, MD[1]

Created: August 25, 2016.

Introduction

Diazepam is a benzodiazepine with several clinical uses, including the management of anxiety, insomnia, muscle spasms, seizures, and alcohol withdrawal. The clinical response to benzodiazepines, such as diazepam, varies widely between individuals (1, 2).

Diazepam is primarily metabolized by CY2C19 and CYP3A4 to the major active metabolite, desmethyldiazepam. Approximately 3% of Caucasians and 15 to 20% of Asians have reduced or absent CYP2C19 enzyme activity ("poor metabolizers"). In these individuals, standard doses of diazepam may lead to a higher exposure to diazepam.

The FDA-approved drug label for diazepam states that "The marked inter-individual variability in the clearance of diazepam reported in the literature is probably attributable to variability of CYP2C19 (which is known to exhibit genetic polymorphism; about 3-5% of Caucasians have little or no activity and are "poor metabolizers") and CYP3A4" (1).

Drug: Diazepam

Diazepam is used in the management of anxiety disorders or for the short-term relief of the symptoms of anxiety. In acute alcohol withdrawal, diazepam may provide symptomatic relief from agitation, tremor, delirium tremens, and hallucinations. Diazepam is also useful as an adjunct treatment for the relief of acute skeletal muscle spasms, as well as spasticity caused by upper motor neuron disorders (3).

There are currently 16 benzodiazepines licensed by the FDA. Diazepam was the second benzodiazepine to be used clinically (after chlordiazepoxide), after being approved for use in 1963. It remains a commonly used drug today, and is included in the World Health Organization's core list of essential medicines needed for a basic healthcare system (4).

The use of benzodiazepines has replaced the use of barbiturates. Although these drug classes share similar therapeutic effects, barbiturates have a narrower therapeutic index, they are more sedative at therapeutic doses, and a barbiturate overdose is more likely to be fatal (5).

Like all benzodiazepines, diazepam is a controlled substance. Chronic use, either at standard therapeutic doses or through recreational abuse, can lead to tolerance and physical dependence. If diazepam treatment is abruptly discontinued, withdrawal symptoms can arise which can be severe and include seizures. Therefore, a gradual tapering of dose is recommended after chronic therapy.

Diazepam has several therapeutic effects—it is a sedative, anxiolytic, anticonvulsant muscle relaxant, and has amnesic effects. Diazepam is thought to exert these effects through an interaction with GABA A-type receptors ($GABA_A$). GABA is the major inhibitory neurotransmitter in the central nervous system. When GABA binds to the $GABA_A$ receptor, the receptor opens, allowing the influx of chloride ions into neurons. This reduces the ability of neurons to depolarize and produce action potentials (excessive action potentials are implicated in seizures). It is thought that diazepam enhances the effects of GABA by increasing the affinity between GABA and its receptor, causing GABA to bind more tightly to the $GABA_A$ receptor (1).

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Diazepam is primarily metabolized via CYP2C19 and CYP3A4 to the major active metabolite (desmethyldiazepam), which is found in the plasma at concentrations equivalent to diazepam. Two minor active metabolites include temazepam and oxazaepam, which are usually not detectable. Other CYP enzymes involved in diazepam metabolism include CYP2C9, CYP2B, and CYP3A5 (2).

It is well documented that wide inter-individual variation in the metabolism of benzodiazepines occurs, which includes diazepam metabolism. This can result in marked differences in drug levels when standard dosing is used, and may potentially influence both therapeutic and adverse effects. It is thought that the variability in clearance of many benzodiazepines, including diazepam, is due to the variability in *CYP2C19* and *CYP3A4* genotypes (2, 3, 6, 7).

Gene: *CYP2C19*

The cytochrome P450 superfamily (CYP) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP genes are very polymorphic and can result in reduced, absent, or increased drug metabolism.

The CYP2C19 enzyme contributes to the metabolism of a range of clinically important drugs, such as antidepressants, several proton pump inhibitors, clopidogrel, and benzodiazepines, including diazepam.

The *CYP2C19* gene is highly polymorphic, as 35 variant star (*) alleles are currently catalogued at the Human Cytochrome P450 (CYP) Allele Nomenclature Database (http://www.cypalleles.ki.se/cyp2c19.htm). The *CYP2C19*1* wild-type allele is associated with normal enzyme activity and the "normal metabolizer" phenotype, whereas the *CYP2C19*17* allele is associated with increased enzyme activity and the "ultrarapid metabolizer" phenotype (8).

The most common loss-of-function variant is *CYP2C19*2*, which contains a c.681G>A variant in exon 5 that results in an aberrant splice site. This leads to the production of a truncated and non-functioning protein. The *CYP2C19*2* allele frequencies are ~15% in Caucasians and Africans, and ~29–35% in Asians (8, 9).

Another commonly tested loss-of-function variant is *CYP2C19*3*, which contains a c.636G>A variant in exon 4 that causes a premature stop codon. The *CYP2C19*3* allele frequencies are ~2–9% in Asian populations, but rare in other racial groups. Other loss-of-function variants occur in less than 1% of the general population, and include *CYP2C19*4-*8* (8, 9).

"Intermediate *CYP2C19* metabolizers" carry one copy of an allele that encodes reduced or absent function (e.g., **1/*2*), whereas "poor metabolizers" are homozygous or compound heterozygous for two loss-of-function alleles (e.g., **2/*2*, **2/*3*) (table 1).

Table 1: *CYP2C19* phenotypes

Phenotype	Phenotype Definition	Genetic Definition	Diplotype Examples
CYP2C19 Ultrarapid metabolizer	Increased enzyme activity compared to rapid metabolizers	Two increased function alleles	**17/*17*
CYP2C19 Rapid metabolizer	Increased enzyme activity compared to normal metabolizers, but less than ultrarapid metabolizers	Combinations of normal function and increased function alleles	**1/*17*
CYP2C19 Normal metabolizer	Fully functional enzyme activity	Two normal function alleles	**1/*1*

Table 1 continued from previous page.

Phenotype	Phenotype Definition	Genetic Definition	Diplotype Examples
CYP2C19 Intermediate metabolizer	Decreased enzyme activity (activity between normal and poor metabolizer)	Combinations of normal function, decreased function, and/or no function alleles	**1/*2* **1/*3* **2/*17* **3/*17*
CYP2C19 Poor metabolizer	Little or no enzyme activity	Combination of no function alleles, and/or decreased function alleles	**2/*2* **2/*3* **3/*3*

Note: The nomenclature used in this table reflects the standardized nomenclature for pharmacogenetic terms proposed by CPIC in a 2016 paper, "Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC)" (10).

Studies have found that individuals who are poor metabolizers have a lower plasma clearance of diazepam compared to normal metabolizers, and that diazepam had a longer plasma half-life (7, 11-13). However, currently, the FDA does not recommend a reduced dose of diazepam in *CYP2C19* poor metabolizers.

One common use of diazepam is to relieve preoperative anxiety in patients. One study found that *CYP2C19* poor metabolizers took a longer period of time to emerge from general anesthesia than normal metabolizers. This study also found that the "slow emergers" had lower levels of CYP3A4 mRNA (14).

Although CYP3A4 is also involved in diazepam metabolism, there have been conflicting results from studies of the impact of *CYP3A4* and *CYP3A5* variants on benzodiazepine metabolism (15-18).

Genetic Testing

Clinical genotyping tests are available for several *CYP2C19* alleles, and a list of test providers is available at the Genetic Testing Registry (GTR) of the National Institutes of Health.

Usually a patient's result is reported as a diplotype, such as *CYP2C19 *1/*1*, and may also include an interpretation of the patient's predicted metabolizer phenotype: ultrarapid, rapid, normal, intermediate, or poor (see table 1).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

Statement from the US Food and Drug Administration (FDA): It has been reported in the literature that diazepam is extensively metabolized to one major active metabolite (desmethyldiazepam) and two minor active metabolites, 3- hydroxydiazepam (temazepam) and 3-hydroxy-N-diazepam (oxazepam) in plasma. At therapeutic doses, desmethyldiazepam is found in plasma at concentrations equivalent to those of diazepam while oxazepam and temazepam are not usually detectable. The metabolism of diazepam is primarily hepatic and involves demethylation (involving primarily CYP2C19 and CYP3A4) and 3-hydroxylation (involving primarily CYP3A4), followed by glucuronidation. The marked inter-individual variability in the clearance of diazepam reported in the literature is probably attributable to variability of CYP2C19 (which is known to exhibit genetic polymorphism; about 3-5% of Caucasians have little or no activity and are "poor metabolizers") and CYP3A4. No inhibition was demonstrated in the presence of inhibitors selective for CYP2A6, CYP2C9,

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

CYP2D6, CYP2E1, or CYP1A2, indicating that these enzymes are not significantly involved in metabolism of diazepam.

Please review the complete therapeutic recommendations that are located here: (1).

Nomenclature

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C19*2*	681G>A Pro227Pro	NM_000769.2:c.681G>A	NP_000760.1:p.Pro227=	rs4244285
*CYP2C19*3*	636G>A Trp212Ter	NM_000769.2:c.636G>A	NP_000760.1:p.Trp212Ter	rs4986893
*CYP2C19*17*	-806C>T	NM_000769.2:c.-806C>T	Not applicable—variant occurs in a non-coding region	rs12248560

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: http://www.cypalleles.ki.se/

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Esomeprazole Therapy and *CYP2C19* Genotype

Laura Dean, MD[1]

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Introduction

Esomeprazole (brand name Nexium) is a proton pump inhibitor (PPI) used to treat gastroesophageal reflux disease (GERD) and to reduce the risk of gastric ulcers associated with nonsteroidal anti-inflammatory drug NSAID use. Esomeprazole is also used in the treatment of hypersecretory conditions, such as Zollinger-Ellison syndrome, and in combination with antibiotics to eradicate *Helicobacter pylori (H. pylori)* infection.

Esomeprazole reduces the acidity (raises the pH) in the stomach by inhibiting the secretion of gastric acid. The level of esomeprazole an individual is exposed to is influenced by several factors, such as the dose used and how quickly the drug is metabolized and inactivated.

Esomeprazole is primarily metabolized by the CYP2C19 enzyme. Individuals with increased CYP2C19 enzyme activity ("CYP2C19 ultrarapid metabolizers") may have an insufficient response to standard doses of esomeprazole, because the drug is inactivated at a faster rate. In contrast, individuals who have reduced or absent CYP2C19 enzyme activity (i.e., CYP2C19 intermediate and poor metabolizers) have a greater exposure to esomeprazole.

The 2018 FDA-approved drug label for esomeprazole states that 3% of Caucasians, and 15–20% of Asians are CYP2C19 poor metabolizers, and that poor metabolizers have approximately twice the level of exposure to esomeprazole, compared with CYP2C19 normal metabolizers. However, the drug label does not include dosing recommendations for CYP2C19 poor metabolizers (1).

Esomeprazole recommendations have been published by the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP), which indicates that no change in dosing is recommended for CYP2C19 poor, intermediate, or ultrarapid metabolizers. The DPWG states that although genetic variation in *CYP2C19* influences the plasma concentration of esomeprazole, there is insufficient evidence to support an effect on treatment outcomes or side effects (2).

Table 1. The FDA (2018) Drug Label for Esomeprazole: *CYP2C19*

Phenotype	Esomeprazole
CYP2C19 poor metabolizer	The CYP2C19 isoenzyme exhibits polymorphism in the metabolism of esomeprazole, since some 3% of Caucasians and 15–20% of Asians lack CYP2C19 and are termed poor metabolizers. At steady state, the ratio of AUC in poor metabolizers to AUC in the rest of the population (normal metabolizers) is approximately 2.

AUC: Area Under the plasma drug concentration-time Curve. AUC reflects the body's exposure to the drug being administered.
Note: "normal metabolizers" were previously termed "extensive metabolizers".
Please see Therapeutic Recommendations based on Genotype for more information from FDA. This FDA table is adapted from (1).

Table 2. The DPWG (2018) Recommendations for Esomeprazole and *CYP2C19* Genotype

Phenotype	Action	Pharmacist text
CYP2C19 poor metabolizer	No action is required for this gene-drug interaction.	Although the genetic variation leads to a higher plasma concentration of esomeprazole, there is insufficient evidence to support an effect on the therapeutic effectiveness and side effects.
CYP2C19 intermediate metabolizer	No action is required for this gene-drug interaction.	

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Table 2. continued from previous page.

Phenotype	Action	Pharmacist text
CYP2C19 ultrarapid metabolizer	No action is required for this gene-drug interaction.	Although the genetic variation may lead to faster inactivation of esomeprazole, there is insufficient evidence to support an effect on the therapeutic effectiveness and side effects.

Please see Therapeutic Recommendations based on Genotype for more information from DPWG. This Dutch Pharmacogenetics Working Group (DPWG) table is adapted from (2).

Drug class: Proton Pump Inhibitors

Proton pump inhibitors block the secretion of gastric acid. They are among the most commonly prescribed drugs in the United States and globally, and some PPI formulations are available without a prescription.

Proton pump inhibitors can be used to treat a number of conditions in adults:

- Active duodenal ulcers
- Active gastric (peptic) ulcers
- *Helicobacter pylori* infection eradication (in combination with antibiotics, to reduce the risk of duodenal ulcer recurrence)
- Hypersecretory conditions (e.g., Zollinger-Ellison syndrome)

Proton pump inhibitors are also used in infants, children, and adults to treat:

- Symptomatic GERD
- Erosive esophagitis (EE) due to acid-mediated GERD
- Maintenance of healing of EE due to acid-mediated GERD

The human stomach contains approximately one billion parietal cells that secrete hydrochloric acid into the stomach (gastric lumen). Gastric acid aids digestion by hydrolyzing dietary protein and facilitating the absorption of calcium, iron, and vitamin B. Gastric acid also helps maintain a sterile environment by suppressing the growth of bacteria (3).

Hydrogen ions (H+) are actively secreted into the gastric lumen in exchange for potassium ions (K+) via an H^+/K^+-ATPase, which is also known as a "proton pump". Located on the luminal surface of gastric parietal cells, the proton pump controls the last step in acid secretion. Proton pump inhibitors potently suppress gastric acid secretion by covalently binding to and irreversibly inactivating this proton pump.

Six PPIs are currently FDA-approved for clinical use: esomeprazole (brand name Nexium), dexlansoprazole (Dexilant, Kapidex), lansoprazole (Prevacid), omeprazole (Prilosec), pantoprazole (Protonix), and rabeprazole (Aciphex). All PPIs are similarly potent at inhibiting gastric acid secretion and are thought to be similarly efficacious (4, 5).

There are a few differences between the indications of different PPIs. For example, for the treatment of GERD in young children, only esomeprazole is indicated for infants from one month old (lansoprazole is indicated from one year of age, omeprazole and dexlansoprazole from 2 years of age, and rabeprazole from age 12) (6).

All 6 PPIs, to varying degrees, are metabolized and inactivated by CYP2C19 (and to a lesser extent by CYP3A4). Additionally, given that PPIs are also inhibitors of CYP2C19 and that CYP2C19 is involved in the metabolism of many drugs, PPI administration can lead to clinically significant drug interactions. For example, the concomitant use of a PPI and clopidogrel, which requires CYP2C19 for bioactivation, has been associated with reduced antiplatelet activity, and thus, the concurrent administration of omeprazole with clopidogrel must balance overall risks and benefits, considering both cardiovascular and gastrointestinal complications (7-11).

Genetic variation in the *CYP2C19* gene influences the clearance of PPIs, which may in turn influence treatment outcomes. Second-generation PPIs are being developed that are not primarily metabolized by CYP2C19, and therefore less likely to be influenced by *CYP2C19* genotype (12-14).

Drug: Esomeprazole

Esomeprazole is a PPI that is available via prescription medication or over-the-counter. It is closely related to omeprazole, which was the first PPI to be licensed in the United States. Esomeprazole is the S-isomer of omeprazole (mirror image of the same chemical structure) whereas omeprazole is a racemic mixture (50:50 mix) of R- and S-isomers.

In adults, esomeprazole is used to reduce the risk of NSAID-associated gastric ulcers and to reduce the risk of recurrence of duodenal ulcers by eradicating *H. pylori* infection. Esomeprazole is also used to treat pathological hypersecretory conditions, including Zollinger-Ellison syndrome.

Esomeprazole is used to treat GERD and to support healing of EE in adults, children, and infants from one month of age.

Esomeprazole is metabolized and inactivated in the liver by the cytochrome P450 system. CYP2C19 is the principal enzyme involved, although other enzymes such as CYP3A4 also contribute to a lesser degree.

The long term use of PPIs has been associated with several adverse effects. Daily treatment with any PPI for longer than 3 years may lead to malabsorption of vitamin B12, caused by hypochlorhydria. Given that prolonged hypochlorhydria also increases the risk of *Clostridium difficile* infection and may increase the risk for osteoporosis-related fractures, the FDA recommends that individuals should use the lowest dose and shortest duration of PPI therapy appropriate for the condition being treated (1).

Studies have not adequately assessed the safety of esomeprazole therapy during pregnancy. For omeprazole use during pregnancy, epidemiology studies failed to find an increased risk of major congenital malformations or other adverse pregnancy outcomes.

Studies have reported that genetic variations in the *CYP2C19* gene influence the plasma concentration of esomeprazole. However, there is insufficient evidence to support that *CYP2C19* genotype influences the efficacy or safety of esomeprazole therapy (15-21). For other PPIs, such as omeprazole, alterations in dose have been recommended by the FDA and the DPWG (1, 2, 16).

Incidental Findings

Genetic variation in the *CYP2C19* gene influences the metabolism of other medications used for the treatment of several conditions:

- Acute coronary syndrome – individuals who are CYP2C19 poor metabolizers and undergoing percutaneous coronary intervention have an increased risk of cardiovascular events if they are treated with the antiplatelet drug clopidogrel (a prodrug that is activated via CYP2C19 metabolism)
- Depression – *CYP2C19* influences the metabolism of tricyclic antidepressants e.g., amitriptyline, imipramine; and selective serotonin reuptake inhibitors (SSRIs) e.g., citalopram. Individuals who are CYP2C19 poor metabolizers may have an increased risk of side effects, whereas there may be an increased risk of treatment failure in ultrarapid metabolizers.

Genetic Testing

Clinical genotyping tests are available for several *CYP2C19* alleles. The NIH Genetic Testing Registry (GTR) provides examples of the genetic tests that are currently available for the esomeprazole response and the

CYP2C19 gene. In addition, variant *CYP2C19* alleles to be included in clinical genotyping assays have been recommended by the Association for Molecular Pathology (22).

Individual results are typically reported as a diplotype, such as *CYP2C19 *1/*1*, and may also include an interpretation with the predicted metabolizer phenotype (ultrarapid, normal, intermediate, or poor).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2018 Statement from the US Food and Drug Administration (FDA)

Metabolism

Esomeprazole is extensively metabolized in the liver by the cytochrome P450 (CYP) enzyme system. The metabolites of esomeprazole lack antisecretory activity. The major part of esomeprazole's metabolism is dependent upon the CYP2C19 isoenzyme, which forms the hydroxy and desmethyl metabolites. The remaining amount is dependent on CYP3A4, which forms the sulphone metabolite. CYP2C19 isoenzyme exhibits polymorphism in the metabolism of esomeprazole, since some 3% of Caucasians and 15 to 20% of Asians lack CYP2C19 and are termed Poor Metabolizers. At steady state, the ratio of AUC in Poor Metabolizers to AUC in the rest of the population (Normal Metabolizers) is approximately 2.

[...]

Interaction with Clopidogrel

Avoid concomitant use of esomeprazole magnesium with clopidogrel. Clopidogrel is a prodrug. Inhibition of platelet aggregation by clopidogrel is entirely due to an active metabolite. The metabolism of clopidogrel to its active metabolite can be impaired by use with concomitant medications, such as esomeprazole, that inhibit CYP2C19 activity. Concomitant use of clopidogrel with 40 mg esomeprazole reduces the pharmacological activity of clopidogrel. When using esomeprazole magnesium consider alternative anti-platelet therapy.

Please review the complete therapeutic recommendations located here: (1).

2018 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

CYP2C19 Poor Metabolizer (PM)

No action is needed for this gene-drug interaction.

Although the genetic variation leads to a higher plasma concentration of esomeprazole, there is insufficient evidence to support an effect on the therapeutic effectiveness and side effects.

CYP2C19 Intermediate Metabolizer (IM)

No action is needed for this gene-drug interaction.

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance with nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

Although the genetic variation leads to a higher plasma concentration of esomeprazole, there is insufficient evidence to support an effect on the therapeutic effectiveness and side effects.

CYP2C19 Ultrarapid Metabolizer (UM)

No action is required for this gene-drug interaction.

Although the genetic variation may lead to faster inactivation of esomeprazole, there is insufficient evidence to support an effect on the therapeutic effectiveness and side effects.

Background information

For more information about the PM, IM, and UM phenotypes: see the general background information about *CYP2C19* on the KNMP Knowledge Bank or on www.knmp.nl (search for *CYP2C19*). Access requires KNMP membership.

Please review the complete therapeutic recommendations that are located here: (2).

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Flibanserin Therapy and *CYP2C19* Genotype

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Introduction

Flibanserin (brand name Addyi) is indicated for the treatment of "hypoactive sexual desire disorder" (HSDD) in premenopausal women. It is the first drug to be approved by the FDA for female sexual dysfunction. Flibanserin acts on central serotonin receptors and was initially developed to be an antidepressant. Although it was not effective for depression, flibanserin did appear to increase sex drive.

The use of flibanserin is limited by modest efficacy and the risk of severe hypotension and syncope (fainting). This risk is increased by alcohol, and by medications that inhibit CYP3A4 (the primary enzyme that metabolizes flibanserin). Consequently, alcohol use is contraindicated during flibanserin therapy, and flibanserin is contraindicated in individuals taking moderate or strong CYP3A4 inhibitors, which include several antibiotics, antiviral agents, cardiac drugs, and grapefruit juice.

The CYP2C19 enzyme also contributes to the metabolism of flibanserin, and individuals who lack CYP2C19 activity ("CYP2C19 poor metabolizers") have a higher exposure to flibanserin than normal metabolizers.

The risk of hypotension, syncope, and CNS depression may be increased in individuals who are CYP2C19 poor metabolizers, according to the FDA-approved drug label, which also states that approximately 2–5% of Caucasians and Africans and 2–15% of Asians are CYP2C19 poor metabolizers. However, the drug label does not provide alternative dosing for poor metabolizers (Table 1). The standard recommended dosage of flibanserin is 100 mg once per day, taken at bedtime (1).

Table 1. The FDA (2015) Drug Label for Flibanserin. Recommendations for CYP2C19 Poor Metabolizers.

Phenotype	Recommendations
CYP2C19 poor metabolizer	Increase monitoring for adverse reactions (e.g., hypotension) in individuals who are CYP2C19 poor metabolizers.

This FDA table is adapted from (1).

Drug: Flibanserin

Flibanserin is the first drug to be approved by the FDA to treat premenopausal women with "acquired, generalized hypoactive sexual desire disorder (HSDD), as characterized by low sexual desire that causes marked distress or interpersonal difficulty". The drug label states that flibanserin should not be used when problems with sexual desire are due to a coexisting medical or psychiatric condition, problems within the relationship, or the effects of medicine or other drugs. In addition, flibanserin should not be used to treat postmenopausal women or men, and it is not indicated to enhance sexual performance (1, 2).

Approximately 10% of adult women in the US are thought to have HSDD, which can significantly affect quality of life. The symptoms of HSDD vary, but may include a lack of sexual desire, impaired arousal, an inability to achieve orgasm, or a general decrease in sexual satisfaction, with accompanying distress (3, 4).

Note: in the 5th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM5), disorders of desire and arousal were joined into one classification titled female sexual interest/excitement and arousal disorder (FSIAD). However, HSDD is still referred to in the literature, and remains a central element of FSAID (5).

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A common reason for sexual problems is insufficient or inadequate sexual stimulation, and the mainstay of treatment is counselling (sex therapy, couples therapy, psychotherapy). Sex therapy includes education on the differences in male and female genital anatomy; e.g., in women, the clitoris is the structure for sexual pleasure, whereas the vagina is a birth canal and not in itself a source of pleasure - therefore, sexual intercourse is unpleasurable and even painful when the woman is insufficiently aroused. Sex therapy provides a safe and respectful space for sexual feelings to emerge and can identify issues such as anxiety and sexual trauma.

In addition to counselling, the management of female sexual dysfunction may also include lifestyle changes (e.g., relaxation techniques, increasing quality time with partner), and physical therapy; e.g., for problems related to pelvic floor hypertonus such as dyspareunia (painful intercourse) and vaginismus (inability for the penis to enter the vagina despite a woman's wish to do so). Medications may include hormone therapy and phosphodiesterase inhibitors (although the latter is not licensed for use in women, and studies report inconsistent results) (6).

Flibanserin is thought to work by targeting central serotonin receptors – it is a postsynaptic 5-HT-1A agonist and 5-HT-2A antagonist. It also has a weak antagonist effect on HT2B, 5-HT2C and dopamine D4 receptors (7-9).

The FDA approval of flibanserin in 2015 was controversial, primarily because of modest efficacy and safety concerns. A daily dose of 100 mg of flibanserin, taken at bedtime, has been associated with a modest increase in sexual desire, and a modest increase of sexually satisfying events – an additional "one half" of an event, per month, on average (9-15). Although only indicated for premenopausal women, one study reported that flibanserin was generally well tolerated and may have efficacy in post-menopausal women (16, 17).

The safety concerns of flibanserin therapy include the risk of severe hypotension, syncope, and CNS depression (e.g., daytime sleepiness). These risks are further increased if flibanserin is taken during the day (it should be taken at bedtime), is taken with alcohol, or taken with CYP3A4 inhibitors (flibanserin is primarily metabolized by CYP3A4). Both alcohol use and the use of strong or moderate CYP3A4 inhibitors are contraindicated with flibanserin use (18, 19). There have been no studies of flibanserin in pregnant women, and it is unknown whether flibanserin causes fetal harm.

Inhibitors of CYP3A4 include antibiotics (e.g., clarithromycin, ciprofloxacin, telithromycin), antifungals (e.g., ketoconazole, itraconazole, posaconazole, fluconazole), HIV drugs – antiretrovirals (e.g., ritonavir, saquinavir, nelfinavir, indinavir, atazanavir) and protease inhibitors – (e.g., amprenavir, fosamprenavir); hepatitis C virus protease inhibitors (e.g., boceprevir, telaprevir), calcium channel blockers (e.g., diltiazem, verapamil), the diuretic conivaptan, the antidepressant nefazodone, and grapefruit juice.

In addition, several drugs can induce CYP3A4 and although concomitant use of these drugs with flibanserin therapy is not contraindicated, it is not recommended. This is because exposure to flibanserin will be decreased, potentially to subtherapeutic levels. The antibiotic rifampin, which is a strong CYP3A4 inducer, decreased concentrations of flibanserin by 95% (20).

The CYP2C19 enzyme has a less prominent role in the metabolism of flibanserin. However, strong CYP2C19 inhibitors may increase flibanserin exposure, and individuals who lack CYP2C19 activity ("CYP2C19 poor metabolizers") may have higher drug levels of flibanserin compared with normal metabolizers (20).

Gene: CYP2C19

The cytochrome P450 superfamily (CYP450) is a large and diverse group of hepatic enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The *CYP450* genes are very polymorphic and can result in reduced, absent, or increased drug metabolism.

The CYP2C19 enzyme contributes to the metabolism of a range of clinically important drugs, such as benzodiazepines, antiplatelet agents, some proton pump inhibitors, antidepressants, and flibanserin –– flibanserin was originally developed to be an antidepressant.

The *CYP2C19* gene is highly polymorphic, as currently there are 35 variant star (*) alleles catalogued by the Pharmacogene Variation (PharmVar) Consortium. The *CYP2C19*1* is considered the wild-type allele when no variants are detected and is associated with normal enzyme activity and the "normal metabolizer" phenotype.

The *CYP2C19*17* allele is associated with increased enzyme activity and is found among individuals with "rapid" (**1/*17*) and "ultrarapid" (**17/*17*) metabolizer phenotypes. Heterozygous carriers of nonfunctional alleles (e.g., **2* and **3*) are classified as "intermediate metabolizers" (e.g. **1/*2*), and individuals who have 2 nonfunctional alleles are classified as "poor metabolizers" (e.g., **2/*2*, **2/*3*) (Table 2).

Table 2. CPIC (2016). Assignment of CYP2C19 Phenotype based on Genotype.

Phenotype	Genotype	Examples of diplotypes
CYP2C19 ultrarapid metabolizer (approximately 2–5% of individuals)[a]	An individual who has 2 increased function alleles	**17/*17*
CYP2C19 rapid metabolizer (approximately 2–30% of individuals)	An individual who has one normal function allele and one increased function allele	**1/*17*
CYP2C19 normal metabolizer (approximately 35–50% of individuals)	An individual who has 2 normal function alleles	**1/*1*
CYP2C19 intermediate metabolizer (approximately 18–45% of individuals)	An individual who has one normal function allele and one no function allele, or one no function allele and one increased function allele	**1/*2* **1/*3* **2/*17*[b]
CYP2C19 poor metabolizer (approximately 2–15% of individuals)	An individual who has 2 no function alleles	**2/*2* **2/*3* **3/*3*

[a] CYP2C19 metabolizer status frequencies are based on average multi-ethnic frequencies. See the *CYP2C19* Frequency Tables for population-specific allele and phenotype frequencies (21).
[b] The predicted metabolizer phenotype for the **2/*17* genotype is a provisional classification. The currently available evidence indicates that the *CYP2C19*17* increased function allele is unable to completely compensate for the *CYP2C19*2* no function allele.
This table is adapted from (21).

Approximately 2% of Caucasians, 4% of African Americans, and 15–25% of East Asians are CYP2C19 poor metabolizers, and up to 45% of individuals are CYP2C19 intermediate metabolizers (2, 22-24).

The most common no function allele is *CYP2C19*2*, which is defined by a c.681G>A variant in exon 5 that creates an aberrant splice site that translates a truncated and nonfunctioning protein. The *CYP2C19*2* allele frequencies are ~15% in Caucasians and Africans, and ~29–35% in Asians (25).

For CYP2C19, another commonly tested no function variant is *CYP2C19*3*, which contains a c.636G>A variant in exon 4 that causes a premature stop codon. The *CYP2C19*3* allele frequencies are ~2-9% in Asian populations, but rare in other racial groups. Other no function variants occur in less than 1% of the general population, and include *CYP2C19*4-*8* (25).

Linking Gene Variation with Treatment Response

Currently, data are lacking on the influence of the CYP2C19 genotype on the efficacy and toxicity of flibanserin.

The drug label for flibanserin cites one study that compared 100 mg daily flibanserin in CYP2C19 poor metabolizers and normal metabolizers. In nine women who were poor metabolizers, the maximum serum concentration of flibanserin was 1.5 times higher, compared with normal metabolizers. In addition, exposure to

flibanserin was 1.3 times higher, and the drug's half-life increased by over 2 hours (from 11.1 hours in normal metabolizers to 13.5 hours in poor metabolizers).

Because CYP2C19 poor metabolizers have increased exposure to flibanserin, the FDA recommends increasing monitoring for adverse reactions (e.g., hypotension) in individuals who are CYP2C19 poor metabolizers.

In contrast to CYP3A4, the concurrent use of strong CYP2C19 inhibitors is not contraindicated with flibanserin therapy. However, the drug label does caution that the concomitant use of strong CYP2C19 inhibitors may increase flibanserin exposure, which in turn increases the risk of hypotension, syncope, and CNS depression. The label recommends discussing the use of a strong CYP2C19 inhibitor with the patient when prescribing flibanserin.

Drugs that are CYP2C19 inhibitors include selective serotonin reuptake inhibitors and other types of antidepressants (e.g., fluoxetine, fluvoxamine, moclobemide), antibiotics (e.g., chloramphenicol, isoniazid), antifungals (e.g., fluconazole, voriconazole), proton pump inhibitors and histamine antagonists (e.g., cimetidine, esomeprazole, omeprazole), HIV drugs (e.g., delavirdine, efavirenz), benzodiazepines, and other types of anti-seizure drugs (e.g., oxcarbazepine, felbamate, topiramate), and antiplatelet agents (e.g., clopidogrel, ticlopidine) (1, 20).

Genetic Testing

Clinical genotyping tests are available for several *CYP2C19* alleles. The NIH's Genetic Testing Registry (GTR) provides examples of the genetic tests that are currently available for the *CYP2C19* gene and flibanserin response. In addition, variant CYP2C19 alleles to be included in clinical genotyping assays have been recommended by the Association for Molecular Pathology (AMP) (27).

Clinical *CYP2C19* genotyping results are reported as a diplotype, such as *CYP2C19 *1/*1*, that typically also include an interpretation of the individual's predicted metabolizer phenotype (ultrarapid, normal, intermediate, or poor). Table 2 summarizes common CYP2C19 phenotypes.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2015 Statement from the US Food and Drug Administration (FDA): CYP2C19 poor metabolizers had increased flibanserin exposures compared to CYP2C19 extensive metabolizers. Additionally, syncope occurred in a subject who was a CYP2C19 poor metabolizer. Therefore, increase monitoring for adverse reactions (e.g., hypotension) in patients who are CYP2C19 poor metabolizers. The frequencies of poor CYP2C19 metabolizers are approximately 2–5% among Caucasians and Africans and approximately 2– 15% among Asians.

Please review the complete therapeutic recommendations that are located here: (1).

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

Nomenclature for selected *CYP2C19* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C19*2*	681G>A Pro227Pro	NM_000769.1:c.681G>A	NP_000760.1:p.Pro227=	rs4244285
*CYP2C19*3*	636G>A Trp212Ter	NM_000769.1:c.636G>A	NP_000760.1:p.Trp212Ter	rs4986893
*CYP2C19*17*	-806C>T	NM_000769.1:c.-806C>T	Not applicable - variant occurs in a non-coding region	rs12248560

Note: the normal "wild type" allele is *CYP2C19*1*.
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines
Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: http://www.cypalleles.ki.se/

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Fluorouracil Therapy and *DPYD* Genotype

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Introduction

Fluorouracil is a chemotherapy agent that belongs to the drug class of fluoropyrimidines. When given as an IV solution, fluorouracil is used in the palliative management of carcinoma of the colon, rectum, breast, stomach, and pancreas (1). When prescribed as a cream for topical use, fluorouracil is used to treat multiple actinic or solar keratoses of the face and scalp (2).

The *DPYD* gene encodes dihydropyrimidine dehydrogenase (DPD), an enzyme that catalyzes the rate-limiting step in fluorouracil metabolism. Individuals who carry at least one copy of no function *DPYD* variants, such as *DPYD*2A*, may not be able to metabolize fluorouracil at normal rates, and are at risk of potentially life-threatening fluorouracil toxicity, such as bone marrow suppression and neurotoxicity. The prevalence of DPD deficiency in Caucasians is approximately 3%-5%.

The FDA-approved drug label for fluorouracil states that "rarely, unexpected, severe toxicity (e.g., stomatitis, diarrhea, neutropenia and neurotoxicity) associated with 5-fluorouracil has been attributed to deficiency of dipyrimidine dehydrogenase activity" (1).

The Clinical Pharmacogenetics Implementation Consortium (CPIC) has published dosing recommendations for fluoropyrimidines (capecitabine, fluorouracil, and tegafur) based on *DPYD* genotype (3) (Table 1). CPIC recommends using an alternative drug for patients who are "poor metabolizers." These individuals carry two copies of no function *DPYD* variants and typically have complete DPD deficiency. CPIC also recommends considering a 50% reduction in starting dose for "intermediate metabolizers." These individuals carry a combination of a normal function and a no function variant and typically have reduced DPD activity (approximately 50% reduced) (3, 4).

Drug Class: Fluoropyrimidines

Fluoropyrimidines are a class of antimetabolite drugs that are widely used in the treatment of cancer. Currently, there are three types of fluoropyrimidines in clinical use: capecitabine, fluorouracil, and tegafur. Capecitabine and tegafur are both active precursors of fluorouracil.

Fluoropyrimidines are thought to exert their chemotherapeutic effects in a number of ways, through several active metabolites. The main mechanism of action is thought to be the inhibition of thymidylate synthase, which plays an important part in the folate-homocysteine cycle, and purine and pyrimidine synthesis pathways. Also, active metabolites can be incorporated into RNA and DNA, ultimately leading to cell death (5).

Approximately 10-40% of patients develop severe and potentially life-threatening toxicity early during treatment with fluoropyrimidines (6). This toxicity typically leads to an interruption or discontinuation of potentially effective anticancer therapy, and often requires hospitalization (7).

The inter-individual variation in the occurrence and severity of adverse events in patients receiving fluoropyrimidines can be partly explained by clinical factors, such as age and sex. However, much of the variability in adverse events remains unexplained (8).

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Of the genetic factors thought to contribute to fluoropyrimidine intolerance, the *DPYD* gene has been the most studied. This gene encodes the primary enzyme involved in breaking down fluoropyrimidines to inactive metabolites. Individuals who have a deficiency of the DPD enzyme have a significantly increased risk of suffering from severe fluoropyrimidine toxicity, and the stratification of patients on the basis of the *DPYD* genotype may help to prevent such adverse events (9-14).

The Clinical Pharmacogenetics Implementation Consortium (CPIC) has published genetics-based dosing recommendations for fluoropyrimidines based on *DPYD* genotype (Table 1).

Table 1. 2013 Recommended dosing of Fluoropyrimidines by DPD phenotype, from Clinical Pharmacogenetics Implementation Consortium (CPIC)

Phenotype	Implications for phenotypic measures	Dosing recommendations	Classification of recommendations[a]
Normal metabolizer	Normal DPD activity and "normal" risk for fluoropyrimidine toxicity	Use label-recommended dosage and administration	Moderate
Intermediate metabolizer	Decreased DPD activity (leukocyte DPD activity at 30–70% that of the normal population) and increased risk for severe or even fatal drug toxicity when treated with fluoropyrimidine drugs	Start with at least a 50% reduction in starting dose, followed by titration of dose based on toxicity[b] or pharmacokinetic test (if available)	Moderate
Poor metabolizer	Complete DPD deficiency and increased risk for severe or even fatal drug toxicity when treated with fluoropyrimidine drugs	Select alternative drug	Strong

Fluoropyrimidines: 5-fluorouracil, capecitabine, and tegafur.
DPD, dihydropyrimidine dehydrogenase.
[a] Rating scheme is described here (3)
[b] Increase the dose in patients experiencing no or clinically tolerable toxicity to maintain efficacy; decrease the dose in patients who do not tolerate the starting dose to minimize toxicities.
Table is adapted from Caudle KE, Thorn CF, Klein TE, Swen JJ, McLeod HL, Diasio RB, Schwab M. Clinical Pharmacogenetics Implementation Consortium guidelines for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing. Clinical pharmacology and therapeutics.2013:94(6):640-5 (3)
Note: The nomenclature used in this table reflects the standardized nomenclature for pharmacogenetic terms proposed by CPIC in a 2016 paper, "Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC)" (15).

Drug: Fluorouracil

Fluorouracil is a form of chemotherapy that when given as an IV solution, is used in the palliative management of carcinoma of the colon, rectum, breast, stomach, and pancreas. Fluorouracil may also be used topically as a cream, for the treatment of multiple actinic or solar keratoses of the face and anterior scalp.

Fluorouracil is structurally similar to pyrimidines, and the enzyme that catalyzes the rate-limiting step in the breakdown of pyrimidines (DPD, dihydropyrimidine dehydrogenase) also catalyzes the rate-limiting step in 5-fluorouracil catabolism. DPD catalyzes the conversion of fluorouracil to the non-cytotoxic dihydrofluorouracil (DHFU) (16).

Fluorouracil is a highly toxic drug with a narrow margin of safety. The FDA-approved label contains the following boxed warning: "It is recommended that Fluorouracil Injection, USP be given only by or under the supervision of a qualified physician who is experienced in cancer chemotherapy and who is well versed in the use of potent antimetabolites. Because of the possibility of severe toxic reactions, it is recommended that patients

be hospitalized at least during the initial course of therapy. These instructions should be thoroughly reviewed before administration of Fluorouracil Injection, USP."

The FDA also states that fluorouracil therapy should be discontinued promptly whenever one of the following signs of toxicity appears:

- Stomatitis or esophageal pharyngitis, at the first visible sign
- Leukopenia (WBC under 3500) or a rapidly falling white blood count
- Vomiting, intractable
- Diarrhea, frequent bowel movements, or watery stools
- Gastrointestinal ulceration and bleeding
- Thrombocytopenia (platelets under 100,000)
- Hemorrhage from any site

Symptomatic DPD deficiency is a rare autosomal recessive disorder with a wide range of symptoms, ranging from no symptoms or signs, to severe neurological problems. In affected individuals, the absent or greatly reduced DPD activity results in uracil and thymine accumulating in the blood, urine, and cerebrospinal fluid. Neurological symptoms typically manifest in early childhood and include seizures, small head size, and delayed cognitive and motor development (17).

Symptomatic DPD deficiency is typically caused by homozygous inactivation of *DPYD*; whereas individuals who are heterozygotes tend to be asymptomatic. However, all patients with less than 70% DPD activity are considered at risk for the development of severe drug toxicity when treated with fluoropyrimidines (18). Signs of fluorouracil toxicity include severe diarrhea, severe mucositis, neutropenia, neurotoxicity, and hand-foot syndrome (redness, swelling, and blisters on the palms of the hands an soles of the feet) (1).

Approximately 3-5% of Caucasians have partial DPD deficiency and 0.2% have complete DPD deficiency (19). Currently, most patients are not screened for DPD deficiency before starting capecitabine therapy (20).

Gene: *DPYD*

The *DPYD* gene encodes the enzyme dihydropyrimidine dehydrogenase (DPD), which catalyzes the first and the rate-limiting step in the breakdown of the pyrimidine nucleotides thymine and uracil. DPD also catalyzes the rate-limiting step in the breakdown of fluoropyrimidines.

Many *DPYD* variants have been described, although only a few have been demonstrated to influence DPD enzyme activity. *DPYD*1* is the wild-type allele and is associated with normal enzyme activity. Individuals who carry two copies of *DPYD* alleles with normal activity are known as "normal metabolizers" and have fully functional DPD enzyme activity (Table 2 and Table 3). Next to *DPYD*1*, the *DPYD* alleles **4*, **5*, **6*, and **9A* are also considered to have normal activity (21).

Table 2. Activity Status of Selected *DPYD* Alleles

Allele type	Alleles
Functional	**1, *4, *5, *6, *9A*
No function	**2A, *13*, rs67376798

Table is adapted from (13, 16) For the nomenclature of human DPYD alleles, please see (22)

The no function *DPYD* variants which have been associated with low DPD activity and an increased risk of toxicity with fluoropyrimidines include **2A*, **13*, and rs67376798 (16). The most well studied variant is *DPYD*2A*, in which a single nucleotide substitution at the invariant splice donor site of intron 14 leads to translation skipping exon 14, resulting in the production of a truncated protein with virtually no enzyme activity.

Individuals who carry combinations of normal function, decreased function, and/or no function *DPYD* alleles are known as "intermediate metabolizers." They have partial DPD deficiency and are at increased risk of capecitabine toxicity. And individuals who carry a combination of no function *DPYD* alleles and/or decreased function *DPYD* alleles are known as "poor metabolizers." They have complete DPD deficiency and are at an even higher risk of capecitabine toxicity. Overall, the prevalence of individuals who are heterozygous for no function variant *DPYD* alleles (partially DPD deficient) that place them at risk of severe drug reactions is estimated to be as high as 3-5%, but this varies in different populations (6, 23-27). For example, in the Dutch population, the *DPYD*2A* had an allele frequency of 0.91% in Caucasians (18).

Table 3 Assignment of likely phenotype based on *DPYD* genotypes

Likely phenotype	Functional definition	Genetic definition	Example diplotypes
Normal metabolizer	Fully functional DPD enzyme activity	Combinations of normal function and decreased function alleles	*DPYD*1/*1*
Intermediate metabolizer (~3–5% of patients)	Decreased DPD enzyme activity (activity between normal and poor metabolizer)	Combinations of normal function, decreased function, and/or no function alleles	**1/*2A*; **1/*13*; or *1/ rs67376798
Poor metabolizer (~0.2% of patients)	Little to no DPD enzyme activity	Combination of no function alleles and/ or decreased function alleles	*2A/*2A; 13/*13; **2/*13*; or rs67376798/ rs67376798

Table is adapted from Caudle KE, Thorn CF, Klein TE, Swen JJ, McLeod HL, Diasio RB, Schwab M. Clinical Pharmacogenetics Implementation Consortium guidelines for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing. Clinical pharmacology and therapeutics.2013:94(6):640-5 (3)
Note: The nomenclature used in this table reflects the standardized nomenclature for pharmacogenetic terms proposed by CPIC in the 2016 paper, "Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC)" (15).

A recent study proposed distinguishing between the various *DPYD* alleles and their functionality by assigning gene activity scores. The use of such scores could result in differentiated individualized dosing advice for fluororpyrimidines, which is essential for reducing toxic side effects while maintaining efficacy (13).

Genetic Testing

The NIH Genetic Testing Registry, GTR, displays genetic tests that are currently available for the *DPYD* gene and the fluorouracil drug response. The *DPYD*2A* variant is the most commonly tested.

Biochemical genetic tests may also be used, which assess the level of activity of the DPD enzyme. These tests include biochemical assays such as analyte testing (e.g., measuring the amount of thymine and uracil in the urine or blood) or an enzyme assay (e.g., directly measuring the activity of DPD using RNA extracted from blood cells and measuring the DPD mRNA copy number) (3, 28, 29).

GTR provides a list of biochemical tests that assess the levels of thymine and uracil analytes, and the activity of the enzyme dihydropyrimidine dehydrogenase.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2016 Statement from the US Food and Drug Administration (FDA): Rarely, unexpected, severe toxicity (e.g., stomatitis, diarrhea, neutropenia and neurotoxicity) associated with 5-fluorouracil has been attributed to

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

deficiency of dipyrimidine dehydrogenase activity. A few patients have been rechallenged with 5-fluorouracil and despite 5-fluorouracil dose lowering, toxicity recurred and progressed with worse morbidity. Absence of this catabolic enzyme appears to result in prolonged clearance of 5-fluorouracil.

Please review the complete therapeutic recommendations that are located here: (1).

2013 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC): [...] Furthermore, patients who are heterozygous for the nonfunctional *DPYD* variants mostly demonstrate partial DPD deficiency (leukocyte DPD activity at 30–70% that of the normal population). Thus, our recommendation is to start with at least a 50% reduction of the starting dose; followed by an increase in dose in patients experiencing no or clinically tolerable toxicity, to maintain efficacy; and a decrease in dose in patients who do not tolerate the starting dose, to minimize toxicities. An alternative is pharmacokinetic-guided dose adjustment (if available). Patients who are homozygous for *DPYD*2A*, **13*, or rs67376798 may demonstrate complete DPD deficiency, and the use of 5-fluouracil or capecitabine is not recommended in these patients. Because capecitabine and tegafur are converted to 5-fluorouracil and then metabolized by DPD, the clearance of and exposure to 5-fluorouracil, in addition to its toxic effects, are similar in patients with these variants.

Please review the complete therapeutic recommendations that are located here: (3).

Nomenclature

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*DPYD**2A	IVS14+1G>A c.1905+1G>A	NM_000110.3:c.1905+1G>A	Not applicable—deletion of exon 14 leads to the production of a truncated protein	rs3918290
*DPYD*13*	1679T>G Ile560Ser	NM_000110.3:c.1679T>G	NP_000101.2:p.Ile560Ser	rs55886062
rs67376798	2846A>T Asp949Val	NM_000110.3:c.2846A>T	NP_000101.2:p.Asp949Val	rs67376798

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

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Flurbiprofen Therapy and *CYP2C9* Genotype

Laura Dean, MD[1]

Created: February 11, 2019.

Introduction

Flurbiprofen (brand name Ansaid) is a nonsteroidal anti-inflammatory drug (NSAID). Tablets and skin patches are used in the management of osteoarthritis and rheumatoid arthritis. Flurbiprofen provides pain relief and reduces inflammation. Flurbiprofen eye drops (brand name Ocufen) may also be used to prevent miosis (excessive constriction of the pupil) during eye operations; e.g., cataract surgery.

Flurbiprofen is primarily metabolized by CYP2C9. Individuals who lack CYP2C9 activity (CYP2C9 poor metabolizers) have an increased exposure to flurbiprofen, and an increased risk of side effects.

Like all NSAIDs, flurbiprofen increases the risk of serious cardiovascular events, including myocardial infarction and stroke, and serious gastrointestinal (GI) adverse events such as bleeding, ulceration, and perforation, which may be fatal.

The recommended starting dose of flurbiprofen tablets in adults is 200–300 mg per day, divided for administration 2, 3, or 4 times a day. But for all patients, the lowest effective dose of flurbiprofen should be used for the shortest length of time, consistent with the treatment goals of each individual.

The FDA-approved drug label for flurbiprofen states that the dose of flurbiprofen should be reduced in "patients who are known or suspected to be poor CYP2C9 metabolizers based on genotype or previous history/experience with other CYP2C9 substrates (such as warfarin and phenytoin)" (Table 1). This dose reduction is to avoid the abnormally high plasma levels of flurbiprofen in these patients caused by reduced metabolic clearance. However, specific dose reductions based on *CYP2C9* phenotype are not provided (1).

As for all NSAIDs, flurbiprofen is contraindicated in patients with a known hypersensitivity; a history of asthma, urticaria, or other allergic-type reactions after taking aspirin or another NSAID; and for coronary artery bypass graft (CABG) surgery. Flurbiprofen should also be avoided by pregnant women starting at 30 weeks gestation (1).

Table 1. The FDA (2017) Drug Label for Flurbiprofen. Poor Metabolizers of CYP2C9 Substrates.

Phenotype	Recommendations
CYP2C9 Poor metabolizers	In patients who are known or suspected to be poor CYP2C9 metabolizers based on genotype or previous history/experience with other CYP2C9 substrates (such as warfarin and phenytoin), reduce the dose of flurbiprofen to avoid abnormally high plasma levels due to reduced metabolic clearance.

This table is adapted from (1).

Drug Class: NSAIDs

NSAIDs are widely used to treat inflammation, fever, and pain. They are one of the most commonly used class of drugs. Worldwide, it is estimated that more than 30 million people receive NSAIDs daily (2).

Currently, more than 20 NSAIDs are licensed for use. Several NSAIDs (e.g., aspirin, ibuprofen, and naproxen) are available over-the-counter, but higher doses and other types of NSAIDs, such as celecoxib, piroxicam, and flurbiprofen, are only available via prescription.

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The main action of NSAIDs is to inhibit cyclooxygenase (COX). Cyclooxygenase is the central enzyme in the synthesis of prostaglandins, prostacyclin, and thromboxanes from arachidonic acid. Prostaglandins can be protective (e.g., protect the gastric mucosal lining and aid platelet aggregation) or inflammatory (e.g., recruiting inflammatory white blood cells).

There are 2 main isoforms of COX, and the safety and effectiveness of NSAIDs may be influenced by the degree they inhibit the 2 different forms. COX-1 is a "housekeeping enzyme" that is expressed in most tissues. It protects the GI tract and induces platelet aggregation in response to injury. In contrast, COX-2 is often undetectable in tissues. However, the expression of COX-2 is increased during inflammation.

Most NSAIDs are non-selective COX inhibitors that inhibit both COX-1 and COX-2. There are exceptions, such as celecoxib, which is a selective COX-2 inhibitor that appears to be associated with fewer adverse GI events. However, GI adverse events still occur.

Approximately 25% of the exposed population in the US has experienced NSAID-related side effects that required medical care (3). All NSAIDs carry a boxed warning regarding the risk of serious GI and cardiovascular adverse events; e.g.,

"*NSAIDs cause an increased risk of serious cardiovascular thrombotic events, including myocardial infarction and stroke, which can be fatal. This risk may occur early in treatment and may increase with duration of use.*

NSAIDs cause an increased risk of serious gastrointestinal adverse events including bleeding, ulceration, and perforation of the stomach or intestines, which can be fatal. These events can occur at any time during use and without warning symptoms. Elderly patients and patients with a prior history of peptic ulcer disease and/or GI bleeding are at greater risk for serious GI events" (1).

Drug: Flurbiprofen

Flurbiprofen is an NSAID used for the relief of the signs and symptoms of osteoarthritis and rheumatoid arthritis. It may also be used for soft tissue injuries, such as bursitis and tendinitis.

The recommended starting dose of flurbiprofen tablets in adults is 200–300 mg per day, divided into doses to be taken 2, 3, or 4 times a day. The largest recommended single dose in a multiple-dose daily regimen is 100 mg (1).

Because of the adverse events associated with any type of NSAID, the lowest effective dose of flurbiprofen should be used, for the shortest duration. And, as for all NSAIDs, flurbiprofen is contraindicated in patients with a known hypersensitivity, or a history of asthma, urticaria, or other allergic-type reactions after taking aspirin or another NSAID. Flurbiprofen is also contraindicated to treat pain in the days following CABG surgery –– this is because NSAIDs increase the risk of myocardial infarction and stroke after surgery. Flurbiprofen should be avoided by pregnant women starting at 30 weeks gestation –– this is because NSAID use in the third trimester causes an increased risk of premature closure of the fetal ductus arteriosus. There are no well-controlled studies of flurbiprofen in pregnant women, but in animal studies, flurbiprofen was lethal to the embryos of pregnant rats and rabbits. Flurbiprofens' safety, efficacy, and pharmacokinetics have not established for pediatric patients.

Flurbiprofen can be taken orally (tablets) or topically (via a skin patch or cream) for the treatment of osteoarthritis and rheumatoid arthritis. Flurbiprofen is also available as an ophthalmic solution (eye drops) –– it is used before eye surgery to prevent miosis (excessive constriction of the pupil), which can occur in surgical procedures such as cataract surgery.

CYP2C9 is the main enzyme involved in the metabolism of flurbiprofen to its inactive metabolite: 4'-hydroxyflurbiprofen. Both flurbiprofen and its metabolite are eliminated as acyl glucuronides. Individuals who have decreased CYP2C9 activity, such as CYP2C9 intermediate and poor metabolizers, have a higher exposure to flurbiprofen (1, 4, 5).

Gene: *CYP2C9*

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs in the liver. The CYP450 genes are very polymorphic and can result in reduced, absent, or increased enzyme activity (6).

The *CYP2C9* gene is highly polymorphic, with approximately 60 known alleles. *CYP2C9*1* is considered the wild-type allele when no variants are detected and is categorized as having normal enzyme activity (7). Individuals who have 2 normal-function alleles (e.g., *CYP2C9 *1/*1*) are classified as "normal metabolizers" (Table 2).

Table 2. Assignment of likely *CYP2C9* Phenotype based on Genotype (CPIC, 2014)

Likely phenotype[a]	Genotype	Examples of diplotypes
Ultrarapid metabolizer (increased activity) (frequency unknown)	Unknown – currently there are no known increased activity alleles	Unknown
Normal metabolizer (normal activity) (approximately 91% of individuals)	An individual with 2 normal-function alleles	*1/*1
Intermediate metabolizer (heterozygote or intermediate activity) (approximately 8% of individuals)[b]	An individual carrying one normal-function allele plus one decreased-function allele	*1/*3, *1/*2
Poor metabolizer (homozygous variant, low or deficient activity) (approximately 1% of individuals)	An individual carrying 2 decreased function alleles	*2/*2, *3/*3, *2/*3

Note: There are no known cases of CYP2C9 ultrarapid metabolizers.
[a] Global frequencies are approximate. Because haplotype frequencies vary considerably among populations, please see (7) for individual population frequencies.
[b] The enzyme activity in this grouping varies widely. Please see (7) for activity ranges.
This table is adapted from (7). Note: The nomenclature used in this table reflects the standardized pharmacogenetic terms proposed by the Clinical Pharmacogenetics Implementation Consortium (CPIC) (8).

Two allelic variants associated with reduced enzyme activity are *CYP2C9*2* and **3*. The **2* allele is more common in Caucasian (10–20%) than Asian (1–3%) or African (0–6%) populations. The **3* allele is less common (<10% in most populations) and is extremely rare in African populations. In African-Americans, the *CYP2C9*5*, **6*, **8* and **11* alleles are more common (9-11).

Linking Gene Variation with Treatment Response

Studies have shown that CYP2C9 intermediate or poor metabolizers have increased drug exposure when taking standard doses of flurbiprofen.

Although data are lacking that link CYP2C9 intermediate or poor metabolizers with an increased risk of the adverse effects associated with NSAID therapy, the dose, and duration of NSAID therapy do influence the risk of adverse effects, such as severe GI bleeding.

Therefore, the FDA drug label for flurbiprofen recommends reducing the dose of flurbiprofen in CYP2C9 poor metabolizers. The FDA label does not however recommend a dose reduction in CYP2C9 intermediate metabolizers, despite the observed high levels of the drug in this genotype group in other studies (1, 4, 5, 12).

Genetic Testing

Clinical genotyping tests are available for several *CYP2C9* alleles. The NIH's Genetic Testing Registry (GTR) displays genetic tests that are currently available for flurbiprofen response and for the *CYP2C9* gene.

The *CYP2C9* variants that are routinely tested for include *CYP2C9*2* and **3*. Usually the results are reported as a diplotype, such as *CYP2C9 *1/*1*, and may also include an interpretation of the patient's predicted metabolizer phenotype (normal, intermediate, or poor). Table 2 summarizes common CYP2C9 phenotypes.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2017 Statement from the US Food and Drug Administration (FDA)

In patients who are known or suspected to be poor CYP2C9 metabolizers based on genotype or previous history/experience with other CYP2C9 substrates (such as warfarin and phenytoin), reduce the dose of flurbiprofen to avoid abnormally high plasma levels due to reduced metabolic clearance.

Please review the complete therapeutic recommendations that are located here:(1)

Nomenclature for selected *CYP2C9* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C9*2*	430C>T Arg144Cys	NM_000771.3:c.430C>T	NP_000762.2:p.Arg144Cys	rs1799853
*CYP2C9*3*	1075A>C Ile359Leu	NM_000771.3:c.1075A>C	NP_000762.2:p.Ile359Leu	rs1057910
*CYP2C9*5*	1080C>G Asp360Glu	NM_000771.3:c.1080C>G	NP_000762.2:p.Asp360Glu	rs28371686
*CYP2C9*6*	818delA Lys273Argfs	NM_000771.3:c.817delA	NP_000762.2:p.Lys273Argfs	rs9332131
*CYP2C9*8*	449G>A Arg150His	NM_000771.3:c.449G>A	NP_000762.2:p.Arg150His	rs7900194
*CYP2C9*11*	1003C>T Arg335Trp	NM_000771.3:c.1003C>T	NP_000762.2:p.Arg335Trp	rs28371685

Note: the normal "wild-type" allele is *CYP2C9*1* and is reported when no variant is detected.
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (13).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for cytochrome P450 enzymes is available from Pharmacogene Variation (PharmVar) Consortium.

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[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

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Gentamicin Therapy and *MT-RNR1* Genotype

Laura Dean, MD[1]

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Introduction

Gentamicin is an aminoglycoside antibiotic that is commonly used to treat sepsis in premature infants. Brand names include Garamycin, Cidomycin, and Setpopal. Gentamicin is administered by injection to treat serious infections caused by Gram-negative bacteria (e.g., *Pseudomonas aeruginosa*, *Proteus* species, *Escherichia coli*, *Klebsiella-Enterobacter-Serratia* species, and *Citrobacter* species), and is used as an adjuvant treatment for infections caused by Gram-positive bacteria (e.g., *Staphylococcus* species) (1). Gentamicin may also be used topically to treat ophthalmic and dermatological infections.

In most patients, prolonged exposure to high gentamicin levels will cause ototoxicity (damage to the inner ear). However, in individuals who carry specific variants in the mitochondrial gene, *MT-RNR1*, a single dose of gentamicin can result in hearing loss. This toxicity occurs in genetically susceptible individuals even though the serum drug level is within therapeutic range (2).

In most studies, 100% of individuals with the *MT-RNR1* variant known as m.1555A>G (NC_012920.1:m.1555A>G) developed hearing loss after receiving aminoglycoside therapy. The onset of hearing loss varies, but once it occurs, the hearing loss is usually moderate to profound, bilateral, and irreversible (3).

Currently, the FDA-approved drug label for gentamicin does not include a statement about *MT-RNR1* However, a 2014 American College of Medical Genetics and Genomics (ACMG) guideline includes the following recommendation: "Single-gene testing may be warranted in cases in which the medical or family history, or presentation of the hearing loss, suggests a specific etiology. For example, testing for mitochondrial DNA mutations associated with aminoglycoside ototoxicity may be considered for individuals with a history of use of aminoglycoside antibiotics" (4, 5).

Drug: Gentamicin

Aminoglycosides such as gentamicin are among the earliest formulations of antibiotics (6). They are effective against most aerobic bacteria, both Gram-positive and Gram-negative. But because they are inactive against anaerobes, they are often used in combination with another antibiotic, such as a beta-lactam antibiotic or a cephalosporin, to increase coverage (7).

Six aminoglycoside drugs are currently approved for use by the FDA: amikacin, gentamicin, neomycin, paromomycin, streptomycin, and tobramycin. The ending of these drug names, -mycin or -micin, reflects from which genus of bacteria the aminoglycoside was derived from, *Streptomyces* or *Micromonospora* respectively (8).

Aminoglycosides exert antibacterial effects by binding to bacterial ribosomes and inhibiting bacterial protein synthesis. They bind to the 30s ribosomal subunit, which interferes with the decoding site—this is where the ribosome has to accurately select tRNA in accordance with the appropriate mRNA codon. Errors here lead to inappropriate translation of the mRNA codons, so that incorrect amino acids are inserted into the polypeptide chain. This can disrupt elongation of the peptide chain (9, 10).

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Like all aminoglycosides, gentamicin is poorly absorbed from the gut, so it is not taken orally. It is either given by injection, with regular blood tests to monitor drug levels, or given topically in the form of drops, cream, or ointment, to treat infections of the eye or skin.

The toxicity of aminoglycosides, along with the discovery of equally potent but less toxic antibiotics, has meant that the use of aminoglycoside injections is reserved for serious infections that are proven, or strongly suspected to be, caused by susceptible bacteria. Aminoglycosides are most commonly used in the treatment of neonatal septicemia, especially in premature babies—over 90% need aminoglycoside therapy during the first weeks of life (11). Aminoglycosides are also used in combination with other antibiotics as surgical prophylaxis in patients who are allergic to penicillin, and for febrile neutropenia, septic shock, and drug-resistant tuberculosis (6).

The main toxicities of aminoglycoside injections are kidney damage (nephrotoxicity) and damage to the inner ear (ototoxicity) (12). Nephrotoxicity primarily involves the proximal tubules and is generally reversible (13). In contrast, aminoglycoside-induced ototoxicity is usually irreversible. Damage may occur to the cochlea—resulting in sensorineural hearing loss—or the vestibular system—causing problems with balance, vertigo, ataxia, nausea, and vomiting. Gentamicin is considered to be more toxic to the vestibular system so is used for vestibular ablation to treat Ménière's disease. Amikacin and neomycin are examples of aminoglycosides that are more toxic to the cochlea (12, 14).

Rarely, neuromuscular blockade can occur after aminoglycoside therapy. The boxed warning on the FDA-approved drug label recommends that aminoglycosides "be used with caution in patients with neuromuscular disorders, such as myasthenia gravis or parkinsonism, because they may aggravate muscle weakness (7)"; whereas the British National Formulary states that aminoglycosides should not be given to patients with myasthenia gravis (15).

Gene: *MT-RNR1*

Mitochondria are the main source of energy in most cells—they use oxygen, sugars, and fats to create energy in the form of ATP. This process is known as oxidative phosphorylation. Any genetic variation that disrupts normal mitochondrial function can have severe effects on health.

Mitochondria have their own genome—it is small, circular, and resembles the bacterial prokaryotes from which they evolved. The mitochondrial genome is passed down from mother to child (maternal inheritance) and contains 37 genes, one of which is the *MT-RNR1* gene ("mitochondrially encoded 12S RNA"). The ribosomal RNA (rRNA) encoded by *MT-RNR1* is essential in the synthesis of the proteins that carry out oxidative phosphorylation.

Consistent with their bacterial origin, mitochondrial rRNA more closely resembles bacterial rRNA than human rRNA. However, at a highly conserved decoding region in the *MR-RNR1* gene, the sequence in humans is distinct from the sequence in bacteria. This difference means that aminoglycosides, which target the decoding region in bacteria, normally do not bind to this region in humans (9).

However, genetic variation in the ribosomal decoding region can result in mitochondrial RNA becoming more similar to bacterial rRNA, thereby facilitating the binding of aminoglycosides. The mechanism is unclear, but aminoglycosides damage the sensory hair cells in the cochlea that mediate hearing (16-18).

The most common *MT-RNR1* variant is a single nucleotide substitution of a guanine at position 1555 for an adenine (m.1555A>G). Individuals with this variant are exquisitely sensitive to aminoglycoside-induced hearing loss, which is moderate to profound, bilateral, irreversible, and may have a rapid onset. Even a single dose of aminoglycoside can be sufficient to cause ototoxicity (2, 19).

Genetically susceptible individuals who are not exposed to aminoglycosides may nonetheless develop hearing loss, referred to as "non-syndromic mitochondrial hearing loss." The course of hearing loss may be affected by

the presence of additional genetic factors as well as environmental factors, such as exposure to loud noise. However, normal hearing is usually preserved until at least 44 years of age (2).

The prevalence of the m.1555A>G variant varies among different populations. In the US, the population prevalence is estimated to be 0.09%, and in the UK, 0.20% (5, 20, 21). In hearing impaired populations, the prevalence is much greater, but the estimates vary widely based on study differences, such as the age of onset of hearing loss and whether there has been exposure to aminoglycosides. Estimates include a prevalence of 3.5% among the hearing impaired population in Japan (22), 5% among deaf individuals in Indonesia (23), and 6% of individuals with post-lingual hearing loss from the UK and Southern Italy (24). Additionally, a prevalence of 15% has been reported in "ethnically diverse patients in the United States with hearing loss after aminoglycoside exposure" (25), and in 15-20% of individuals from Spain with hearing loss (26).

The m.1555A>G variant is the most well studied *MT-RNR1* variant with regards to aminoglycoside ototoxicity, but other mitochondrial variants are also strongly associated with hearing loss. In 10 small studies, all individuals with the 1494C > T (NC_012920.1:m.1494C>T) variant developed hearing loss after receiving an aminoglycoside antibiotic. A 827A>G (NC_012920.1:m.827A>G) variant, and variants at position 961, have also been associated with non-syndromic hearing loss, both with and without the use of aminoglycosides (3).

Several studies have highlighted the complex issues raised by screening for pathogenic *MT-RNR1* variants. The aim of screening is to prevent avoidable hearing loss in genetically susceptible individuals by administering an alternative antibiotic whenever possible. Issues include the costs of universal screening, for example, as part of the newborn screening program—given that the prevalence of m.1555A>G is thought to be 1 in 385 Caucasians (2, 27, 28) —versus limiting genetic testing to a case-by-case basis (e.g., patients with tuberculosis, children with leukemia, individuals with cystic fibrosis, and surgical patients allergic to beta-lactam antibiotics)(5, 29).

In the US, aminoglycosides are most commonly used in the neonatal intensive care unit, where acute, life-threatening sepsis means that aminoglycoside therapy cannot be delayed to wait for the results of genetic testing (30). However, recent advances in screening has allowed for rapid, accurate and inexpensive testing (31-33). One potential alternative would be to screen all pregnant women, because mitochondrial variants are maternally inherited and m.1555A>G is almost always homoplasmic ("homoplasmy" is when all the mitochondrial DNAs in a cell are identical; when they are not, it is called "heteroplasmy") (5, 19, 34).

Genetic screening may provide more benefit if aminoglycoside use becomes more widespread because of growing resistance to other antibiotics. Or screening could be reserved for countries where aminoglycosides are still commonly used, despite their toxicity, because they are inexpensive (35). In countries where the use of aminoglycosides is more common, a quarter of people with aminoglycoside-induced hearing loss have maternal relatives who also have drug-related hearing loss (36, 37).

A report from the World Health Organization's Essential Medicines and Pharmaceutical Policies comments that "pre-treatment screening is an important consideration to prevent aminoglycoside related hearing loss but given cost and access issues, asking about a maternal family history of deafness may be more practical" (38).

Genetic Testing

The NIH's Genetic Testing Registry (GTR) provides examples of the genetic tests that are currently available for the *MT-RNR1* gene. Targeted mutation panels vary among testing laboratories, but most laboratories routinely test for m.1555A>G.

MT-RNR1 variants are associated with two conditions: aminoglycoside hypersensitivity resulting in post-exposure deafness and nonsyndromic mitochondrial hearing loss that tends to develop gradually over time. While the presence of an *MT-RNR1* variant indicates a high risk of aminoglycoside ototoxicity, the test results do not predict the age of onset or severity of nonsyndromic mitochondrial hearing loss (19).

Therapeutic Recommendations based on Genotype

Excerpt from the American College of Medical Genetics and Genomics (ACMG) Guideline for the Clinical Evaluation and Etiologic Diagnosis of Hearing Loss:

For individuals lacking physical findings suggestive of a known syndrome and having medical and birth histories that do not suggest an environmental cause of hearing loss, a tiered diagnostic approach should be implemented.

Pretest genetic counseling should be provided, and, with patient's informed consent, genetic testing should be ordered.

Single-gene testing may be warranted in cases in which the medical or family history, or presentation of the hearing loss, suggests a specific etiology. For example, testing for mitochondrial DNA mutations associated with aminoglycoside ototoxicity may be considered for individuals with a history of use of aminoglycoside antibiotics.

Please review the complete therapeutic recommendations that are located here: (4).

Nomenclature

Common allele name	Alternative names	HGVS reference sequence			dbSNP reference identifier for allele location
		Genomic	Coding	Protein	
m.1555A>G	A1555G	NC_012920.1:m.1555A>G	NA	NA (encodes ribosomal RNA)	rs267606617
m.1494C>T	C1494T	NC_012920.1:m.1494C>T	NA	NA (encodes ribosomal RNA)	rs267606619
m. 827A>G	A827G	NC_012920.1:m.827A>G	NA	NA (encodes ribosomal RNA)	rs28358569

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Version History

To view the 2015 version of this summary (created: April 29, 2015) please click here.

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Imipramine Therapy and *CYP2D6* and *CYP2C19* Genotype

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Introduction

Imipramine is a tricyclic antidepressant used in the treatment of several psychiatric disorders including major depression, obsessive-compulsive disorder, generalized anxiety disorder, post-traumatic stress disorder, and bulimia. Imipramine may also be useful as an adjunctive treatment in the management of panic attacks, neuropathic pain, attention-deficit disorder, and childhood enuresis (bedwetting) (1).

Tricyclic antidepressants (TCAs) primarily mediate their therapeutic effect by inhibiting the reuptake of both serotonin and norepinephrine, leaving more neurotransmitter in the synaptic cleft stimulating the neuron. Because tricyclics can also block different receptors (histamine H1, α1-adrenergic, and muscarinic receptors), side effects are common. As such, more specific selective serotonin reuptake inhibitors (SSRIs) have largely replaced the use of them. However, TCAs still have an important use in specific types of depression and other conditions.

Imipramine is primarily metabolized via CYP2C19 to active metabolites, including desipramine, also a tricyclic antidepressant. Further metabolism is catalyzed by CYP2D6. Individuals who are "CYP2D6 ultrarapid metabolizers" carry more than two normal function alleles (i.e., multiple copies) (Table 1, 2), whereas individuals who are "CYP2C19 ultrarapid metabolizers" carry two increased function alleles (Table 3, 4). Individuals who are CYP2D6 or CYP2C19 "poor metabolizers" carry two no function alleles for *CYP2D6* or *CYP2C19*, respectively.

The FDA-approved drug label for imipramine states that CYP2D6 poor metabolizers have higher than expected plasma concentrations of tricyclic antidepressants when given usual doses. Their recommendations include monitoring tricyclic antidepressant plasma levels whenever a tricyclic antidepressant is going to be co-administered with another drug known to be an inhibitor of CYP2D6 (1).

In 2016, the Clinical Pharmacogenetics Implementation Consortium (CPIC) made dosing recommendations for tricyclic antidepressants based on *CYP2C19* and *CYP2D6* genotypes. Amitriptyline and nortriptyline were used as model drugs for this guideline because the majority of pharmacogenomic studies have focused on these two drugs. According to the CPIC guideline, because TCAs have comparable pharmacokinetic properties, it may be reasonable to apply the recommendations to other tricyclics, including imipramine (2).

For CYP2D6 ultrarapid metabolizers, CPIC recommends avoiding the use of a tricyclic due to the potential lack of efficacy, and to consider an alternative drug not metabolized by CYP2D6. If a TCA is still warranted, CPIC recommends considering titrating the TCA to a higher target dose (compared to normal metabolizers) and using therapeutic drug monitoring to guide dose adjustments. For CYP2D6 intermediate metabolizers, CPIC recommends considering a 25% reduction of the starting dose, and for CYP2D6 poor metabolizers, to avoid the use of tricyclics because of the potential for side effects. If a tricyclic is still warranted for CYP2D6 poor metabolizers, CPIC recommends considering a 50% reduction of the starting dose while monitoring drug plasma concentrations to avoid side effects.

For CYP2C19 ultrarapid metabolizers, CPIC recommends avoiding the use of tertiary amines (e.g., imipramine) due to the potential for a sub-optimal response, and to consider an alternative drug not metabolized by CYP2C19, such as the secondary amines nortriptyline or desipramine. For CYP2C19 poor metabolizers, CPIC

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recommends avoiding tertiary amine use due to the potential for sub-optimal response, and to consider an alternative drug not metabolized by CYP2C19. If a tertiary amine is still warranted for CYP2C19 poor metabolizers, CPIC recommends considering a 50% reduction of the starting dose while monitoring drug plasma concentrations to avoid side effects (2).

Drug Class: Tricyclic Antidepressants

Tricyclic antidepressants (TCAs) are mixed serotonin-norepinephrine reuptake inhibitors. They increase the amount of neurotransmitter in the synaptic cleft, thought to mediate their antidepressant effects.

From the 1960s to the 1980s, tricyclics were the first-line treatment for depression, until the introduction of SSRIs, which have fewer side effects and are safer. The common side effects of tricyclics include anticholinergic side effects (e.g., blurred vision, dry mouth, constipation, and sedation), cardiac effects, and orthostatic hypotension.

Today, the main therapeutic use of tricyclics is chronic pain management, such as neuropathic pain. However, tricyclics are still used in the treatment of depression as well as other psychiatric disorders including obsessive-compulsive disorder, panic attacks, generalized anxiety disorder, post-traumatic stress disorder, bulimia nervosa, smoking cessation, and enuresis (bedwetting).

Tricyclics are named after their chemical structure of three central rings and a side chain important for their function and activity. Its structure determines whether a drug is classified a tertiary amine (amitriptyline, clomipramine, doxepin, imipramine, and trimipramine) or secondary amine (desipramine and nortriptyline).

Whereas tertiary amines are generally more potent in blocking reuptake of serotonin, the secondary amines are more potent in blocking the reuptake of norepinephrine. Secondary amines are better tolerated and are also associated with fewer anticholinergic side effects.

The CYP2C19 enzyme metabolizes tertiary amines to active metabolites, which include desipramine (the active metabolite of imipramine) and nortriptyline (the active metabolite of amitriptyline). Both the tertiary and secondary amines are metabolized by CYP2D6 to less active metabolites.

The effectiveness and tolerability of tricyclics are affected by CYP2D6 metabolism and partially by CYP2C19 metabolism. Individuals who carry *CYP2D6* or *CYP2C19* variants that influence enzyme activity may be at an increased risk of treatment failure (if plasma drug levels are decreased) or drug toxicity (if plasma drug levels are increased).

Drug: Imipramine

Imipramine was the first tricyclic used in the treatment of depression in the late 1950s. Imipramine is still used to relieve the symptoms of major depressive disorder, and it may be useful too as temporary adjunctive therapy in reducing enuresis (bedwetting) in children aged 6 years and older. Off-label uses of imipramine also include the treatment of neuropathic pain and attention deficit disorder.

Imipramine is a tertiary amine and is similar in structure to amitriptyline, another tertiary amine. Both drugs potently block the reuptake of serotonin and to a lesser degree norepinephrine. Imipramine has also strong affinities for alpha-1 adrenergic, histamine H1, and muscarinic M1 receptors, which account for its side effects of orthostatic hypotension, sedation, weight gain, and anticholinergic effects. However, the intensity of these side effects is generally less than it is for amitriptyline (3).

Imipramine is metabolized by CYP2C19 to desipramine, which is also a tricyclic antidepressant with distinct clinical features that differ from the imipramine. Desipramine is then metabolized by CYP2D6 to the less active

hydroxy-imipramine. For therapeutic drug monitoring, the levels of imipramine and hydroxy-imipramine should be monitored (4).

The optimal therapeutic range for imipramine is well-defined (5). Most individuals display an optimal response to imipramine when combined serum levels of imipramine and desipramine are between 175 and 300 ng/mL (6). However, individuals who are carriers of certain *CYP2D6* and/or *CYP2C19* variants may have drug levels that are outside this range even after being treated with standard doses of imipramine. As a result, they may have an increased risk of side effects (if the level of imipramine and its active metabolites are too high) or treatment failure (if drug levels are too low).

Gene: *CYP2D6*

The cytochrome P450 superfamily (CYP) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP genes are very polymorphic and can result in reduced, absent, or increased drug metabolism.

CYP2D6 is responsible for the metabolism of many commonly prescribed drugs, including antipsychotics, analgesics, beta-blockers, and TCAs such as imipramine.

CYP2D6 is highly polymorphic, with over 100 star (*) alleles described and currently catalogued at the Human Cytochrome P450 (CYP) Allele Nomenclature Database (7).

CYP2D6 is a particularly complex gene that is difficult to genotype, partly because of the large number of variants, but also because of the presence of gene deletions, duplications, and its neighboring pseudogenes. The complexity of genetic variation at this locus complicates the ability to interrogate *CYP2D6*.

There is substantial variation in *CYP2D6* allele frequencies among different populations (8). *CYP2D6*1* is the wild-type allele and is associated with normal enzyme activity and the "normal metabolizer" phenotype. The *CYP2D6* alleles **2*, **33*, and **35* are also considered to have normal activity.

Other alleles include no function variants that produce a non-functioning enzyme (e.g., **3*, **4*, **5*, **6*, **7*, **8*, and **12*) or an enzyme with decreased activity (e.g., **10*, **17*, *29, and **41*) (see Table 1) (9). There are large inter-ethnic differences in the frequency of these alleles, with **3*, **4*, **5*, **6*, and **41* being more common in the Caucasian population, **17* more common in Africans, and **10* more common in Asians (10).

Table 1: 2016 Assignment of *CYP2D6* phenotypes by CPIC

Phenotype	Activity Score	Genotypes	Examples of diplotypes
CYP2D6 ultrarapid metabolizer (approximately 1–20% of patients)[a]	Greater than 2.0	An individual carrying duplications of functional alleles	*(*1/*1)xN* *(*1/*2)xN* *(*2/*2)xN*[b]
CYP2D6 normal metabolizer (approximately 72–88% of patients)	1.0 – 2.0[c]	An individual carrying two normal function alleles or two decreased function alleles or one normal and no function allele or one normal function and decreased function allele or combinations of duplicated alleles that result in an activity score of 1.0 to 2.0	**1/*1* **1/*2* **2/*2* **1/*9* **1/*41* **41/*41* **1/*5* **1/*4*
CYP2D6 intermediate metabolizer (approximately 1–13% of patients)	0.5	An individual carrying one decreased function and one no function allele	**4/*41* **5/*9* **4/*10*

Table 1 continued from previous page.

Phenotype	Activity Score	Genotypes	Examples of diplotypes
CYP2D6 poor metabolizer (approximately 1–10% of patients)	0	An individual carrying two no function alleles	**4/*4* **4/*4xN* **3/*4* **5/*5* **5/*6*

a For population-specific allele and phenotype frequencies, please see (2).
b Where xN represents the number of *CYP2D6* gene copies (N is 2 or more).
c Patients with an activity core of 1.0 may be classified as intermediate metabolizers by some reference laboratories.
For more information about activity scores, please see the Genetic Testing section.
This table has been adapted from Hicks J.K., Sangkuhl K., Swen J.J., Ellingrod V.L., Müller D.J., Shimoda K., Bishop J.R., Kharasch E.D., Skaar T.C., Gaedigk A., Dunnenberger H.M., Klein T.E., Caudle K.E. Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC®) for *CYP2D6* and *CYP2C19* Genotypes and Dosing of Tricyclic Antidepressants: 2016 Update. Clinical pharmacology and therapeutics. 2016 Dec 20 [Epub ahead of print] (2).

Individuals who are intermediate or poor metabolizers carry copies of reduced-activity or no function *CYP2D6* alleles, respectively (Table 1). Approximately 30% of Asians and individuals of Asian descent are intermediate metabolizers. In these populations, only half of *CYP2D6* alleles are fully functional, with the reduced function **10* variant being very common (~40%, compared to ~2% in Caucasians) (11). As a result, Asians are more likely to be intermediate metabolizers than Caucasians (12). Similarly, in Africans and African Americans, only half of *CYPD6* alleles are functional; however, a wider range of variants account for the remaining alleles (12-14).

Approximately 6-10% of European Caucasians and their descendants are poor metabolizers, mainly due to no function **4* and **5* alleles (12). Notably, less than 40% are homozygous normal metabolizers (carrying two copies of **1* allele) (15-17).

Individuals who are CYP2D6 poor metabolizers require a lower dose of imipramine to be in therapeutic range than CYP2D6 normal metabolizers (18). When treated with standard doses of imipramine, individuals who are CYP2D6 poor metabolizers will also have higher plasma concentrations of imipramine and desipramine compared to CYP2D6 normal metabolizers (19).

Because adverse effects are more likely due to elevated tricyclic plasma concentrations, CPIC recommends alternative agents for individuals who are CYP2D6 poor metabolizers. If a tricyclic is warranted, CPIC recommends considering a 50% reduction of the usual starting dose, and they strongly recommend therapeutic drug monitoring (4).

Individuals who have more than two copies of normal function *CYP2D6* alleles are *CYP2D6* ultrarapid metabolizers. These individuals require higher doses of imipramine to be within therapeutic range compared to normal metabolizers (18). However, increasing the dose of imipramine can lead to high plasma concentrations of desipramine, which may increase the risk for cardiotoxicity. Therefore, CPIC recommends that an alternative agent be used for CYP2D6 ultrarapid metabolizers. However, if a tricyclic is warranted, there is insufficient evidence to calculate a starting dose, and so therapeutic drug monitoring is strongly recommended (4) (Table 2).

Table 2. 2016 CPIC Dosing recommendations for tricyclic antidepressants based on *CYP2D6* phenotype

Phenotype	Implication	Therapeutic recommendation
CYP2D6 ultrarapid metabolizer	Increased metabolism of TCAs to less active compounds compared to normal metabolizers	Avoid tricyclic use due to potential lack of efficacy. Consider alternative drug not metabolized by CYP2D6
	Lower plasma concentrations of active drugs will increase probability of pharmacotherapy failure	If a TCA is warranted, consider titrating to a higher target dose (compared to normal metabolizers)[a]. Utilize therapeutic drug monitoring to guide dose adjustments.
CYP2D6 normal metabolizer	Normal metabolism of TCAs	Initiate therapy with recommended starting dose[b].
CYP2D6 intermediate metabolizer	Reduced metabolism of TCAs to less active compounds compared to normal metabolizers	Consider a 25% reduction of recommended starting dose[b]. Utilize therapeutic drug monitoring to guide dose adjustments[a].
	Higher plasma concentrations of active drug will increase the probability of side effects	
CYP2D6 poor metabolizer	Greatly reduced metabolism of TCAs to less active compounds compared to normal metabolizers	Avoid tricyclic use due to potential for side effects. Consider alternative drug not metabolized by CYP2D6
	Higher plasma concentrations will increase the probability of side effects	If a TCA is warranted, consider a 50% reduction of recommended starting dose[b]. Utilize therapeutic drug monitoring to guide dose adjustments[a].

TCAs: Tricyclic Antidepressants
Dosing recommendations only apply to higher initial doses of TCAs for treatment of conditions such as depression.
The therapeutic recommendations for amitriptyline and nortriptyline are classified as "moderate" for intermediate CYP2D6 metabolizers, and "strong" for ultrarapid, normal, and poor CYP2D6 metabolizers. CPIC state that it may be reasonable to apply these recommendations to other TCAs also metabolized by CYP2D6, including clomipramine, desipramine, doxepin, imipramine, and trimipramine.
[a] Titrate dose to observed clinical response with symptom improvement and minimal (if any) side effects.
[b] Patients may receive an initial low dose of tricyclic, which is then increased over several days to the recommended steady-state dose. The starting dose in this guideline refers to the recommended steady-state dose.
Table has been adapted from Hicks J.K., Sangkuhl K., Swen J.J., Ellingrod V.L., Müller D.J., Shimoda K., Bishop J.R., Kharasch E.D., Skaar T.C., Gaedigk A., Dunnenberger H.M., Klein T.E., Caudle K.E. Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC®) for *CYP2D6* and *CYP2C19* Genotypes and Dosing of Tricyclic Antidepressants: 2016 Update. Clinical pharmacology and therapeutics. 2016 Dec 20 [Epub ahead of print] (2).

Gene: CYP2C19

The CYP2C19 enzyme contributes to the metabolism of a range of clinically important drugs, such as several proton pump inhibitors, clopidogrel, benzodiazepines, and several tricyclic antidepressants, including imipramine.

The *CYP2C19* gene is highly polymorphic as 35 variant star (*) alleles are currently catalogued at the Human Cytochrome P450 (CYP) Allele Nomenclature Database: (http://www.cypalleles.ki.se/cyp2c19.htm).

The *CYP2C19*1* wild-type allele is associated with normal enzyme activity and the "normal metabolizer" phenotype, whereas the *CYP2C19*17* allele is associated with increased enzyme activity and the "rapid" and "ultrarapid" metabolizer phenotypes (20).

The most common no function variant is *CYP2C19*2*, which is characterized by c.681G>A in exon 5 that results in an aberrant splice site and the production of a truncated and non-functioning protein. The *CYP2C19*2* allele frequencies are ~15% in Caucasians and Africans, and ~29–35% in Asians (20, 21).

Another commonly tested no function variant is *CYP2C19*3*, which is characterized by c.636G>A in exon 4 that causes a premature stop codon. The *CYP2C19*3* allele frequencies are ~2–9% in Asian populations, but rare in other racial groups. Other no function variants occur in less than 1% of the general population, and include *CYP2C19*4-*8* (20, 21).

"CYP2C19 intermediate metabolizers" carry one copy of a no function allele (e.g. **1/*2*), whereas "poor metabolizers" are homozygous or compound heterozygous for two no function alleles (e.g., **2/*2*, **2/*3*) (Table 3).

Table 3: 2016 Assignment of *CYP2C19* phenotypes by CPIC

Phenotype	Genotypes	Examples of diplotypes
CYP2C19 ultrarapid metabolizer (approximately 2–35% of patients)[a]	An individual carrying two increased function alleles	**17/*17*
CYP2C19 rapid metabolizer (approximately 2–30% of patients)	An individual carrying one normal function allele and one increased function allele	**1/*17*
CYP2C19 normal metabolizer (approximately 35–50% of patients)	An individual carrying two normal function alleles	**1/*1*
CYP2C19 intermediate metabolizer (approximately 18–45% of patients)	An individual carrying one normal function and one no function allele or one no function allele and one increased function allele	**1/*2* **1/*3* **2/*17*[b]
CYP2C19 poor metabolizer (approximately 2–15% of patients)	An individual carrying two no function alleles	**2/*2* **2/*3* **3/*3*

[a] For population-specific allele and phenotype frequencies, please see (2).
[b] The predicted metabolizer phenotype for the *2/*17 genotype is a provisional classification.
Table has been adapted from Hicks J.K., Sangkuhl K., Swen J.J., Ellingrod V.L., Müller D.J., Shimoda K., Bishop J.R., Kharasch E.D., Skaar T.C., Gaedigk A., Dunnenberger H.M., Klein T.E., Caudle K.E. Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC®) for *CYP2D6* and *CYP2C19* Genotypes and Dosing of Tricyclic Antidepressants: 2016 Update. Clinical pharmacology and therapeutics. 2016 Dec 20 [Epub ahead of print] (2).

Studies have found that individuals who are CYP2C19 poor metabolizers have a lower plasma clearance of imipramine compared to normal metabolizers. When given standard doses of imipramine, CYP2C19 poor metabolizers have greater concentrations of imipramine and its active metabolite desipramine (22-24). Increased drug levels could potentially lead to an increased risk of adverse events. CPIC recommends considering a 50% reduction in the starting dose of tricyclics for CYP2C19 poor metabolizers (4)

Individuals who are CYP2C19 ultrarapid metabolizers may require an increased dose of tricyclics (25).

One study found that the imipramine plasma concentration was significantly lower in ultrarapid metabolizers (i.e., *CYP2C19*17/*17*) when compared to normal metabolizers (i.e., *CYP2C19*1/*1*) patients. However, the imipramine + desipramine plasma concentrations were not significantly different between *CYP2C19* genotypes (26). Because of the possibility of altered tricyclic plasma concentrations, CPIC recommends an alternative tricyclic or other drug for ultrarapid metabolizers (4) (Table 4).

Table 4. 2016 CPIC Dosing recommendations for tertiary amines based on *CYP2C19* phenotype

Phenotype	Implication	Therapeutic recommendation
CYP2C19 ultrarapid metabolizer and CYP2C19 rapid metabolizer	Increased metabolism of tertiary amines as compared to normal metabolizers Greater conversion of tertiary amines to secondary amines may affect response or side effects	Avoid tertiary amine use due to potential for sub-optimal response. Consider alternative drug not metabolized by CYP2C19. TCAs without major CYP2C19 metabolism include the secondary amines nortriptyline and desipramine.
		If a tertiary amine is warranted, utilize therapeutic drug monitoring to guide dose adjustments[a].
CYP2C19 normal metabolizer	Normal metabolism of tertiary amines	Initiate therapy with recommended starting dose[b].
CYP2C19 intermediate metabolizer	Reduced metabolism of tertiary amines compared to normal metabolizers	Initiate therapy with recommended starting dose[b].
CYP2C19 poor metabolizer	Greatly reduced metabolism of tertiary amines compared to normal metabolizers	Avoid tertiary amine use due to potential for sub-optimal response. Consider alternative drug not metabolized by CYP2C19. TCAs without major CYP2C19 metabolism include the secondary amines nortriptyline and desipramine. For tertiary amines, consider a 50% reduction of recommended starting dose[b]. Utilize therapeutic drug monitoring to guide dose adjustments[a].
	Decreased conversion of tertiary amines to secondary amines may affect response or side effects	

Dosing recommendations apply only to higher initial doses of amitriptyline for treatment of conditions such as depression. The therapeutic recommendations for amitriptyline are classified as "strong" for normal and intermediate CYP2C19 metabolizers, "moderate" for poor metabolizers, and "optional" for ultrarapid metabolizers. CPIC state that it may be reasonable to apply these recommendations to other TCAs also metabolized by CYP2C19, including clomipramine, doxepin, imipramine, and trimipramine.
[a] Titrate dose to observed clinical response with symptom improvement and minimal (if any) side effects).
[b] Patients may receive an initial low dose of tricyclic, which is then increased over several days to the recommended steady-state dose. The starting dose in this guideline refers to the recommended steady-state dose.
Table has been adapted from Hicks J.K., Sangkuhl K., Swen J.J., Ellingrod V.L., Müller D.J., Shimoda K., Bishop J.R., Kharasch E.D., Skaar T.C., Gaedigk A., Dunnenberger H.M., Klein T.E., Caudle K.E. Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC®) for *CYP2D6* and *CYP2C19* Genotypes and Dosing of Tricyclic Antidepressants: 2016 Update. Clinical pharmacology and therapeutics. 2016 Dec 20 [Epub ahead of print] (2).

Genetic Testing

Clinical genotyping tests are available for many *CYP2D6* and *CYP2C19* alleles. The NIH's Genetic Testing Registry (GTR) provides a list of test providers for "imipramine response," and the CYP2D6 and CYP2C19 genes.

Results are typically reported as a diplotype, such as *CYP2D6 *1/*1*. A result for copy number, if available, is also important when interpreting *CYP2D6* results (27). However, it is important to note that the number of variants tested can vary among laboratories, which can result in diplotype result discrepancies between testing platforms and laboratories (28).

If the test results include an interpretation of the patient's predicted metabolizer phenotype, this should be confirmed by checking the diplotype and assigning an activity score to each allele (e.g., 0 for nonfunctional, 0.5 for reduced function, and 1 for each copy of a functional allele). The phenotype is defined by the sum of the two scores:

- A normal (previously referred to as "extensive") metabolizer phenotype has an activity score of 1 to 2
- An intermediate metabolizer has an activity score of 0.5
- A poor metabolizer has an activity score of 0
- An ultrarapid metabolizer has an activity score greater than 2 (2, 29)

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2016 Statement from the US Food and Drug Administration (FDA): The biochemical activity of the drug metabolizing isozyme cytochrome P450 2D6 (debrisoquin hydroxylase) is reduced in a subset of the Caucasian population (about 7% to 10% of Caucasians are so-called "poor metabolizers"); reliable estimates of the prevalence of reduced P450 2D6 isozyme activity among Asian, African, and other populations are not yet available. Poor metabolizers have higher than expected plasma concentrations of tricyclic antidepressants (TCAs) when given usual doses. Depending on the fraction of drug metabolized by P450 2D6, the increase in plasma concentration may be small, or quite large (8-fold increase in plasma AUC of the TCA).

In addition, certain drugs inhibit the activity of this isozyme and make normal metabolizers resemble poor metabolizers. An individual who is stable on a given dose of TCA may become abruptly toxic when given one of these inhibiting drugs as concomitant therapy. The drugs that inhibit cytochrome P450 2D6 include some that are not metabolized by the enzyme (quinidine; cimetidine) and many that are substrates for P450 2D6 (many other antidepressants, phenothiazines, and the Type 1C antiarrhythmics propafenone and flecainide). While all the selective serotonin reuptake inhibitors (SSRIs), e.g., fluoxetine, sertraline, and paroxetine, inhibit P450 2D6, they may vary in the extent of inhibition. The extent to which SSRI-TCA interaction may pose clinical problems will depend on the degree of inhibition and the pharmacokinetics of the SSRI involved. Nevertheless, caution is indicated in the co-administration of TCAs with any of the SSRIs and also in switching from one class to the other. Of particular importance, sufficient time must elapse before initiating TCA treatment in a patient being withdrawn from fluoxetine, given the long half-life of the parent and active metabolite (at least 5 weeks may be necessary).

Concomitant use of tricyclic antidepressants with drugs that can inhibit cytochrome P450 2D6 may require lower doses than usually prescribed for either the tricyclic antidepressant or the other drug. Furthermore, whenever one of these other drugs is withdrawn from co-therapy, an increased dose of tricyclic antidepressant may be required. It is desirable to monitor TCA plasma levels whenever a TCA is going to be co-administered with another drug known to be an inhibitor of P450 2D6.

Please review the complete therapeutic recommendations that are located here: (1).

2016 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC):

Because the TCAs have comparable pharmacokinetic properties, it may be reasonable to extrapolate this guideline to other TCAs including clomipramine, desipramine, doxepin, imipramine, and trimipramine, with the acknowledgement that there are fewer data supporting dose adjustments for these drugs than for amitriptyline or nortriptyline. […]

CYP2D6 dosing recommendations.

[…]. The recommended starting dose of amitriptyline or nortriptyline does not need adjustment for those with genotypes predictive of CYP2D6 normal metabolism. A 25% reduction of the recommended dose may be considered for CYP2D6 intermediate metabolizers. The strength of this recommendation is classified as "moderate" because patients with a CYP2D6 activity score of 1.0 are inconsistently categorized as intermediate or normal metabolizers in the literature, making these studies difficult to evaluate.

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

CYP2D6 ultrarapid metabolizers have a higher probability of failing amitriptyline or nortriptyline pharmacotherapy due to subtherapeutic plasma concentrations, and alternate agents are preferred. There are documented cases of CYP2D6 ultrarapid metabolizers receiving large doses of nortriptyline in order to achieve therapeutic concentrations. However, very high plasma concentrations of the nortriptyline hydroxy-metabolite were present, which may increase the risk for cardiotoxicity. If a tricyclic is warranted, there are insufficient data in the literature to calculate a starting dose for a patient with CYP2D6 ultrarapid metabolizer status, and therapeutic drug monitoring is strongly recommended. Adverse effects are more likely in CYP2D6 poor metabolizers due to elevated tricyclic plasma concentrations; therefore, alternate agents are preferred. If a tricyclic is warranted, consider a 50% reduction of the usual dose, and therapeutic drug monitoring is strongly recommended.

CYP2C19 dosing recommendations.

[…]. The usual starting dose of amitriptyline may be used in CYP2C19 normal and intermediate metabolizers. Although CYP2C19 intermediate metabolizers would be expected to have a modest increase in the ratio of amitriptyline to nortriptyline plasma concentrations, the evidence does not indicate that CYP2C19 intermediate metabolizers should receive an alternate dose.

Patients taking amitriptyline who are CYP2C19 rapid or ultrarapid metabolizers may be at risk for having low plasma concentrations and an imbalance between parent drug and metabolites causing treatment failure and/or adverse events. Although the CYP2C19*17 allele did not alter the sum of amitriptyline plus nortriptyline plasma concentrations, it was associated with higher nortriptyline plasma concentrations, possibly increasing the risk of adverse events. For patients taking amitriptyline, extrapolated pharmacokinetic data suggest that CYP2C19 rapid or ultrarapid metabolizers may need a dose increase. Due to the need for further studies investigating the clinical importance of CYP2C19*17 regarding tricyclic metabolism and the possibility of altered concentrations, we recommend to consider an alternative tricyclic or other drug not affected by CYP2C19. This recommendation is classified as optional due to limited available data. If amitriptyline is administered to a CYP2C19 rapid or ultrarapid metabolizer, therapeutic drug monitoring is recommended.

CYP2C19 poor metabolizers are expected to have a greater ratio of amitriptyline to nortriptyline plasma concentrations. The elevated amitriptyline plasma concentrations may increase the chance of a patient experiencing side effects. Use an alternative agent not metabolized by CYP2C19 (e.g., nortriptyline and desipramine) or consider a 50% reduction of the usual amitriptyline starting dose along with therapeutic drug monitoring.

Please review the complete therapeutic recommendations that are located here: (2).

2011 Summary of recommendations from the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP):

For CYP2D6 poor metabolizers, defined as patients carrying two inactive alleles, reduce the dose of imipramine by 70% and monitor imipramine and desipramine plasma concentrations.

For CYP2D6 intermediate metabolizers, defined as patients carrying two decreased-activity alleles or one active/decreased-activity allele and one inactive allele, reduce the dose of imipramine by 30% and monitor imipramine and desipramine plasma concentrations.

For CYP2D6 ultrarapid metabolizers, defined as patients carrying a gene duplication in the absence of inactive or decreased-activity alleles, select an alternative drug (e.g., citalopram, sertraline) or increase dose by 70% and monitor imipramine and desipramine plasma concentration (Table 5).

For CYP2C19 poor metabolizers, reduce the dose of imipramine by 30% and monitor plasma concentration of imipramine and desipramine or select an alternative drug (e.g., fluvoxamine, mirtazapine).

For CYP2C19 intermediate metabolizers, there is insufficient data to allow calculation of dose adjustment for imipramine, select an alternative drug (e.g., fluvoxamine, mirtazapine)

There are no data for dose recommendations for CYP2C19 ultrarapid metabolizers (Table 6).

Table 5. *CYP2D6* phenotypes and the therapeutic recommendations for imipramine therapy, from The Dutch Pharmacogenetics Working Group (2011)

Phenotype	Recommendations for imipramine therapy
Ultrarapid metabolizer	Select alternative drug (e.g., citalopram, sertraline) or increase dose by 70% and monitor imipramine and desipramine plasma concentration
Intermediate metabolizer	Reduce dose by 30% and monitor imipramine and desipramine plasma concentrations
Poor metabolizer	Reduce dose by 70% and monitor imipramine and desipramine plasma concentrations

The level of evidence for the therapeutic (dose) recommendations is 4/4 ("good quality") for all metabolizer types. There are no data for ultrarapid metabolizers. The Table is adapted from Swen J.J., Nijenhuis M., de Boer A., Grandia L. et al. Pharmacogenetics: from bench to byte - an update of guidelines. Clinical pharmacology and therapeutics. 2011;89(5):662–73 (30).

Table 6. *CYP2C19* phenotypes and the therapeutic recommendations for imipramine therapy, from The Dutch Pharmacogenetics Working Group (2011)

Phenotype	Recommendations for imipramine therapy
Ultrarapid metabolizer	No dose recommendations
Intermediate metabolizer	No dose recommendations
Poor metabolizer	Reduce dose by 70% and monitor plasma concentration of imipramine and desipramine or select alternative drug (e.g., fluvoxamine, mirtazapine)

The level of evidence for the therapeutic (dose) recommendations is 4/4 ("good quality") for all metabolizer types. The table is adapted from (31)

Please review the complete therapeutic recommendations that are located here: (30, 31).

Nomenclature

Nomenclature for selected *CYP2D6* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*4*	1846G>A	NM_000106.5:c.506-1G>A	Not applicable - variant occurs in a non-coding region	rs3892097
*CYP2D6*5*	Not applicable - variant results in a whole gene deletion			
*CYP2D6*6*	1707 del T Trp152Gly	NM_000106.5:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655
*CYP2D6*10*	100C>T Pro34Ser	NM_000106.5:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
*CYP2D6*17*	Includes at least two functional variants*: 1023C>T (Thr107Ile) 2850C>T (Cys296Arg)	NM_000106.5:c.320C>T NM_000106.5:c.886T>C	NP_000097.3:p.Thr107Ile NP_000097.3:p.Cys296Arg	rs28371706 rs16947
*CYP2D6*41*	2988G>A	NM_000106.5:c.985+39G>A	Not applicable – variant occurs in a non-coding region	rs28371725

* In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.

Nomenclature for selected *CYP2C19* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C19*2*	681G>A Pro227Pro	NM_000769.1:c.681G>A	NP_000760.1:p.Pro227=	rs4244285
*CYP2C19*3*	636G>A Trp212Ter	NM_000769.1:c.636G>A	NP_000760.1:p.Trp212Ter	rs4986893
*CYP2C19*17*	-806C>T	NM_000769.2:c.-806C>T	Not applicable—variant occurs in a non-coding region	rs12248560

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: http://www.cypalleles.ki.se/

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Irinotecan Therapy and *UGT1A1* Genotype

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Introduction

Irinotecan (brand name Camptosar) is a topoisomerase I inhibitor widely used in the treatment of cancer. It is most frequently used in combination with other drugs to treat advanced or metastatic colorectal cancer. However, irinotecan therapy is associated with a high incidence of toxicity, including severe neutropenia and diarrhea (1).

Irinotecan is converted in the body to an active metabolite known as SN-38, which is then inactivated and detoxified by a UDP-glucuronosyltransferase (UGT) enzyme encoded by the *UGT1A1* gene. The UGT enzymes are responsible for glucuronidation, a process that transforms lipophilic metabolites into water-soluble metabolites that can be excreted from the body.

The risk of irinotecan toxicity increases with genetic variants associated with reduced UGT enzyme activity, such as *UGT1A1*28*. The presence of this variant results in reduced excretion of irinotecan metabolites, which leads to increased active irinotecan metabolites in the blood. Approximately 10% of North Americans carry 2 copies of the *UGT1A1*28* allele (homozygous, *UGT1A1 *28/*28*), and are more likely to develop neutropenia following irinotecan therapy (1-3).

The FDA-approved drug label for irinotecan states that "when administered as a single-agent, a reduction in the starting dose by at least one level of irinotecan hydrochloride injection should be considered for patients known to be homozygous for the *UGT1A1*28* allele. However, the precise dose reduction in this patient population is not known and subsequent dose modifications should be considered based on individual patient tolerance to treatment" (Table 1) (1).

The Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP) recommends starting with 70% of the standard dose for homozygous carriers of the *UGT1A1*28* allele. If the patient tolerates this initial dose, the dose can be increased guided by the neutrophil count. They state that no action is needed for heterozygous carriers of the *UGT1A1*28* allele (e.g., *UGT1A1 *1/*28*) (Table 2) (4). In addition, the French National Network of Pharmacogenetics (RNPGx) has proposed a decision tree for guiding irinotecan prescribing based on the *UGT1A1* genotype and irinotecan dose (Table 3) (5).

Table 1. FDA (2017) Drug Label for Irinotecan. Therapeutic Recommendations based on *UGT1A1* Genotype. Dosage and Administration.

Genotype	Recommendations
*UGT1A1 *28/*28*	When administered as a single-agent, a reduction in the starting dose by at least one level of irinotecan hydrochloride injection, USP[1] should be considered for patients known to be homozygous for the *UGT1A1*28* allele. However, the precise dose reduction in this patient population is not known and subsequent dose modifications should be considered based on individual patient tolerance to treatment.

Please see Therapeutic Recommendations based on Genotype for more information from the FDA. Table adapted from (1).

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[1] USP stands for the United States Pharmacopeia. The USP establishes standards that promote safe medication use (e.g., procurement, prescribing, transcribing, order entry, preparation, dispensing, administration, and monitoring of medications).

Table 2. DPWG (2014) Recommendations for Irinotecan and *UGT1A1* Genotype.

Phenotype / genotype	Recommendations
UGT1A1 intermediate metabolizer (IM)	NO action is needed for this gene-drug interaction.
UGT1A1 poor metabolizer (PM)	Start with 70% of the standard dose If the patient tolerates this initial dose, the dose can be increased, guided by the neutrophil count.
*UGT1A1 *1/*28*	NO action is needed for this gene-drug interaction.
*UGT1A1 *28/*28*	Start with 70% of the standard dose If the patient tolerates this initial dose, the dose can be increased, guided by the neutrophil count.

Please see Therapeutic Recommendations based on Genotype for more information from the DPWG. Table is adapted from (4).

Table 3. RNPGx (2017) Recommendations for Irinotecan and *UGT1A1* Genotype.

Dose of irinotecan	Recommendation
low doses (<180 mg/m^2/week)	Presence of the *UGT1A1*28* allele is not a major risk factor (little difference in risk of hematological or digestive toxicity irrespective of the genotype)
180—230 mg/m^2 spaced by 2—3-week intervals	Patients who are homozygous for the *UGT1A1*28* allele have a higher risk of hematological and/or digestive toxicity than patients who are heterozygous or non-carriers. For these *28/*28* patients, a 25-30% dose reduction is recommended, especially if the patient presents other risk factors for toxicity. Dose can be adjusted for subsequent cycles depending on the tolerance.
240 mg/m^2 or higher spaced by 2—3-week intervals	Homozygous *UGT1A1*28* patients have a greatly increased risk of hematological toxicity (neutropenia) compared with other genotypes, contraindicating administration at this higher dose and leading to discussion of a standard dose depending on the associated risk factors. Administration of an intensive dose (240 mg/m^2) is recommended only for *1/*1* patients, or for *1/*28* patients who have no other risk factors and who benefit from intensive surveillance.

Please see Therapeutic Recommendations based on Genotype for more information from the RNPGx. Table is adapted from (5).

Drug: Irinotecan

Irinotecan is used to treat colorectal cancer, which is the third most common cancer worldwide (6). It is often used in combination with other drugs to treat patients with advanced or metastatic colorectal cancer, when the cancer has recurred, or has progressed following initial treatment. A common irinotecan-based combination therapy is referred to as FOLFIRI (FOLinic acid [also known as leucovorin], Fluorouracil, IRInotecan).

Irinotecan is a semisynthetic derivative of the antineoplastic agent camptothecin, which derives its name from the Camptotheca tree where it was first isolated. Like camptothecin, irinotecan is an inhibitor of the nuclear enzyme, topoisomerase I. This enzyme catalyzes a number of nuclear processes; regulation of DNA supercoiling, replication, recombination, and repair.

Topoisomerase I decreases the torsional strain in the helical strands of DNA by making single strand breaks in the DNA. Single strands of DNA pass through the breaks and bind to the topoisomerase to form a cleavable complex. Once the DNA is sufficiently relaxed and the passage of strands has been completed, topoisomerase re-ligates the broken DNA strands and allows for transcription to proceed (7, 8).

Irinotecan is a pro-drug, and is converted by carboxylesterase enzymes to the active metabolite SN-38, which is 100–1000 times more potent that its parent drug, after administration by intravenous injection (9). The SN-38 metabolite exerts its cytotoxic effects by binding to the cleavable complex to form a ternary drug-topoisomerase-DNA complex. This complex is thought to prevent the re-ligation of the single strand breaks, which interrupts the moving DNA replication fork. The arrest of replication and the interaction between replication enzymes and

the ternary complex introduces lethal double-stranded breaks in DNA causing irreparable DNA damage and subsequent cell apoptosis. (10, 11).

SN-38 is lipophilic, and it needs to be inactivated by undergoing phase II metabolism (glucuronidation). The resulting conjugated SN-38 glucuronide is water-soluble, and is mainly excreted through the bile, with about 30% excreted by the kidneys (12).

Irinotecan-based combination therapy has been found to be superior in overall response and survival when compared with the use of 5-fluorouracil/leucovorin therapy alone for patients with metastatic colorectal cancer (3). However, the use of irinotecan is limited by a high incidence of unpredictable and severe dose-limiting toxicity, including severe neutropenia, fever, and diarrhea (13). The rate of grade 3 or 4 adverse events is around 20-25% (14). Approximately 7% of patients who present with severe neutropenia and fever following treatment with irinotecan will die from these complications (3, 15-18).

Gene: *UGT1A1*

The UGT enzymes (uridine diphosphate-glucuronosyltransferase, or UDP-glucuronosyltransferase) are a superfamily of enzymes that metabolize a wide range of lipophilic molecules such as bilirubin, steroids, toxins, and drugs—including irinotecan's active metabolite, SN-38. These enzymes mediate the process of glucuronidation, which is a phase II metabolic pathway during which glucuronic acid is conjugated to specific targets to convert them to water-soluble metabolites that can then be eliminated from the body.

The UGT genes are polymorphic, and genomic processes, such as copy-number variations, variant splicing, and epigenetic factors, likely contribute to their diversity. As a result, the substrates that the UGT enzymes catalyze are particularly variable (19).

The UGT superfamily contains at least 117 enzymes divided into 4 families, of which UGT1A is a member (20). The *UGT1A* gene locus, located on chromosome 2q37, is complex—it encodes multiple genes and pseudogenes, and alternatively spliced isoforms also exist (21).

The *UGT1A* locus contains multiple alternative first coding exons, each of which has its own promoter site, enabling the transcription of 9 unique UGT1A enzymes (22). One of these transcripts is *UGT1A1*, which encodes UGT1A1, the bilirubin-UGT enzyme. Whereas many UGT enzymes overlap in the substrates they glucuronidate, UGT1A1 is the only enzyme that glucuronidates bilirubin (23).

Bilirubin is a yellow waste product produced during the catabolism of heme, a constituent of hemoglobin. When old or damaged red blood cells are broken down in the spleen, their hemoglobin is broken down to heme, which is then converted into bilirubin. The UGT1A1 enzyme converts this toxic, insoluble form of bilirubin (unconjugated bilirubin) to its nontoxic form (conjugated bilirubin). Because conjugated bilirubin is water-soluble, it can be dissolved in bile and eliminated with solid waste. If bilirubin is not eliminated and instead accumulates to high levels (hyperbilirubinemia), it can cause a yellowish discoloration of the skin and eyes, a condition known as jaundice.

Variants of the *UGT1A1* gene that decrease UGT1A1 enzyme activity can lead to jaundice. The data suggests that one copy of *28 allele results in about a 35% decrease in transcriptional activity, and 2 copies (**28/*28*, homozygous) results in about a 70% decrease (24, 25).

The jaundice may be mild, as seen in Gilbert syndrome, or severe, as seen in Crigler-Najjar syndrome. Crigler-Najjar syndrome presents in 2 forms called type 1 and type 2. Type 1 is the extremely severe form where affected individuals can die in childhood due to kernicterus (bilirubin-induced brain injury), although they may survive for longer with treatment. Type 2 is less severe; the affected individuals are less likely to develop kernicterus and most survive into adulthood.

Currently, over 135 genetic variants of *UGT1A1* have been reported (23, 26). *UGT1A1*1* is the wild-type allele associated with normal enzyme activity. The most common variant allele is *UGT1A1*28*, which is commonly found in African-Americans (0.42–0.45 allele frequency) and Caucasians (0.26–0.31), and is less common in Asian populations (0.09–0.16) (27, 28). Within Caucasian and African American populations, the *UGT1A1*28* variant is a common cause of Gilbert syndrome and is also a cause of Crigler-Najjar syndrome types 1 and 2 (19, 27).

The *UGT1A1*28* [$(TA)_7TAA$] variant contains an extra thymine-adenine (TA) repeat within the TATA box promoter region (7 TA repeats compared with 6 in the wild-type allele) (29). This extra (TA) repeat decreases the rate of transcription initiation of the *UGT1A1* gene, leading to decreased enzyme activity and decreased glucuronidation of bilirubin to about 30% of wild-type levels (30).

Another variant allele, UGT1A1*37 [$(TA)_8TAA$], has 8 TA repeats at this site, and results in reduced promoter activity of the gene to levels lower than the UGT1A1*28 allele. In contrast, the UGT1A1*36 [$(TA)_5TAA$] allele only has 5 repeats and is associated with increased promoter activity and a reduced risk of neonatal hyperbilirubinemia (a common, and typically benign condition). Both UGT1A1*36 and UGT1A1*37 occur almost exclusively in populations of African origin, with estimated allele frequencies of 0.03–0.10 and 0.02–0.07, respectively.

The *UGT1A1*28* variant is also associated with drug toxicity. Approximately 10% of the North American population is homozygous for the **28* allele (**28/*28* genotype, also known as *UGT1A1 7/7* genotype) and are at an increased risk of neutropenia following intravenous irinotecan therapy (28). The rate of severe neutropenia in **28/*28* homozygous patients is as high as 36%, and is strongly associated with a higher hospitalization rate (7, 31, 32).

There is less evidence to support a link between *UGT1A1* genotype and irinotecan treatment-related diarrhea, and there is conflicting data on whether an individual's *UGT1A1* genotype influences their response to irinotecan therapy (8, 33).

Another variant allele, *UGT1A1*6*, is more prevalent in Asian populations, with an allele frequency of around 15–30% in Chinese, Korean, and Japanese populations (24, 34, 35). In this variant, there is a switch of amino acids, from a glycine to an arginine at position 71 within a coding region (p.Arg71Gly). Individuals who are homozygous for this allele have reduced UGT1A1 enzyme activity, which can cause Gilbert syndrome and prolonged neonatal jaundice (36-39). This variant also appears to be an important predictor of severe toxicity to irinotecan therapy in Asian populations (35, 40-47).

In addition to genetic variations in the *UGT1A1* gene, several other genetic markers may influence the risk of irinotecan toxicity. These include genetic variation in the adenosine triphosphate (ATP)-binding cassette (ABC) transporter genes, *ABCC1* and *ABCB2* (43, 48, 49), the solute carrier (*SLC*) transporter genes (48, 50, 51), the transforming growth factor (*TGFB*) gene (52), and the xenobiotic-sensing receptor, *NR1I2* (53).

The emerging data suggests that other variant alleles may have a protective effect. The newly discovered marker rs11563250 (NM_001287395.1:c.-1068A>G), located in the 3′-flanking region of *UGT1A1*, has a major *A* allele (rs11563250A) and a relatively common variant *G* allele (rs11563250G, found in 12% of the population). Carriers of the *G* allele have a lower risk of irinotecan-induced neutropenia. They also tend to have lower total plasma bilirubin levels, suggesting that this variant is associated with an enhanced capacity for glucuronidation. Evidence suggests that carriers of rs11563250G could tolerate a higher dose of irinotecan, especially if they also have the *UGT1A1*1/*1* genotype (54).

Genetic Testing

The NIH's Genetic Testing Registry provides examples of the genetic tests currently available for irinotecan response and for the *UGT1A1* gene.

Genetic testing can be used to optimize irinotecan dosing. For example, the use of genotyping in selective cases may make the following patient choices possible:

- If the patient prefers aggressive treatment: genotyping might allow higher dosing for **1/*1* and **1/*28* genotypes (55-59).
- If the patient prefers maximizing quality of life: genotyping might allow lower dosing for **28/*28* genotype (7, 31, 32).

Genotyping may also enable irinotecan to be added to the treatment of other gastrointestinal tumors without the risk of hematologic toxicity (60). Genotyping may also be used as part of the management of Gilbert syndrome (15).

In the USA, the common **1* and **28 UGT1A1* alleles comprise 98–99% of genotypes (61). Routine genotyping typically tests for *UGT1A1 *1/*1, *1/*28*, and **28/*28 genotypes (*also known as *6/6*, *6/7*, and *7/7*, respectively).

Routine screening does not rule out other *UGT1A1* polymorphisms that are more common in specific populations (7). For example, the *UGT1A1*6* allele is common in Asian populations, and in Japan, a reduced dose of irinotecan is recommended for individuals with *UGT1A1 *6/*6*, **6/*28*, and **28/*28* genotypes (62). In addition, routine screening does not identify patients who are being under-dosed and could potentially tolerate a much higher dose of irinotecan.

The adoption of preemptive *UGT1A1*28* genotyping to increase irinotecan safety and efficacy in clinical practice is still limited and often not covered by health insurance, despite the significant costs of treating irinotecan-related toxicities (63, 64).

Part of the reason that healthcare providers forgo testing may be because the standard dose of irinotecan used in FOLFIRI is low (180 mg/m^2). A phase II trial is currently determining whether dosing irinotecan based on genotype, as part of a FOLFIRI treatment regime, is effective and safe. The standard irinotecan dose of 180 mg/m^2 is being used for patients with the *28/*28* genotype, a dose of 260 mg/m^2 is being used for patients with the **1/*28* genotype, and a dose of 310 mg/m^2 is being used for patients with the **1/*1* genotype (65).

Therapeutic Recommendations based on Genotype

This section contains excerpted[2] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2017 Statement from the US Food and Drug Administration (FDA)

Individuals who are homozygous for the *UGT1A1*28* allele (*UGT1A1 7/7* genotype) are at increased risk for neutropenia following initiation of Irinotecan Hydrochloride Injection, USP treatment.

In a study of 66 patients who received single-agent Irinotecan Hydrochloride Injection, USP (350 mg/m^2 once-every-3-weeks), the incidence of grade 4 neutropenia in patients homozygous for the *UGT1A1*28* allele was 50%, and in patients heterozygous for this allele (*UGT1A1 6/7* genotype) the incidence was 12.5%. No grade 4 neutropenia was observed in patients homozygous for the wild-type allele (*UGT1A1 6/6* genotype).

[2] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

When administered as a single-agent, a reduction in the starting dose by at least one level of Irinotecan Hydrochloride Injection, USP should be considered for patients known to be homozygous for the *UGT1A1*28* allele. However, the precise dose reduction in this patient population is not known and subsequent dose modifications should be considered based on individual patient tolerance to treatment.

UGT1A1 Testing

A laboratory test is available to determine the *UGT1A1* status of patients. Testing can detect the *UGT1A1 6/6*, *6/7* and *7/7* genotypes.

Please review the complete therapeutic recommendations that are located here: (1).

2017 Recommendations from the French National Network of Pharmacogenetics (RNPGx)

Interpreting Results

The RNPGx has proposed a decision tree for guiding irinotecan prescription based on the *UGT1A1* genotype and the protocol's theoretical dose:

- for low doses (< 180 mg/m^2 /week), presence of the *UGT1A1*28* allele is not a major risk factor (little difference in risk of hematological or digestive toxicity irrespective of the genotype);
- for doses in the 180—230mg/m^2 spaced by 2—3-week intervals, patients who are homozygous for the *UGT1A1*28* allele have a higher risk of hematological and/or digestive toxicity than patients who are heterozygous or non-carriers. For these **28/*28* patients, a 25% to 30% dose reduction is recommended, especially if the patient presents other risk factors for toxicity. Dose can be adjusted for subsequent cycles depending on the tolerance;
- for doses of 240mg/m^2 or higher spaced by 2—3 weeks intervals, homozygous *UGT1A1*28* patients have a greatly increased risk of hematological toxicity (neutropenia) compared with other genotypes, contraindicating administration at this higher dose and leading to discussion of a standard dose depending on the associated risk factors. Administration of an intensive dose (240 mg/m^2) is recommended only for *1/*1 patients, or for *1/*28 patients who have no other risk factors and who benefit from intensive surveillance.

[...]

The first-intention of this strategy for analysis of *UGT1A1* status is to detect the *28 variant, the most common deficiency variant observed in the Caucasian population, to be performed before initiating treatment. Referring to the level of evidence classification for RNPGx recommendations detailed in the article by Picard et al. in this issue, *UGT1A1* genotyping is advisable for a standard dose (180—230mg/m^2) and essential for intensified dose (> 240 mg/m^2).

Thus, individualized treatment can be proposed based on the *UGT1A1* genotype, with either a dose reduction for **28/*28* homozygous patients, or possibly dose intensification for non-carriers of the *28 allele.

For the other *UGT1A1* alleles, genotyping is performed by a limited number of laboratories and is considered a second- intention test.

Moreover, the RNPGx suggests that this analysis could be performed concomitantly with other genetic explorations for colorectal cancer patients (search for *KRAS*, *BRAF* mutations. . .) and constitutional (search for *DYPD* variants) in order to guarantee optimal irinotecan therapy within adequate delay for optimal hospital practices.

Please review the complete therapeutic recommendations that are located here: (5).

2014 Recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

UGT1A1 Intermediate Metabolizers (IM)
NO action is needed for this gene-drug interaction.
This genetic variation (IM) is more common in Western populations than the wild-type (*1/*1). This means that treatment is largely geared to patients with this genetic variation. Adjustment of the treatment is therefore not useful.

UGT1A1 Poor Metabolizers (PM)
Genetic variation reduces conversion of irinotecan to inactive metabolites. This increases the risk of serious, life-threatening adverse events.
Recommendation:
1. Start with 70% of the standard dose
If the patient tolerates this initial dose, the dose can be increased, guided by the neutrophil count.

UGT1A1 *1/*28
NO action is needed for this gene-drug interaction.
This genetic variation (*1/*28) is more common in Western populations than the wild-type (*1/*1). This means that treatment is largely geared to patients with this genetic variation. Adjustment of the treatment is therefore not useful.

UGT1A1 *28/*28
Genetic variation reduces conversion of irinotecan to inactive metabolites. This increases the risk of serious, life-threatening adverse events.
Recommendation:
1. Start with 70% of the standard dose
If the patient tolerates this initial dose, the dose can be increased, guided by the neutrophil count.

Please review the complete therapeutic recommendations that are located here: (4).

Nomenclature of selected *UGT1A1* variants

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*UGT1A1*1*	$(TA)_6TAA$	NM_000463.2:c.-53_-52TA[7]	Not applicable—variant occurs in a non-coding (TATA box promoter) region	rs8175347
*UGT1A1*6*	211G>A Gly71Arg	NM_000463.2:c.211G>A	NP_000454.1:p.Gly71Arg	rs4148323
*UGT1A1*36*	$(TA)_5TAA$	NM_000463.2:c.-53_-52TA[6]	Not applicable—variant occurs in a non-coding (TATA box promoter) region	rs8175347
*UGT1A1*28*	$(TA)_7TAA$	NM_000463.2:c.-53_-52[8]	Not applicable—variant occurs in a non-coding (TATA box promoter) region	rs8175347

Table continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*UGT1A1*37*	$(TA)_8TAA$	NM_000463.2:c.-53_-52TA[9]	Not applicable—variant occurs in a non-coding (TATA box promoter) region	rs8175347

*UGT1A1*1* is the wild-type allele and is associated with normal enzyme activity.
Note: The *UGT1A1*28* variant has 8 TA repeats, as shown by the "[8]" in the official HGVS term, "NM_000463.2:c.-53_-52[8]". In the medical literature, the term "$(TA)_7TAA$" is commonly used. Here, 7 of the TA repeats are shown in parentheses "$(TA)_7$", followed by the 8th repeat "(TAA)".
For an overview of the haplotypes for *UGT1A1*, please see the PharmGKB's Haplotype Translation Table.
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).

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Lacosamide Therapy and *CYP2C19* Genotype

Laura Dean, MD[1]

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Introduction

Lacosamide (brand name Vimpat) is an antiseizure drug indicated for adjunctive therapy for partial-onset seizures in pediatric and adult patients with epilepsy. Lacosamide is thought to work by selectively enhancing slow inactivation of voltage-dependent sodium channels. This stabilizes the neuronal membrane and suppresses the repetitive neuronal firing associated with seizures.

Several cytochrome P450 (CYP) enzymes are involved in metabolizing active lacosamide to an inactive metabolite, including CYP2C19. Individuals who have no CYP2C19 enzyme activity are known as "CYP2C19 poor metabolizers".

The FDA-approved drug label for lacosamide cites a small study that found plasma levels of lacosamide were similar in CYP2C19 poor metabolizers (n=4) and normal (extensive) metabolizers (n=8) (Table 1). Therefore, the recommended standard doses of lacosamide may be used for CYP2C19 poor metabolizers (1).

Table 1. FDA (2016) Drug Label for Lacosamide. Recommendations for CYP2C19 Phenotype. Pharmacokinetics.

Phenotype	Recommendations
CYP2C19 Poor metabolizer	There are no clinically relevant differences in the pharmacokinetics of lacosamide between CYP2C19 poor metabolizers and extensive metabolizers.

This table is adapted from (1).

Drug: Lacosamide

Lacosamide is an antiseizure drug that is used in the treatment of partial-onset (focal) seizures. It may be used as monotherapy, or as an adjunctive therapy. When lacosamide is taken orally, it can be used in pediatric patients (from age 4), or if given intravenously, it is indicated for adult patients (from age 17) (1).

Over 50 million people worldwide suffer from epilepsy, which is characterized by spontaneous recurrent epileptic seizures classified as generalized or focal. Generalized seizures appear to originate in all regions of the cortex simultaneously and include absence seizures (sudden impaired consciousness and staring) and general tonic-clonic seizures (loss of consciousness, stiffening of limbs in the tonic phase, and twitching or jerking muscles in the clonic phase). In contrast, symptoms of focal seizures depend upon where the focus of the seizure originates in the brain (e.g., jerking of a limb indicates a focus in the contralateral motor cortex).

Most currently available antiseizure medications target sodium channels (e.g., carbamazepine, phenytoin), calcium channels (e.g., ethosuximide), or the gamma-aminobutyric acid (GABA) system (e.g., clobazam). However, up to one-third of patients may not achieve seizure control, or they may not be able to tolerate the side effects. This has led to the development of newer antiseizure drugs with unconventional targets.

Lacosamide is a third-generation antiseizure drug that was designed to have a novel mechanism of action — it selectively enhances the slow inactivation of voltage-gated sodium channels. This leads to a stabilization of neuronal membranes and an inhibition of repetitive neuronal firing. This mode of action is fundamentally different to traditional sodium channel blocking drugs, which affect the fast inactivation of voltage-gated sodium channels (2, 3).

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In adult patients (aged 17 and older), the recommended initial dose for lacosamide monotherapy is 100 mg twice daily (50 mg twice daily for adjunctive therapy), which may be increased to a maximum dose of 200 mg twice daily, for both monotherapy and adjunctive therapy. At these doses, randomized controlled trials have reported that lacosamide reduces the frequency of focal seizures significantly more than placebo, while also being well tolerated (4-7). The most common adverse drug effects of lacosamide are diplopia (double vision), headache, dizziness, and nausea (2, 3, 8, 9).

Lacosamide is metabolized to a major inactive O-desmethyl metabolite by several cytochrome P (CYP) enzymes, including CYP3A4, CYP2C9, and CYP2C19. The role of CYP2C19 in lacosamide metabolism has been the most thoroughly studied. According to the FDA drug label for lacosamide, individuals who harbor 2 nonfunctional *CYP2C19* variant alleles ("CYP2C19 poor metabolizers") have lower concentrations of the inactive O-desmethyl metabolite in their plasma, compared to normal (extensive) metabolizers with 2 functional *CYP2C19* alleles.

However, the plasma concentration of active lacosamide was similar in both poor metabolizers and normal (extensive) metabolizers. Therefore, the label states that there are no clinically relevant differences in the pharmacokinetics of lacosamide between CYP2C19 poor and normal metabolizers (1).

The Cytochrome P450 Superfamily

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP450 genes are highly polymorphic and can result in no, decreased, normal, or increased enzyme activity.

Enzymes CYP2C19, CYP2C9, and CYP3A4 are involved in the metabolism of lacosamide to a major inactive O-desmethyl metabolite. The role of CYP2C19 in lacosamide metabolism has been the most thoroughly studied (10). According to the FDA drug label for lacosamide, individuals who harbor 2 nonfunctional *CYP2C19* variant alleles ("CYP2C19 poor metabolizers") have lower concentrations of the inactive O-desmethyl metabolite in their plasma and excreted in the urine compared with normal (extensive) metabolizers with 2 functional *CYP2C19* alleles.

Genetic Testing

The National Institutes of Health (NIH) Genetic Testing Registry (GTR) displays genetic tests that are currently available for the *CYP2C19* gene.

Given that currently there are no clinically significant differences in lacosamide pharmacokinetics between CYP2C19 poor and normal metabolizers, there is no evidence supporting clinical CYP2C19 pharmacogenetic testing prior to initiating lacosamide, and testing has not been addressed by currently available professional society practice guidelines.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2016 Statement from the US Food and Drug Administration (FDA):

CYP2C19 Polymorphism

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

There are no clinically relevant differences in the pharmacokinetics of lacosamide between CYP2C19 poor metabolizers and extensive metabolizers. Results from a trial in poor metabolizers (PM) (N=4) and extensive metabolizers (EM) (N=8) of cytochrome P450 (CYP) 2C19 showed that lacosamide plasma concentrations were similar in PMs and EMs, but plasma concentrations and the amount excreted into urine of the O-desmethyl metabolite were about 70% reduced in PMs compared to EMs.

Please review the complete therapeutic recommendations that are located here: (1).

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Lesinurad Therapy and *CYP2C9* Genotype

Laura Dean, MD[1]

Created: February 11, 2019.

Introduction

Lesinurad (brand name Zurampic) is a urate transport inhibitor used in the treatment of gout. Gout is one of the most common types of inflammatory arthritis, affecting approximately 3% of adults worldwide. It is caused by the accumulation of urate crystals in joints. The long-term management of gout includes reducing risk factors (e.g., obesity, alcohol use, diuretic use, poor renal function), and medication to lower uric acid levels.

Lesinurad reduces the high level of uric acid (hyperuricemia) associated with gout. Lesinurad should only be used in combination with a xanthine oxidase inhibitor (e.g., allopurinol, febuxostat) –– the risk of acute renal failure is increased if lesinurad is used alone.

The addition of lesinurad to gout treatment is reserved for patients who have failed to achieve their target uric acid level despite being treated with a xanthine oxidase inhibitor. Xanthine oxidase inhibitors reduce uric acid by inhibiting its production, whereas lesinurad reduces uric acid by blocking its reabsorption in the kidney.

Lesinurad is primarily metabolized by CYP2C9 to several inactive metabolites. Individuals who lack CYP2C9 activity ("CYP2C9 poor metabolizers") have an increased exposure to lesinurad, and an increased risk of side effects. Adverse reactions of lesinurad therapy include kidney stones and other kidney problems. Lesinurad is also associated with an increased risk of cardiovascular events.

The FDA-approved drug label for lesinurad states that lesinurad should be used with caution in CYP2C9 poor metabolizers, but does not provide specific dose adjustments in this group (Table 1). The standard dose of lesinurad is 200 mg daily (1). Lesinurad is contraindicated in patients with severe impairment of kidney function (e.g., kidney transplant and hemodialysis patients) as well as individuals with tumor lysis syndrome or Lesch-Nyhan syndrome.

Table 1. The FDA (2018) Drug Label for Lesinurad. CYP2C9 Inhibitors, CYP2C9 Poor Metabolizers, and CYP2C9 Inducers.

Phenotype	Recommendations
CYP2C9 Poor metabolizer	Lesinurad exposure is increased when lesinurad is co-administered with inhibitors of CYP2C9 and in CYP2C9 poor metabolizers. Lesinurad should be used with caution in patients taking moderate inhibitors of CYP2C9 (e.g., fluconazole, amiodarone), and in CYP2C9 poor metabolizers.

This table is adapted from (1).

Drug: Lesinurad

Lesinurad is a urate transporter inhibitor used to treat the increased level of uric acid in patients with gout. Lesinurad should only be used for patients with gout who have high levels of uric acid despite being treated with a xanthine oxidase inhibitor, and lesinurad should only be used in combination with a xanthine oxidase inhibitor (1).

Gout is one of the most common types of inflammatory arthritis. It affects approximately 3% of adults worldwide, and the prevalence is increasing (2-5). Gout is caused by the body's inflammatory response to an accumulation of urate crystals. A high level of uric acid in the blood (above 6.8 mg/dl indicates hyperuricemia)

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always precedes gout. However, the majority of individuals with hyperuricemia do not develop urate crystal deposits and gout, and lesinurad should not be used to treat asymptomatic hyperuricemia.

Patients with gout usually have an extremely painful, swollen joint –– this is known as acute gouty arthritis. A single joint in the lower limb is most commonly affected (e.g., the base of the big toe, knee), and the joint will remain painful for at least several days. In a minority, persistent hyperuricemia leads to chronic gout, which is associated with deposits of urate crystals known as tophi.

Medications for gout focus on lowering uric acid levels. There are 3 main types of drugs:

- xanthine oxidase inhibitors that decrease the production of uric acid (e.g., allopurinol, febuxostat)
- uricosuric drugs that inhibit the reabsorption of uric acid in the kidneys (e.g., benzbromarone, probenecid, and lesinurad)
- uricase drugs that convert uric acid to a more soluble metabolite (e.g., pegloticase, rasburicase)

Lesinurad is the newest uricosuric drug to be approved for gout. However, since the introduction of allopurinol in the 1960s, uricosuric drugs have not been commonly used. This is because they are associated with numerous drug interactions and side effects (6-8).

Like other uricosuric drugs, lesinurad inhibits the urate transporter 1 (URAT1), which mediates reabsorption of uric acid in the kidney, and the organic anion transporter 4 (OAT4), which is implicated with hyperuricemia associated with diuretic use. But unlike probenecid, lesinurad does not appear to inhibit OAT1 or OAT3, and this may result in fewer drug interactions and adverse events (9). However, like all uricosuric agents, lesinurad is associated with the development of kidney stones (10-12).

There are many risk factors that may contribute to triggering a gout attack. These include dietary factors, dehydration, and alcohol use. In addition, medications that alter serum concentrations of uric acid, such as diuretics and gout medications, can trigger gout. Therefore, when starting medical therapy for gout, it is recommended that urate levels are reduced slowly (e.g., 1–2 mg/dl per month). To prevent flare-ups, an additional drug such as colchicine may be used to reduce swelling and pain until target serum levels have been achieved and maintained (13).

Allopurinol is the mainstay treatment for gout –– it is effective in lowering uric acid levels, reduces the frequency of gout attacks, and contributes to resolving tophi. However, in individuals who are carriers of the genetic variant *HLA-B*58:01*, allopurinol is associated with severe cutaneous adverse reactions (SCAR) (14, 15). For these individuals, febuxostat may be the safer choice –– it is a structurally different xanthine oxidase inhibitor that is not associated with SCAR (13).

In general, when allopurinol is used at an adequate dose, levels of uric acid can reach the target range of below 6 mg/dl (16, 17). If uric acid levels stay in this range, subsequent attacks of gout are unlikely. However, allopurinol therapy is needed long term; compliance is often poor; therefore, patient education is important (11, 18).

Several trials have shown that the addition of lesinurad to allopurinol therapy leads to a greater reduction in uric acid levels, and at the recommended dose of 200 mg daily, lesinurad is generally well tolerated (19-21).

Adverse effects associated with lesinurad therapy include rising creatinine levels, which are often reversible, nephrolithiasis (kidney stones), urolithiasis (stones in the bladder or urinary tract), and acute renal failure – which is associated more with lesinurad monotherapy (not recommended by the FDA) at the higher drug dose of 400 mg (twice the FDA-approved dose). Lesinurad is also associated with an increased risk of cardiovascular events (22).

Patients with moderate renal impairment experience a 150% increase in exposure to lesinurad, which should be used with caution in these patients (23). Lesinurad is contraindicated in individuals with severe renal

impairment, end stage renal disease, kidney transplant recipients, or patients on dialysis as well as individuals with tumor lysis syndrome or Lesch-Nyhan syndrome.

Lesinurad is primarily metabolized by CYP2C9 to several inactive metabolites. The co-administration of lesinurad with moderate inducers of CYP2C9 (e.g., rifampin, carbamazepine), may decrease the therapeutic effect of lesinurad by reducing its exposure.

In contrast, the co-administration of lesinurad with drugs that are CYP2C9 inhibitors (e.g., fluconazole, amiodarone), or the administration of lesinurad to patients who lack CYP2C9 activity ("CYP2C9 poor metabolizers"), will increase exposure to lesinurad. This may increase the risk of adverse reactions.

Therefore, lesinurad should be used with caution in patients with moderate kidney disease, patients taking CYP2C9 inhibitors, and patients who are CYP2C9 poor metabolizers (1, 24).

Gene: CYP2C9

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs in the liver. The CYP450 genes are very polymorphic and can result in reduced, absent, or increased enzyme activity (25).

The *CYP2C9* gene is highly polymorphic, with approximately 60 known alleles. *CYP2C9*1* is considered the wild-type allele when no variants are detected and is categorized as normal enzyme activity (26). Individuals who have 2 normal-function alleles (e.g., *CYP2C9 *1/*1*) are classified as "normal metabolizers" (Table 2).

Table 2. Assignment of likely *CYP2C9* Phenotype based on Genotype (CPIC, 2014)

Likely phenotype[a]	Genotype	Examples of diplotypes
Ultrarapid metabolizer (increased activity) (frequency unknown)	Unknown – currently there are no known increased activity alleles	Unknown
Normal metabolizer (normal activity) (approximately 91% of individuals)	An individual with 2 normal-function alleles	*1/*1
Intermediate metabolizer (heterozygote or intermediate activity) (approximately 8% of individuals)[b]	An individual with one normal-function allele plus one decreased-function allele	*1/*3, *1/*2
Poor metabolizer (homozygous variant, low or deficient activity) (approximately 1% of individuals)	An individual with 2 decreased-function alleles	*2/*2, *3/*3, *2/*3

Note: There are no known cases of CYP2C9 ultrarapid metabolizers.
a Global frequencies are approximate. Because haplotype frequencies vary considerably among popualations, please see (26) for individual population frequencies.
[b] The enzyme activity in this grouping varies widely. Please see (26) for activity ranges.
This table is adapted from (26). Note: The nomenclature used in this table reflects the standardized pharmacogenetic terms proposed by the Clinical Pharmacogenetics Implementation Consortium (CPIC) (27).

Two allelic variants associated with reduced enzyme activity are *CYP2C9*2* and **3*. The **2* allele is more common in Caucasian (10-20%) than Asian (1-3%) or African (0-6%) populations. The **3* allele is less common (<10% in most populations) and is extremely rare in African populations. In African-Americans, the *CYP2C9*5*, **6*, **8* and **11* alleles are more common (28-30).

Linking Gene Variation with Treatment Response

Currently, data are limited on the relationship between an individual's *CYP2C9* status and their response to lesinurad therapy.

The lesinurad drug label discusses an analysis of a small group of patients –– 2 patients were CYP2C9 poor metabolizers, and 41 were normal CYP2C9 metabolizers. At the 400 mg dose of lesinurad (which is twice the recommended dose of 200 mg daily), exposure to lesinurad was approximately 1.8 times higher in poor metabolizers compared to normal metabolizers. Therefore, the label states that lesinurad should be used with caution in CYP2C9 poor metabolizers.

The drug label also states that lesinurad should be used with caution in patients taking drugs that are CYP2C9 inhibitors (because of increased exposure and risk of side effects) and in patients taking drugs that are CYP2C9 inducers (because of decreased exposure and risk of reduced therapeutic effect). Drugs that inhibit CYP2C9 include fluconazole (antifungal agent) and amiodarone (antiarrhythmic); and drugs that induce CYP2C9 include rifampin (antibiotic) and carbamazepine (anti-seizure drug) (1).

Genetic Testing

Clinical genotyping tests are available for several *CYP2C9* alleles. The NIH Genetic Testing Registry (GTR) displays genetic tests that are currently available for lesinurad response and for the *CYP2C9* gene.

The *CYP2C9* variants that are routinely tested for include *CYP2C9*2* and **3*. Usually the results are reported as a diplotype, such as *CYP2C9 *1/*1*, and may also include an interpretation of the patient's predicted metabolizer phenotype (normal, intermediate, or poor). Table 2 summarizes common CYP2C9 phenotypes.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2018 Statement from the US Food and Drug Administration (FDA)

Lesinurad exposure is increased when lesinurad is co-administered with inhibitors of CYP2C9, and in CYP2C9 poor metabolizers. Lesinurad should be used with caution in patients taking moderate inhibitors of CYP2C9 (e.g., fluconazole, amiodarone), and in CYP2C9 poor metabolizers.

Lesinurad exposure is decreased when lesinurad is co-administered with moderate inducers of CYP2C9 (e.g., rifampin, carbamazepine), which may decrease the therapeutic effect of lesinurad.

[…]

Patients who are CYP2C9 poor metabolizers are deficient in CYP2C9 enzyme activity. A cross-study pharmacogenomic analysis assessed the association between CYP2C9 polymorphism and lesinurad exposure in patients receiving single or multiple doses of lesinurad at 200 mg, 400 mg or 600 mg. At the 400 mg dose, lesinurad exposure was approximately 1.8-fold higher in CYP2C9 poor metabolizers (i.e., subjects with *CYP2C9 *2/*2* [N=1], and **3/*3* [N=1] genotype) compared to CYP2C9 extensive metabolizers (i.e., *CYP2C9 *1/*1* [N=41] genotype). Use with caution in CYP2C9 poor metabolizers, and in patients taking moderate inhibitors of CYP2C9.

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labelled all formulations containing the generic drug.

Please review the complete therapeutic recommendations that are located here: (1).

Nomenclature for selected *CYP2C9* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C9*2*	430C>T Arg144Cys	NM_000771.3:c.430C>T	NP_000762.2:p.Arg144Cys	rs1799853
*CYP2C9*3*	1075A>C Ile359Leu	NM_000771.3:c.1075A>C	NP_000762.2:p.Ile359Leu	rs1057910
*CYP2C9*5*	1080C>G Asp360Glu	NM_000771.3:c.1080C>G	NP_000762.2:p.Asp360Glu	rs28371686
*CYP2C9*6*	818delA Lys273Argfs	NM_000771.3:c.817delA	NP_000762.2:p.Lys273Argfs	rs9332131
*CYP2C9*8*	449G>A Arg150His	NM_000771.3:c.449G>A	NP_000762.2:p.Arg150His	rs7900194
*CYP2C9*11*	1003C>T Arg335Trp	NM_000771.3:c.1003C>T	NP_000762.2:p.Arg335Trp	rs28371685

Note: the normal "wild-type" allele is *CYP2C9*1* and is reported when no variant is detected.
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (31).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for cytochrome P450 enzymes is available from Pharmacogene Variation (PharmVar) Consortium.

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Maraviroc Therapy and *CCR5* Genotype

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Introduction

Maraviroc is a chemokine receptor antagonist that is used in combination with other antiretroviral agents to treat human immunodeficiency virus type 1 (HIV-1) infection. Maraviroc exerts its therapeutic activity by blocking entry of the HIV-1 virus into immune cells—specifically the CD4-expressing T-helper cells, which play a major role in protecting the body from infection—precursor cells, and dendritic cells.

HIV-1 infection is classified in two major forms according to the co-receptor it employs to gain entry in to the cell, namely the chemokine receptor 5 (CCR5) or the CXC chemokine receptor 4 (CXCR4). These co-receptors are expressed on different types of cells, and HIV tropism refers to the types of cells and tissues in which the virus infects and replicates. A tropism assay is conducted to determine which co-receptor the HIV-1 virus uses, i.e., whether the virus is CCR5-tropic, CXCR4-tropic, dual tropic (i.e., HIV-1 virus that is able to use both receptors), or mixed tropic (i.e., a mixture of HIV-1 viruses, some of which use CCR5 and others that use CXCR4).

Maraviroc is only indicated for treatment of adults with CCR5 tropic HIV-1 and is not recommended when the CXCR4-tropic virus has been detected. The FDA-approved drug label for maraviroc states that "prior to initiation of maraviroc, test all patients for CCR5 tropism using a highly sensitive tropism assay" (1).

Drug: Maraviroc

Maraviroc is the first FDA-approved drug in a class of HIV drugs called entry and fusion inhibitors. Maraviroc blocks the interaction between HIV-1 and CCR5 in healthy immune cells, preventing certain strains (CCR5-tropic) of HIV from entering and infecting the cell. Maraviroc must be taken twice daily and must always be used with other HIV drugs. Taken in combination with these drugs, maraviroc may lower the HIV virus load in the blood.

Currently, maraviroc is the only CCR5 co-receptor inhibitor that has been approved for clinical use (2). It is used to treat HIV-1-infected patients who have a virus that uses CCR5 for entry, and either never received antiretroviral treatment before, or have experienced therapeutic failure following traditional antiretroviral therapies (3). Among other CCR5 antagonists currently under investigation is cenicriviroc, which is in Phase II trials and appears to block the CCR2 receptor (4, 5).

Maraviroc treatment regimens may be used less often than other regimens. Possible reasons include the requirement to test for tropism, which is time-consuming and expensive (see Genetic Testing). Furthermore, there is a large selection of potent and tolerable treatment regimens currently available that do not require genotyping prior to use. These treatment regimens may be based on nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse-transcriptase inhibitors (NNRTI), boosted protease inhibitors (PI), and integrase inhibitors (2, 6).

The entry of HIV-1 into a host cell is a complex process, which begins when the viral envelope glycoprotein, gp120, binds to the cellular protein, CD4. Binding induces conformational changes in gp120 resulting in the exposure of gp4, another viral envelope protein that helps mediate the interaction between the virus and cellular co-receptors, and the fusion of viral and cellular membranes.

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The CD4 count is often used to determine the stages of HIV disease. CD4 is a glycoprotein found on the surface of T helper immune cells. HIV-1 infection leads to a progressive reduction in the number of T cells that express CD4, and a CD4 count of less than 200 cells/mm^3 is one of the qualifications for a diagnosis of AIDS (7, 8).

Measurement of the CD4 count is useful before HIV treatment is started because the CD4 count provides information on the overall immune function of the patient. In the United States, antiretroviral therapy (ART) is now recommended for all HIV-infected patients, regardless of their CD4 count or viral load (9), to keep viral loads at undetectable levels for as long as possible. In adults receiving optimized background treatment for infection with CCR5-tropic HIV-1, the addition of maraviroc leads to a greater increase in CD4 counts compared to the addition of placebo (1).

HIV-1 most commonly uses either the CCR5 or CXCR4 co-receptors to enter its target cells (10). Maraviroc is an effective antiretroviral agent in individuals who only harbor the CCR5-tropic HIV-1 virus. It is incapable of inhibiting infection against viruses that do not use CCR5 (i.e., CXCR-using virus or dual/mixed virus) (1).

Maraviroc is metabolized by the cytochrome P450 system, mainly CYP3A, in the liver to inactive metabolites (11, 12). As noted above, maraviroc must be used in combination with other antiretroviral medications; the recommended dosage of maraviroc depends on whether the co-medications are inhibitors or inducers of CYP3A (1).

Gene: *CCR5*

The chemokine (CC motif) receptor 5 (CCR5) is primarily expressed on the surface of white blood cells. Chemokines are a type of cytokine—they are small, secreted proteins that have a crucial role in the inflammatory response by helping immune cells migrate to areas of tissue damage. Other functions of chemokines include influencing the maturation of various immune cells and promoting the growth of new blood vessels.

Most chemokines have four characteristic cysteine residues in a conserved location, and they are classified into four families by the location of the first two cysteine residues: CXC, CC, C, and CX3C. For example, members of the "CC" cytokine family have two adjacent cysteine residues near their amino terminus.

The receptors for chemokines are G-protein coupled, seven-transmembrane domain receptors. Two of these receptors, CCR5 (binds CC chemokines) and CXCR4 (binds CXC chemokines), are also co-receptors used by HIV to enter human white blood cells. CCR5 is expressed on fewer cells (e.g., specific T cells, precursor cells (or macrophages) and dendritic cells) than CXCR4 (e.g., most immune cells, vascular endothelial cells, and neurons).

HIV-1 virus that uses the CCR5 co-receptor (CCR5-tropic) is more commonly found in the early stages of infection. It is also more common among individuals who have yet to receive treatment, and at least half of all infected individuals harbor only CCR5-tropic viruses throughout the course of infection. The CXCR4-tropic virus is more commonly found during later stages of disease and among individuals who have received HIV treatment. The presence of CXCR4-tropic virus is a predictor of lower CD4 count, a higher viral load, and a more rapid progression to AIDS (7).

A variant of *CCR5*, *CCR5-Δ32* (NM_000579.3:c.554_585del32), contains a 32 bp deletion and codes a nonfunctional receptor that hinders the entry of CCR5-tropic virus in to cells. Individuals who have two copies of this allele are highly resistant to HIV infection, and although individuals who have one copy of the allele remain susceptible to HIV infection, the progression of HIV infection to AIDS is delayed (13).

The *CCR5-Δ32* allele occurs at high frequency in European Caucasians (5%–14%) but is rare among African, Native American, and East Asian populations, suggesting that the allele may have conferred an evolutionary survival advantage (14). Possible causes of a positive selection pressure include protection against the bubonic

plague (*Yersinia pestis*) or smallpox (*Variola virus*) during the Middle Ages. However, other studies have found that the *CCR5-Δ32* allele arose long before this time and underwent neutral evolution (15).

Genetic Testing

Testing of the HIV-1 virus (i.e., the virus, not the patient) should be carried out prior to initiation of treatment with maraviroc. A tropism assay is needed to identify individuals with CCR5-tropic HIV-1. The assay must be highly sensitive to detect low levels of CXCR4-tropic viruses. Maraviroc should not be prescribed if non-CCR5 variants (CXCR4-tropic or dual/mixed-tropic) are detected (1, 11). HIV tropism can be determined by phenotype or genotype testing. Phenotypic assays can be performed using plasma RNA (if viral load is greater than 1000 copies/ml) or cell-associated DNA (if viral load is less than 1000 copies/ml). Phenotypic assays use replication-defective laboratory viruses that carry the complete cloned viral envelope proteins gp120 and gp41 derived from the patient. Phenotypic assays measure the ability of these pseudoviruses to infect CD4+ target cells that express either CCR5 or CXCR4 (9).

Genotyping methods are used to predict which co-receptors on the cell are used by the virus rather than directly assessing tropism. Genotyping methods involve sequencing the third variable region (V3) of gp120 and using algorithms to predict co-receptor usage.

While phenotypic assays are still considered to be the gold standard, the use of genotyping to determine patient eligibility for maraviroc is increasing due to low cost, greater accessibility, and faster turnaround time for the results as compared to the other methods (16, 17). Although there can be discrepancies between the results from phenotypic and genotypic assays, the correlation between genotypic assays and the clinical efficacy of maraviroc is improving (18).

The NIH's Genetic Testing Registry (GTR) displays genetic testing information for human genes and conditions, including tests for maraviroc response. These tests investigate the human genes that contribute to the pharmacokinetics of maraviroc, as opposed to the FDA-recommended genetic tests, which are tests for viral genes.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2016 Statement from the US Food and Drug Administration (FDA): Prior to initiation of maraviroc, test all patients for CCR5 tropism using a highly sensitive tropism assay. Maraviroc is recommended for patients with only CCR5-tropic HIV-1 infection. Outgrowth of pre-existing low-level CXCR4- or dual/mixed-tropic HIV-1 not detected by tropism testing at screening has been associated with virologic failure while on maraviroc.

Please review the complete therapeutic recommendations that are located here: (1).

Nomenclature

Allele name	Other name(s)	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
CCR5delta32		NM_000579.3:c.554_585del32 NM_001100168.1:c.554_585del32	NP_000570.1:p.Ser185Ilefs NP_001093638.1:p.Ser185Ilefs	rs333

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labelled all formulations containing the generic drug.

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

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Mercaptopurine Therapy and *TPMT* Genotype

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Introduction

Mercaptopurine is an immunosuppressant and antineoplastic agent that belongs to the drug class of thiopurines. It is used in combination with other drugs to treat acute lymphoblastic leukemia, which is the most common form of cancer in children (1). In addition, off-label uses include the treatment of inflammatory bowel disease (IBD).

Mercaptopurine is a prodrug that must first be activated to form thioguanine nucleotides (TGNs), the major active metabolites. Thiopurine S-methyltransferase (TPMT) inactivates mercaptopurine, leaving less parent drug available to form TGNs.

An adverse effect of mercaptopurine therapy is bone marrow suppression, which can occur in any patient, is dose-dependent, and may be reversed by reducing the dose of mercaptopurine. However, patients who carry two nonfunctional *TPMT* alleles universally experience life-threatening myelosuppression when treated with mercaptopurine, due to high levels of TGNs. Patients who carry one nonfunctional *TPMT* allele may also be unable to tolerate conventional doses of mercaptopurine (2, 3).

The FDA-approved drug label for mercaptopurine states that heterozygous patients with low or intermediate TPMT activity accumulate higher concentrations of active TGNs than people with normal TPMT activity and are more likely to experience mercaptopurine toxicity; and that TPMT genotyping or phenotyping (red blood cell TPMT activity) can identify patients who are homozygous deficient or have low or intermediate TPMT activity (1).

The Clinical Pharmacogenetics Implementation Consortium (CPIC) has published dosing recommendations for *TPMT* genotype-based mercaptopurine dosing. These recommendations include:

Start with reduced doses of mercaptopurine for patients with one nonfunctional *TPMT* allele, or drastically reduced doses for patients with malignancy and two nonfunctional alleles; adjust dose based on degree of myelosuppression and disease-specific guidelines. Consider alternative nonthiopurine immunosuppressant therapy for patients with nonmalignant conditions and two nonfunctional alleles (see Table 1) (2-4).

Table 1. *TPMT* phenotypes and the therapeutic recommendations for mercaptopurine therapy, adapted from CPIC

Phenotype	Phenotype details	*TPMT* Genotype	Examples of diplotypes	Therapeutic recommendations for mercaptopurine (MP)
Homozygous wild-type ("normal")	High enzyme activity. Found in ~86-–97% of patients.	Two or more functional *TPMT* alleles	**1/*1*	Start with normal starting dose (e.g., 75 mg/m^2/d or 1.5 mg/kg/d) and adjust doses of MP (and of any other myelosuppressive therapy) without any special emphasis on MP compared to other agents. Allow 2 weeks to reach steady state after each dose adjustment.

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Table 1. continued from previous page.

Phenotype	Phenotype details	*TPMT* Genotype	Examples of diplotypes	Therapeutic recommendations for mercaptopurine (MP)
Heterozygous	Intermediate enzyme activity. Found in ~3-–14% of patients.	One functional *TPMT* allele plus one nonfunctional *TPMT* allele	**1/*2* **1/*3A* **1/*3B* **1/*3C* **1/*4*	Start with reduced doses (start at 30–70% of full dose: e.g., at 50 mg/m^2/d or 0.75 mg/kg/d) and adjust doses of MP based on degree of myelosuppression and disease-specific guidelines. Allow 2–4 weeks to reach steady state after each dose adjustment. In those who require a dosage reduction based on myelosuppression, the median dose may be ~40% lower (44 mg/m2) than that tolerated in wild-type patients (75 mg/ m^2). In setting of myelosuppression, and depending on other therapy, emphasis should be on reducing MP over other agents.
Homozygous variant	Low or deficient enzyme activity. Found in ~1 in 178 to 1~3736 patients.	Two nonfunctional *TPMT* alleles	**3A/*3A* **2/*3A* **3C/*3A* **3C/*4* **3C/*2* **3A/*4*	For malignancy, start with drastically reduced doses (reduce daily dose by 10-fold and reduce frequency to thrice weekly instead of daily, e.g., 10 mg/m^2/d given just 3 days/week) and adjust doses of MP based on degree of myelosuppression and disease-specific guidelines. Allow 4–6 weeks to reach steady state after each dose adjustment. In setting of myelosuppression, emphasis should be on reducing MP over other agents. For nonmalignant conditions, consider alternative nonthiopurine immunosuppressant therapy.

MP: Mercaptopurine
The strength of therapeutic recommendations is "strong" for all phenotypes.
Table is adapted from Relling M.V. et al. Clinical Pharmacogenetics Implementation Consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing. Clinical pharmacology and therapeutics. 2011;89(3):387–91 (2, 3).

Drug Class: Thiopurines

Thiopurines are used as anticancer agents and as immunosuppressants in inflammatory bowel disease, rheumatoid arthritis, and other autoimmune conditions. Three thiopurines are used clinically: thioguanine, mercaptopurine, and azathioprine (a prodrug for mercaptopurine). All three agents have similar effects but are typically used for different indications. Thioguanine is most commonly used in the treatment of myeloid leukemias, mercaptopurine is used for lymphoid malignancies, and mercaptopurine and azathioprine are used for immune conditions.

Thiopurines are either activated to form TGNs (the major active metabolite) or deactivated by TPMT. Individuals who carry two non-functional *TPMT* alleles ("*TPMT* homozygotes") universally experience life-threatening bone marrow suppression because of high levels of TGNs when treated with conventional doses. Individuals who carry one non-functional *TPMT* allele ("*TPMT* heterozygotes") may also be unable to tolerate conventional doses of thiopurines due to increased levels of TGNs.

Drug: Mercaptopurine

Mercaptopurine is a neoplastic agent and an immunosuppressive agent that is used in the treatment of acute lymphoblastic leukemia (ALL) as part of a combination regimen. ALL is the most common form of cancer in

children, accounting for approximately 30% of childhood malignancies with a peak incidence occurring at 3 to 5 years of age (5).

An off-label use of mercaptopurine is in the treatment of inflammatory bowel disease (IBD). Along with the closely related azathioprine (which is metabolized to mercaptopurine), mercaptopurine is used as an "immunomodulator" and as a "steroid-sparing agent" in the treatment of Crohn's disease and ulcerative colitis.

Mercaptopurine is a slow-acting drug and for IBD, it typically takes at least three months of therapy before a therapeutic effect is observed. Therefore, mercaptopurine is used for the induction and maintenance of IBD remission rather than as a monotherapy for acute relapses (6). Because the discontinuation of mercaptopurine is associated with a high rate of relapse of IBD, mercaptopurine is usually continued long-term if there are no adverse effects (7, 8).

The use of mercaptopurine or the related drug azathioprine, has been associated with a 4-fold increased risk of developing lymphoma, which does not persist after discontinuation of therapy (9, 10).

Like all thiopurines, mercaptopurine is a purine analogue, and acts as an antimetabolite by interfering with nucleic acid synthesis and inhibiting purine metabolism. Activation of mercaptopurine occurs via HPRT1 (hypoxanthine phosphoribosyltransferase) followed by a series of reactions to form TGNs. The cytotoxicity of mercaptopurine is due, in part, to the incorporation of TGNs into DNA.

Inactivation of mercaptopurine occurs via two different pathways, via methylation (by TPMT) or via oxidation (by xanthine oxidase). TPMT activity is highly variable in patients because of genetic polymorphism in the *TPMT* gene.

One of the most frequent adverse reactions to mercaptopurine is myelosuppression, which can occur in any patient, and can usually be reversed by decreasing the dose of mercaptopurine. However, all patients who carry two nonfunctional *TPMT* alleles (approximately 0.3%) experience life-threatening myelosuppression after starting treatment with conventional doses of mercaptopurine, due to high levels of TGNs.

Individuals who are heterozygous for nonfunctional *TPMT* alleles (approximately 10%) are at a significantly higher risk for toxicity than individuals with two functional alleles. However, some of these individuals, approximately 40–70%, can tolerate the full dose of mercaptopurine. This may be because heterozygous-deficient individuals have lower concentrations of less active metabolites, such as MeMPN (methylmercaptopurine nucleotides), than homozygous-deficient individuals (2, 3).

Approximately 90% of individuals have normal TPMT activity with two functional alleles; however, all individuals receiving mercaptopurine require close monitoring (2, 3, 11, 12). One study reports that in patients with IBD receiving thiopurine therapy, TPMT polymorphisms are associated with the overall incidence of adverse reactions and with bone marrow toxicity, but not with other adverse reactions, such as liver damage and pancreatitis. Therefore, although determining *TPMT* genotype is helpful before initiating therapy, regular blood tests to monitor for side effects are needed during therapy (1, 13).

The other mercaptopurine inactivation pathway is via oxidation, which is catalyzed by xanthine oxidase. If this pathway is inhibited, for example, in patients taking allopurinol (an inhibitor of xanthine oxidase), the decreased break down of mercaptopurine can lead to mercaptopurine toxicity (1). However, some studies have found that the co-administration of allopurinol, with a reduced dose of mercaptopurine (or azathioprine), can help optimize the treatment response in patients with IBD (14, 15).

Gene: *TPMT*

The *TPMT* gene encodes one of the important enzymes of phase II metabolism, thiopurine S-methyltransferase. TPMT is one of the main enzymes involved in the metabolism of thiopurines, such as mercaptopurine. TPMT

activity is inherited as a co-dominant trait, as the *TPMT* gene is highly polymorphic with over 40 reported variant alleles (16-19).

The wild-type *TPMT*1* allele is associated with normal enzyme activity. Individuals who are homozygous for *TPMT*1* (TPMT normal metabolizers) are more likely to have a typical response to mercaptopurine and a lower risk of myelosuppression. This accounts for the majority of patients (~86–97%) (2, 3).

Individuals who are TPMT poor (approximately 0.3%) or intermediate (approximately 3–14%) metabolizers carry variant *TPMT* alleles that encode reduced or absent enzyme activity. Three variant *TPMT* alleles account for over 90% of the reduced or absent activity *TPMT* alleles (20, 21):

- *TPMT*2* (c.238G>C)
- *TPMT*3A* (c.460G>A and c.719A>G)
- *TPMT*3B* (c.460G>A)
- *TPMT*3C* (c.719A>G)

The frequency of *TPMT* alleles varies among different populations. In the United States, the most common low-activity allele in the Caucasian population is *TPMT*3A* (~5%). This allele is also found in individuals who originate from India and Pakistan, but less frequently (16, 20).

In East Asian, African-American, and some African populations, the most common variant is *TPMT*3C* (~2%), although *TPMT*8* may be more common in African populations than previously thought (~2%). In general, *TPMT*2* occurs much less commonly, and *TPMT*3B* occurs rarely (16, 22).

Genetic Testing

Genetic testing is available for several *TPMT* variant alleles, which most commonly includes *TPMT*2*, **3A*, and **3C* as they account for >90% of inactivating alleles. Of note, rare and/or previously undiscovered variants will not be detected by variant-specific genotyping methods (2, 3, 23-26).

TPMT phenotype enzyme activity testing is also available by measuring TPMT activity in red blood cells directly (11). In adult patients taking mercaptopurine as an immunosuppressive agent, there is strong evidence of a near 100% concordance between phenotype and genotype testing. Inflammatory disease processes do not interfere with the accuracy of TPMT activity measurements if the blood sample is taken under standard conditions (e.g., not within two months of a blood transfusion).

However, in patients with leukemia, the concordance between TPMT phenotype and genotype is poor (27). By the time of diagnosis, red cell TPMT activity is typically greatly reduced because of atypical hematopoiesis. Therefore, phenotype testing may wrongly identify an individual as having a TPMT deficiency, e.g., a patient who has two functional copies of the *TPMT* gene (homozygous wild-type) may be determined as having only one functional copy and one nonfunctional variant (*TPMT* heterozygous); and a patient who is *TPMT* heterozygous may be wrongly determined to be *TPMT* homozygous (two copies of nonfunctional *TPMT* variants). In addition, during the course of chemotherapy, *TPMT* phenotype testing may reveal excessively high TPMT activity. This is thought to be due to an excess of young red blood cells with their associated higher level of TPMT enzyme activity. Therefore, to avoid an incorrect TPMT status, genotype testing is recommended for patients with leukemia (27).

Finally, one study reported that *TPMT* genotyping was more reliable than phenotyping in identifying patients at risk of adverse reactions from thiopurine treatment (28), and several studies reported that the *TPMT* genotype is a better indicator than TPMT activity for predicting TGN accumulation or treatment outcome (12, 29-31).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2015 Statement from the US Food and Drug Administration (FDA): Individuals who are homozygous for an inherited defect in the TPMT (thiopurine-S-methyltransferase) gene are unusually sensitive to the myelosuppressive effects of mercaptopurine and prone to developing rapid bone marrow suppression following the initiation of treatment. Laboratory tests are available, both genotypic and phenotypic, to determine the TPMT status. Substantial dose reductions are generally required for homozygous-TPMT deficient patients (two non-functional alleles) to avoid the development of life threatening bone marrow suppression. Although heterozygous patients with intermediate TPMT activity may have increased mercaptopurine toxicity, this is variable, and the majority of patients tolerate normal doses of mercaptopurine. If a patient has clinical or laboratory evidence of severe toxicity, particularly myelosuppression, TPMT testing should be considered. In patients who exhibit excessive myelosuppression due to 6-mercaptopurine, it may be possible to adjust the mercaptopurine dose and administer the usual dosage of other myelosuppressive chemotherapy as required for treatment.

Please review the complete therapeutic recommendations that are located here: (1).

2013 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC): Testing for *TPMT* status is recommended prior to starting mercaptopurine therapy so that the starting dosages can be adjusted accordingly—see Table 1 for dosing recommendations. In homozygous variant individuals, consider an alternative agent for nonmalignant conditions and drastically reduce doses in malignant conditions. In heterozygous individuals, depending on the disease being treated, starting doses should be reduced. In both patient groups, a longer period of time should be left after each dose adjustment to allow for a steady state to be reached.

Please review the complete therapeutic recommendations that are located here: (2, 3).

Nomenclature

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*TPMT*2*	238G>C Ala80Pro	NM_000367.2:c.238G>C	NP_000358.1:p.Ala80Pro	rs1800462
*TPMT*3A*	This allele contains two variants in cis: c.460G>A and c.719A>G			
*TPMT*3B*	460G>A Ala154Thr	NM_000367.2:c.460G>A	NP_000358.1:p.Ala154Thr	rs1800460
*TPMT*3C*	719A>G Tyr240Cys	NM_000367.2:c.719A>G	NP_000358.1:p.Tyr240Cys	rs1142345

The TPMT Nomenclature Committee defines the nomenclature and numbering of novel TPMT variants: http://www.imh.liu.se/tpmtalleles

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labelled all formulations containing the generic drug.

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Metoprolol Therapy and *CYP2D6* Genotype

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Introduction

Metoprolol is a beta blocker used in the treatment of hypertension, angina, and heart failure. Metoprolol selectively blocks $beta_1$ adrenoreceptors mainly expressed in cardiac tissue. Blockade of these receptors reduces the heart rate and decreases the force of heart contractions.

Metoprolol is primarily metabolized by the CYP2D6 enzyme. Approximately 8% of Caucasians and 2% of most other populations have absent CYP2D6 activity and are known as "*CYP2D6* poor metabolizers." In addition, a number of drugs inhibit CYP2D6 activity, such as quinidine, fluoxetine, paroxetine, and propafenone.

The FDA-approved drug label for metoprolol states that *CYP2D6* poor metabolizers, and normal metabolizers who concomitantly take drugs that inhibit CYP2D6, will have increased (several-fold) metoprolol blood levels, decreasing metoprolol's cardioselectivity (1).

The Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP) has published metoprolol dosing recommendations based on *CYP2D6* genotype. For individuals who have a *CYP2D6* gene variation that reduces the conversion of metoprolol to inactive metabolites, DPWG states that the clinical consequences are limited mainly to the occurrence of asymptomatic bradycardia. For CYP2D6 poor metabolizers, if a gradual reduction in heart rate is desired, or in the event of symptomatic bradycardia, DPWG recommends increasing the dose of metoprolol in smaller steps and/or prescribing no more than 25% of the standard dose. For other cases, no action is required (2).

Please note: Beta blockers such as metoprolol have been demonstrated in several large trials to be safe and effective for treatment of patients with cardiovascular disease. As a mainstay of therapy associated with improvements in quality of life, hospitalization rates, and survival (3, 4), clinical care pathways that might lead to underutilization of beta blockers require scrutiny. FDA points out that CYP2D6 poor metabolizers will have decreased cardioselectivity for metoprolol due to increased metoprolol blood levels. Yet, it is common clinical practice to adjust the dose of metoprolol according to the patient's heart rate. FDA does not specifically comment on the role of genetic testing for initiating therapy.

Drug: Metoprolol

Metoprolol is a commonly prescribed drug that belongs to the drug class of beta-adrenoreceptor antagonists, also known as "beta blockers." Metoprolol is indicated to treat hypertension, angina, and heart failure (stable, symptomatic (NYHA Class II or III) heart failure). Metoprolol selectively blocks the $beta_1$ adrenoreceptor (1).

There are two main types of adrenoreceptors, alpha and beta, each of which have numbered subtypes. The beta adrenoreceptors have three subtypes, $beta_1$, $beta_2$, and $beta_3$. All three subtypes are coupled to the G_s protein, which in turn activates adenylate cyclase enzyme, which catalyzes the production of cyclic AMP (cAMP).

The binding of an agonist, such as the catecholamines adrenaline and noradrenaline, to beta receptors leads to a rise in the intracellular concentration of cAMP, which triggers signaling pathways. Stimulation of the $beta_1$ receptor, which is predominantly expressed in cardiac tissue, leads to an increase in heart rate and an increase in

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the contractility of the atria and ventricles. It also leads to the increased secretion of hormones from other tissues —renin (from the kidneys), ghrelin (from the stomach), and amylase (from the salivary glands).

In the treatment of heart failure, beta blockers such as extended-release metoprolol are thought to protect the heart from increased catecholamine stimulation. In the short term, adrenergic activation can help the heart maintain cardiac performance, but over time, continued activation can be detrimental. Harmful effects include a persistently increased heart rate, down-regulation and impaired functioning of the beta receptors, and myocyte hypertrophy and death—which leads to adverse remodeling of heart tissue (5, 6).

Metoprolol exerts its therapeutic effects by reducing the impact of catecholamine stimulation. Metoprolol reduces the heart rate, improves contractile function by stimulating the upregulation of beta-1 receptors, reduces vasoconstriction, and possibly also reduces the risk of arrhythmias (3, 5, 7, 8).

Metoprolol is a racemic mixture of R- and S-enantiomers (an equal amount of left- and right-handed enantiomers, which are molecules that are mirror images of each other, but are not superimposable on one another).

Metoprolol is primarily metabolized by CYP2D6, an enzyme which is absent in about 8% of Caucasians (poor metabolizers) and about 2% of most other populations. Individuals who lack CYP2D6 activity will have higher plasma concentrations of metoprolol, almost 5-fold higher, and may be at an increased risk of side effects (9-12).

In addition, at higher plasma concentrations, metoprolol is less cardio-selective. Metoprolol can inhibit $beta_2$ receptors, which are mainly located in the bronchial and vascular musculature.

Genetic variants of the *CYP2D6* gene have been found to influence the ratio of enantiomers, the dose and dose titration of metoprolol, and to influence heart rate—CYP2D6 poor metabolizers have an increased risk of bradycardia (13-16). However, *CYP2D6* does not appear to influence the efficacy of metoprolol when used to treat hypertension (17).

Variants within the $beta_1$ receptor have also been found to influence the treatment response to specific beta blockers. The most commonly studied is a reduced function variant, Arg389Gly, which leads to reduced levels of cAMP and diminished $beta_1$ receptor signaling cascades (18). Individuals who are homozygous Arg389 carriers may have a more favorable response to metoprolol treatment than individuals who are homozygous for Gly389 (18-21).

The Cytochrome P450 Superfamily

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The *CYP450* genes are very polymorphic and can result in reduced, absent, or increased enzyme activity.

Gene: *CYP2D6*

CYP2D6 is highly polymorphic, with over 100 star (*) alleles described (22). *CYP2D6*1* is the reference (or wild-type) allele encoding enzyme with normal activity. The *CYP2D6*2*, **33*, and **35* alleles are also considered to confer normal activity (Table 1).

Table 1. Activity status of selected *CYP2D6* alleles

Allele type	*CYP2D6* Alleles
Normal function	**1, *2, *33, *35*
Decreased function	**9, *10, *17, *29, *36, *41*

Table 1. continued from previous page.

Allele type	*CYP2D6* Alleles
No function	**3-*8, *11-*16, *19-*21, *38, *40, *42*

For a detailed list of *CYP2D6* alleles, please see (22).

Individuals who have more than two normal function copies of the *CYP2D6* gene are "ultrarapid metabolizers," whereas individuals who carry two normal or one normal and one decreased function allele are classified as "normal metabolizers."

Individuals with one normal and one no function allele or two decreased function alleles are categorized as "normal metabolizers" by recent nomenclature guidelines (23), but have also been categorized as "intermediate metabolizers" in the literature. Subjects with one decreased and one no function allele are predicted to be intermediate metabolizers and those with two no function alleles, poor metabolizers.

The most common no function alleles include *CYP2D6*3*, **4*, **5*, and **6* (24-27), and the most common decreased function alleles include *CYP2D6*9, *10, *17, *29* and **41* (28-32) (Table 1).

There are large inter-ethnic differences in the frequency of these alleles. For example, *CYP2D6*4* is the most common no function allele in Caucasians, but is less abundant in subjects with African ancestry, and is rare in Asians. In contrast, the decreased function allele *CYP2D6*10* is the most common allele in Asians, and *CYP2D6*17* is almost exclusively found in individuals with African ancestry (33).

Consequently, the phenotype frequencies also vary substantially among the major ethnicities and may vary among populations. Approximately 6-8% of European Caucasians and their descendants are poor metabolizers, mainly due to the prevalent no function *CYP2D6*4* and **5* alleles (34, 35).

Genetic Testing

The NIH's Genetic Testing Registry provides examples of the genetic tests that are currently available for metoprolol response and the CYP2D6 gene.

Results are typically reported as a diplotype, such as *CYP2D6 *1/*1*. A result for copy number, if available, is also important when interpreting *CYP2D6* results (36).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2016 Statement from the US Food and Drug Administration (FDA): Metoprolol is metabolized predominantly by CYP2D6, an enzyme that is absent in about 8% of Caucasians (poor metabolizers) and about 2% of most other populations. CYP2D6 can be inhibited by a number of drugs. Poor metabolizers and extensive metabolizers who concomitantly use CYP2D6 inhibiting drugs will have increased (several-fold) metoprolol blood levels, decreasing metoprolol's cardioselectivity.

Please review the complete therapeutic recommendations that are located here: (1).

2016 Summary of recommendations from the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP):

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

CYP2D6 Poor Metabolizers:

The gene variation reduces the conversion of metoprolol to inactive metabolites. However, the clinical consequences are limited mainly to the occurrence of asymptomatic bradycardia.

Recommendation:

If a gradual reduction in heart rate is desired, or in the event of symptomatic bradycardia:

1 Increase the dose in smaller steps and/or prescribe no more than 25% of the standard dose

Other cases:

1 No action required

CYP2D6 Intermediate Metabolizers:

The gene variation reduces the conversion of metoprolol to inactive metabolites. However, the clinical consequences are limited mainly to the occurrence of asymptomatic bradycardia.

Recommendation:

If a gradual reduction in heart rate is desired, or in the event of symptomatic bradycardia:

1 Increase the dose in smaller steps and/or prescribe no more than 50% of the standard dose

Other cases:

1 No action required

CYP2D6 Ultrarapid Metabolizers:

The gene variation increases the conversion of metoprolol to inactive metabolites. This can increase the dose requirement. However, with a target dose of 200 mg/day, there was no effect on the blood pressure and hardly any effect on the reduction of the heart rate.

Recommendation:

1. Use the maximum dose for the relevant indication as a target dose
2. If the effectiveness is still insufficient: increase the dose based on effectiveness and side effects to 2.5 times the standard dose or select an alternative

Possible alternatives include:

- Heart failure: bisoprolol or carvedilol. Bisoprolol: advantage: not metabolised by CYP2D6; disadvantage: elimination depends on the kidney function. Carvedilol: advantage: elimination does not depend on the kidney function; disadvantage: is metabolised (to a lesser extent than metoprolol) by CYP2D6.
- Other indications: atenolol or bisoprolol. Neither is metabolised by CYP2D6.

Please review the complete therapeutic recommendations that are located here: (2)

Nomenclature of selected *CYP2D6* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*4*	1846G>A	NM_000106.5:c.506-1G>A	Variant occurs in a non-coding region (splice variant causes a frameshift)	rs3892097

Table continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*5*	Variant results in a whole gene deletion			
*CYP2D6*6*	1707 del T Trp152Gly CYP2D6T	NM_000106.5:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655
*CYP2D6*10*	100C>T (Pro34Ser)	NM_000106.5:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
*CYP2D6*17*	1023C>T[1] (Thr107Ile)	NM_000106.5:c.320C>T	NP_000097.3:p.Thr107Ile	rs28371706
	2850C>T[2] (Cys296Arg)	NM_000106.5:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
*CYP2D6*41*	2850C>T[2] (Cys296Arg)	NM_000106.5:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
	2988G>A	NM_000106.5:c.985+39 G>A	Variant occurs in a non-coding region (impacts slicing).	rs28371725

[1] In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.
[2] In the literature, 2850C>T is also referred to as 2938C>T.

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: http://www.cypalleles.ki.se/

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Omeprazole Therapy and *CYP2C19* Genotype

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Introduction

Omeprazole blocks the secretion of gastric acid and belongs to the drug class of proton pump inhibitors. It is used to treat gastroesophageal reflux disease (GERD), gastric ulcers, duodenal ulcers, erosive esophagitis, and other acid-related disorders. It is also used in the treatment of hypersecretory conditions, such as Zollinger-Ellison syndrome, and is used in combination with antibiotics to eradicate *Helicobacter pylori (H. pylori)* infection (1).

CYP2C19 is the principal enzyme that metabolizes omeprazole to inactive metabolites. Approximately 3% of Caucasians and 15 to 20% of Asians have reduced or absent CYP2C19 enzyme activity ("poor metabolizers"). In these individuals, standard doses of omeprazole may lead to higher exposure to the drug and improved treatment outcomes (2). In contrast, individuals with increased CYP2C19 activity ("ultrarapid metabolizers") may have an insufficient response to treatment as the active drug is inactivated at a faster rate.

The FDA-approved drug label for omeprazole states that a dose reduction should be considered in the Asian population, particularly for the maintenance of healing of erosive esophagitis (1). The Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP) has published dose alterations based on *CYP2C19* genotype. For *CYP2C19* poor metabolizers, they do not recommend altering the dose; however for ultrarapid metabolizers, they recommend being extra alert to an insufficient response to treatment. For the eradication of *H. pylori* in ultrarapid metabolizers, they recommend increasing the dose of omeprazole by 100–200%, and to consider the same dose increase for other conditions (see Table 1) (3, 4).

Table 1. *CYP2C19* phenotypes and the therapeutic recommendations for omeprazole therapy, adapted from the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP).

Phenotype	Phenotype details	Examples of diplotypes	Therapeutic (dose) recommendations for omeprazole
Ultrarapid metabolizer	Normal or increased CYP2C19 activity	**17/*17*	Be extra alert to insufficient response. For the eradication of *H. pylori*, increase dose by 100–200%. For other conditions, consider dose increase by 100–200%.
Extensive metabolizer	Normal CYP2C19 activity	**1/*1*	No recommendations
Intermediate metabolizer	Decreased CYP2C19 activity	**1/*2* **1/*3* **2/*17* **3/*17*	No recommendations
Poor metabolizer	Markedly reduced or absent CYP2C19 activity	**2/*2* **2/*3* **3/*3*	No recommendations

Good quality evidence supports the dose recommendations for poor and intermediate metabolizers; moderate quality evidence supports the dose recommendations for ultrarapid metabolizers.
Table is adapted from Swen J.J., Nijenhuis M., de Boer A., Grandia L. et al. Pharmacogenetics: from bench to byte - an update of guidelines. Clinical pharmacology and therapeutics. 2011;89(5):662–73 (3).

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Drug class: Proton Pump Inhibitors

Proton pump inhibitors (PPIs) are inhibitors of gastric acid secretion that are used in the treatment of stomach-acid related disorders. PPIs are also used to prevent and treat ulcers associated with nonsteroidal anti-inflammatory drugs (NSAIDs), and can be used in combination with antibiotics to eradicate *H. pylori* infection.

Six PPIs are currently FDA-approved for clinical use: esomeprazole, dexlansoprazole, lansoprazole, omeprazole, pantoprazole, and rabeprazole. All PPIs are similarly potent at inhibiting gastric acid secretion and are thought to be similarly efficacious (5, 6).

PPIs are metabolized and inactivated by a number of CYP enzymes, including CYP2C19, which has a principal role in the metabolism of omeprazole. The increased function *CYP2C19*17* variant allele may enhance PPI clearance (7) resulting in less active PPI available to inhibit gastric acid secretion. In contrast, the *CYP2C19*2* loss-of-function allele is associated with decreased PPI clearance, resulting in more active PPI available and enhanced treatment. For several PPIs, including omeprazole and lansoprazole, higher drug levels in patients with low or absent CYP2C19 activity have been associated with increased drug efficacy and improved treatment outcomes (2, 8).

Drug: Omeprazole

Omeprazole was the first PPI to be introduced to the US market in 1989. Today, omeprazole is one of the PPIs that are available both as prescription and over-the-counter (OTC) medications.

In adults, omeprazole is used in the treatment of ulcers (gastric and duodenal), GERD, and to maintain healing of erosive esophagitis. Omeprazole is also used in the long-term treatment of hypersecretory conditions such as Zollinger-Ellison syndrome, multiple endocrine adenomas, and systemic mastocytosis. In children, omeprazole is used in the treatment of GERD and erosive esophagitis (1).

The human stomach contains approximately one billion parietal cells that secrete hydrochloric acid (HCl) into the gastric lumen. Gastric acid aids digestion by hydrolyzing dietary protein and facilitating the absorption of calcium, iron, and vitamin B. Gastric acid also helps maintain a sterile environment by suppressing the growth of bacteria (9).

Hydrogen ions (H+) are actively secreted in to the gastric lumen in exchange for potassium ions (K+) via an H^+/K^+-ATPase, which is also known as a "proton pump". Located on the surface of gastric parietal cells, the proton pump controls the last step in acid secretion, and by targeting this step, omeprazole and the other PPIs are able to potently inhibit gastric acid secretion.

Omeprazole is metabolized and inactivated in the liver by the cytochrome P450 system. CYP2C19 is the principal enzyme involved, although other enzymes such as CYP3A4 may also contribute. Omeprazole is metabolized to hydroxy and desmethyl metabolites, which have no effect on gastric acid secretion (10).

Individuals with reduced CYP2C19 enzyme activity may experience twice the exposure to omeprazole compared to individuals with normal enzyme function. This reduced enzyme activity has a positive effect on clinical outcomes, and because PPIs are generally regarded as safe drugs, especially in the short-term (less than 6 months), this can have a beneficial effect without an increased risk of omeprazole toxicity (11, 12).

One study reported that when using omeprazole as part of the treatment to eradicate *H. pylori*, success was achieved in all patients who had little or no CYP2C19 activity, but in only 29% of patients who had "normal" CYP2C19 activity. Similar results were found in another study that evaluated lansoprazole in the treatment of GERD: the cure rate was 85% for patients with little or no CYP2C19 activity, compared to 16% for patients with normal CYP219 activity (13-15).

The FDA-approved drug label for omeprazole does not comment on dose adjustments based on CYP2C19 status. However, guidelines from KNMP recommend that patients with increased CYP2C19 activity ("ultrarapid metabolizers") should receive an increased dose of omeprazole for the eradication of *H. pylori*, and that an increased dose should be considered for other indications (Table 1).

The long-term use of PPIs has been associated with several adverse effects. Daily treatment with any PPI for longer than three years may lead to malabsorption of vitamin B12, caused by hypochlorhydria. Because prolonged hypochlorhydria also increases the risk of *Clostridium difficile* infection, and may increase the risk for osteoporosis-related fractures, the FDA recommends that patients should use the lowest dose and shortest duration of PPI therapy appropriate to the condition being treated (1).

Gene: *CYP2C19*

The cytochrome P450 superfamily (CYP) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP genes are very polymorphic and can result in reduced, absent, or increased drug metabolism.

The CYP2C19 enzyme contributes to the metabolism of a range of clinically important drugs, such as antidepressants, benzodiazepines, and some of the PPIs, including omeprazole.

CYP2C19 is highly polymorphic, as 35 variant star (*) alleles are currently catalogued at the Human Cytochrome P450 (CYP) Allele Nomenclature Database (http://www.cypalleles.ki.se/cyp2c19.htm). The *CYP2C19*1* wild-type allele is associated with normal enzyme activity and the "extensive metabolizer" phenotype (16).

The most common loss-of-function variant is *CYP2C19*2*, which contains a c.681G>A variant in exon 5 that results in an aberrant splice site. This leads to the production of a truncated and non-functioning protein. The *CYP2C19*2* allele frequencies are ~15% in Caucasians and Africans, and ~29–35% in Asians (17). "Intermediate metabolizers" carry one copy of an allele that encodes reduced or absent function (e.g. **1/*2*), whereas "poor metabolizers" are homozygous or compound heterozygous for two loss-of-function alleles (e.g., **2/*2*, **2/*3*).

Another commonly tested loss-of-function variant is *CYP2C19*3*, which contains a c.636G>A variant in exon 4 that causes a premature stop codon. The *CYP2C19*3* allele frequencies are ~2–9% in Asian populations, but rare in other racial groups. Other loss-of-function variants occur in less than 1% of the general population, and include *CYP2C19*4-*8* (17, 18).

In contrast to non-functional alleles, the *CYP2C19*17* allele (c.-806C>T) is associated with increased enzyme activity. Allele frequencies range from 3 to 21% across different populations (19). Individuals who are homozygous for the **17* allele are known as "ultrarapid metabolizers", and it is this patient group who may benefit from an increased dose of omeprazole. However, not all studies have identified a significant effect of *CYP2C19*17* on the metabolism of PPIs and treatment outcomes (15, 20, 21).

Genetic Testing

Currently, the FDA does not provide recommendations about the use of *CYP2C19* genetic testing for omeprazole treatment (1).

Clinical genotyping tests are available for several *CYP2C19* alleles, and a list of some test providers is available at the Genetic Testing Registry (GTR) of the National Institutes of Health: http://www.ncbi.nlm.nih.gov/gtr/tests/?term=1557[geneid].

Usually a patient's result is reported as a diplotype, such as *CYP2C19 *1/*1*, and may also include an interpretation of the patient's predicted metabolizer phenotype (ultrarapid, extensive, intermediate, or poor).

Table 1 summarizes common *CYP2C19* phenotypes with recommendations developed by the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2014 Statement from the US Food and Drug Administration (FDA): Asian Population: In pharmacokinetic studies of single 20 mg omeprazole doses, an increase in AUC of approximately four-fold was noted in Asian subjects compared with Caucasians. Dose reduction, particularly where maintenance of healing of erosive esophagitis is indicated, for Asian subjects should be considered.

Please review the complete therapeutic recommendations that are located here: (1)

2011 Summary of recommendations from the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP): For individuals who are ultrarapid metabolizers, an increase in the dose of omeprazole by 100–200% is recommended for the eradication of *H.pylori*, and the physician should be extra alert to an insufficient response. For other conditions, the physician should remain extra alert to an insufficient response, and consider a dose increase by 100–200%.

There are no therapeutic (dose) recommendations for individuals who are either poor or intermediate metabolizers.

Please review the complete therapeutic recommendations that are located here: (3).

Nomenclature

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C19*2*	681G>A Pro227Pro	NM_000769.1:c.681G>A	NP_000760.1:p.Pro227=	rs4244285
*CYP2C19*3*	636G>A Trp212Ter	NM_000769.1:c.636G>A	NP_000760.1:p.Trp212Ter	rs4986893
*CYP2C19*17*	-806C>T	NM_000769.2:c.-806C>T	Not applicable—variant occurs in a non-coding region	rs12248560

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: http://www.cypalleles.ki.se/

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[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labelled all formulations containing the generic drug.

Version History

To view an earlier version of this summary (update: 18 March 2013), please click here.

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Pertuzumab Therapy and *ERBB2 (HER2)* Genotype

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Created: September 10, 2015.

Introduction

Pertuzumab is a monoclonal antibody used in the treatment of breast cancer. It targets a receptor in the epidermal growth factor family encoded by the *ERBB2* gene, which is commonly referred to as the *HER2* gene.

The *HER2* gene is overexpressed in 15-20% of breast cancers and is also overexpressed in some cases of other cancer types (gastric, colon, head and neck). Overall, "HER2 positive" tumors are associated with a faster rate of growth and a poorer prognosis. The use of pertuzumab in treatment regimens for breast cancer improves outcomes, but adverse effects of therapy include cardiac toxicity.

The FDA-approved drug label for pertuzumab states that pertuzumab should only be used to treat patients with tumors which have either HER2 protein overexpression or *HER2* gene amplification, as determined by an accurate and validated FDA-approved assay. This is because these are the only patients studied for whom benefit has been shown (1) .

A guideline from ASCO/CAP states that oncologists must request HER2 testing on every primary invasive breast cancer (and on a metastatic site, if stage IV and if specimen available) from a patient with breast cancer to guide decision to pursue HER2-targeted therapy. This should be especially considered for a patient who previously tested HER2 negative in a primary tumor and presents with disease recurrence with clinical behavior suggestive of HER2-positive or triple-negative disease (2).

Drug: Pertuzumab

Pertuzumab (brand name, Perjeta) is a monoclonal antibody that targets ERBB2 (a tyrosine kinase receptor, also known as HER2 or HER-2/neu). Pertuzumab is only used to treat specific tumors that overexpress ERBB2; these tumors are known as "HER2-positive" tumors.

Pertuzumab is used in the treatment of HER2-positive metastatic breast cancer to increase the chance of long-term disease-free survival. Pertuzumab is used in combination with trastuzumab (another monoclonal antibody that targets ERBB2) and docetaxel (a chemotherapy drug) (1).

Recently, HER2 targeted therapy has been approved by the FDA for use in the neoadjuvant setting. Neoadjuvant therapy is given before surgical therapy in women with early stage breast cancer. In the neoadjuvant setting, pertuzumab, along with trastuzumab and docetaxel, is used to treat HER2-positive breast cancer, which may be at an early stage, locally advanced, or inflammatory (1, 3, 4).

Before treatment with pertuzumab begins, overexpression of the HER-2 protein or amplification of the *HER-2* gene must first be determined. In clinical studies of pertuzumab, patients with breast cancer were required to have evidence of HER-22 overexpression defined as 3+ IHC or FISH amplification ratio of 2 or greater (see Genetic Testing) (1). The FDA recommends that testing be performed using an FDA-approved test, in a laboratory with demonstrated proficiency with the technology being used. This is because the benefits of pertuzumab have only been proven in patients with tumors that overexpress HER2. In addition, although pertuzumab is generally well tolerated, the risks of treatment include infusion reactions, and rarely pulmonary toxicity, and cardiomyopathy that can result in cardiac failure.

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Pertuzumab targets the HER2 receptor by binding to a specific region in its extracellular domain. The HER2 receptor is an epidermal growth factor receptor, consisting of an intracellular tyrosine kinase domain, a single transmembrane spanning region, and an extracellular domain, comprised of four subdomains (I – IV). Pertuzumab binds to subdomain II and trastuzumab binds to subdomain IV. This binding limits the receptor's ability to activate its intrinsic kinase, which in turn, limits the activation of numerous signaling pathways that can promote cell growth.

A number of proposed mechanisms may underlie the anti-tumor effects of pertuzumab and trastuzumab. One such mechanism is that these drugs block the HER3 receptor from binding to HER2. The HER2-HER3 dimerized receptor is thought to be highly active, triggering many signaling cascades in the absence of a "true" ligand (5-8).

Another proposed mechanism is antibody-dependent cellular cytotoxicity (ADCC). Once pertuzumab or trastuzumab have bound to a cancer cell, immune cells (typically activated natural killer cells) bind to the drug and initiate lysis of the cancer cell (9). Trastuzumab may also mediate the enhanced internalization and degradation of the HER2 receptor, inhibit angiogenesis, and inhibit HER2 shedding by preventing the cleavage of HER2 and the subsequent release of its extracellular domain (10, 11).

Unfortunately, breast cancer may start to progress again during HER2 targeted therapy. Possible mechanisms that may facilitate drug resistance and disease progression during treatment include increased signaling from the HER family of receptors, an upregulation of downstream signaling pathways, and an increased level of insulin growth factor -1 receptor (12, 13).

At the time of writing, four drugs have been approved to target HER2 (pertuzumab, trastuzumab, lapatinib, and T-DM1), with more drugs in clinical trials.

Gene: ERBB2 (HER2)

The human epidermal growth factor receptor (HER) family consists of four members: the epidermal growth factor receptor (EGFR), HER2, HER3, and HER4 (see Nomenclature). All four members are transmembrane tyrosine kinase receptors, and they regulate a number of important cellular processes, such as cell growth, survival, and differentiation (14).

HER2, along with *EGFR*, are proto-oncogenes. Proto-oncogenes are a group of genes that, when mutated or expressed at abnormally high levels, can contribute to abnormal cell growth. The mutated version of the proto-oncogene is called an oncogene. Proto-oncogenes typically encode proteins that stimulate cell division, inhibit cell differentiation, and halt cell death. All these are important biological processes. However, the increased production of these proteins, caused by oncogenes, can lead to the proliferation of poorly differentiated cancer cells (15).

The official gene symbol for *HER2* is *ERBB2*, which is derived from a viral oncogene with which the receptor shares homology; "v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2." However, clinicians commonly refer to the *ERBB2* gene as "*HER2*" (Human Epidermal growth factor Receptor 2) or "*HER2/neu*" (neu was the name given to the gene that caused cancer derived from a rodent neuro/glioblastoma). *HER2* is an alternate gene symbol for *ERBB2* and is more commonly used by the community in clinical care.

One unique feature of ERBB2 compared to the other receptors in the HER family is the absence of a known ligand. It is therefore thought that this receptor may permanently be in an activated state, or it may become activated during heterodimerization with one of the other members of the HER family (11). And, one unique feature of HER3 is that it has very little enzymatic activity compared to the other tyrosine kinase receptors in the HER family. It is therefore thought that an important role of HER3 is to act as a heterodimerization partner for ERBB2 (16, 17).

When a partner such as HER3 binds to ERBB2, the heterodimer undergoes activation, which stimulates the intrinsic tyrosine kinase activity of the receptor. Autophosphorylation of several key residues of the receptor triggers the downstream activation of many commonly used growth factor signaling pathways, such as the PI3K/AKT/mTOR pathway and the RAS/RAF/MEK/ERK pathway (18, 19). Impaired ERBB2 signaling is associated with the development of neurodegenerative diseases, such as multiple sclerosis and Alzheimer disease, whereas excessive ERBB2 signaling is associated with the development of cancers.

ERBB2 is overexpressed in approximately 15-20% of breast tumors, as a result of amplification of the *ERBB2* gene, and tumors with increased ERBB2 usually have a higher growth rate and more aggressive clinical behavior (2, 20-22). Although gene amplification is frequently seen in cancer and other degenerative disorders, the underlying basis for amplification remain largely unknown (23). And in the case of ERBB2, although sequence variants have been identified, it is nearly always the wildtype *ERBB2* gene that is overexpressed in tumors (24). In about 1% of breast cancers, activating mutations in *ERBB2* can be identified that are likely to drive tumorigenesis, without *ERBB2* amplification (25).

Tumor Testing for *ERBB2 (HER2)*

There are two main methods used for HER2 testing: testing for overexpression of the HER2 protein using immunohistochemistry (IHC), or testing for gene amplification using in-situ hybridization (ISH). Each assay type has diagnostic pitfalls that must be avoided, and so the pathologist who reviews the histologic findings should determine the optimal assay (IHC or ISH) for the determination of HER2 status (2, 22).

In an IHC assay, a slice of tumor tissue is stained, along with a control sample that contains high levels of HER2. The tumor sample is then examined by light microscopy to assess the intensity of membrane staining—the amount of staining correlates with the quantity of HER2 protein and is typically graded from 0 to 3+:

- IHC 0 means no visible staining and is an "HER2 negative" result
- IHC 1+ is also an "HER2 negative" result—there is a staining pattern with weak and incomplete staining, or weak and complete staining of very few tumor cells
- IHC 2+ is an "HER2 equivocal result"—there is a staining pattern with moderately intense staining, or intense staining of very few tumor cells
- IHC 3+ is an "HER2 positive result"—there is a staining pattern with intense membrane staining on more than 10% of tumor cells, indicating a higher than normal level of HER2

For an equivocal (IHC 2+) result, either a reflex test must be ordered (same specimen using ISH), or a new test must be ordered (using a new specimen, if available, using IHC or ISH) to confirm the results.

The ISH assay, or FISH assay (fluorescence in situ hybridization), measures *HER2* gene amplification by measuring *HER2* DNA—the actual number of copies of the *HER2* genes are counted. Under the microscope, the genes appear as red signals or dots, in a blue-stained cancer cell nucleus. The result is usually either FISH negative (normal level of *HER2* gene) or FISH positive (at least twice as much as normal level of *HER2* gene), but in a small number of cases the FISH result will be equivocal due to a low level of *HER2* amplification. The use of a control helps distinguish between a negative result and a non-informative result caused by an error. Approximately 25% of patients who have an IHC 2+ result will have a FISH positive result (26).

For the complete algorithms for evaluation of HER2 protein expression using IHC or ISH, please see the American Society of Clinical Oncology (ASCO) guidelines, located here: (27)

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

Statement from the US Food and Drug Administration (FDA):

Detection of HER2 protein overexpression is necessary for selection of patients appropriate for pertuzumab therapy because these are the only patients studied and for whom benefit has been shown. Patients with breast cancer were required to have evidence of HER2 overexpression defined as 3+ IHC or FISH amplification ratio ≥ 2.0 in the clinical studies. Only limited data were available for patients whose breast cancer was positive by FISH, but did not demonstrate protein overexpression by IHC.

Assessment of HER2 status should be performed by laboratories using FDA-approved tests with demonstrated proficiency in the specific technology being utilized. Improper assay performance, including use of sub-optimally fixed tissue, failure to utilize specified reagents, deviation from specific assay instructions, and failure to include appropriate controls for assay validation, can lead to unreliable results.

Please review the complete therapeutic recommendations that are located here: (1).

FDA-approved medical devices for HER2 are listed here.

Excerpted recommendations from the American Society of Clinical Oncology / College of American Pathologists 2013 clinical practice guideline update:

Key Recommendations for Oncologists

- Must request HER2 testing on every primary invasive breast cancer (and on metastatic site, if stage IV and if specimen available) from a patient with breast cancer to guide decision to pursue HER2-targeted therapy. This should be especially considered for a patient who previously tested HER2 negative in a primary tumor and presents with disease recurrence with clinical behavior suggestive of HER2-positive or triple-negative disease.
- Should recommend HER2-targeted therapy if HER2 test result is positive, if there is no apparent histopathologic discordance with HER2 testing and if clinically appropriate.
- Must delay decision to recommend HER2-targeted therapy if initial HER2 test result is equivocal. Reflex testing should be performed on the same specimen using the alternative test if initial HER2 test result is equivocal or on an alternative specimen.
- Must not recommend HER2-targeted therapy if HER2 test result is negative and if there is no apparent histopathologic discordance with HER2 testing.
- Should delay decision to recommend HER2-targeted therapy if HER2 status cannot be confirmed as positive or negative after separate HER2 tests (HER2 test result or results equivocal). The oncologist should confer with the pathologist regarding the need for additional HER2 testing on the same or another tumor specimen.
- If the HER2 test result is ultimately deemed to be equivocal, even after reflex testing with an alternative assay (i.e., if neither test is unequivocally positive), the oncologist may consider HER2-targeted therapy. The oncologist should also consider the feasibility of testing another tumor specimen to attempt to definitely establish the tumor HER2 status and guide therapeutic decisions. A clinical decision to ultimately consider HER2-targeted therapy in such cases should be individualized on the basis of patient status (comorbidities, prognosis, and so on) and patient preferences after discussing available clinical evidence.

Please review the complete therapeutic recommendations, including Key Recommendations for Pathologists that are located here (2).

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

Nomenclature

Common gene symbols	Alternative gene symbols
EGFR	*ERBB1* *ERBB* *HER1*
ERBB2	*HER2* *HER-2* *HER-2/neu* *NEU*
ERBB3	*HER3*
ERBB4	*HER4*

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Phenytoin Therapy and *HLA-B*15:02* and *CYP2C9* Genotypes

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Introduction

Phenytoin is an antiseizure medication used for the prevention of focal seizures and generalized tonic-clonic convulsions (1).

Phenytoin has a narrow therapeutic index—patients that have toxic blood concentrations of phenytoin have inreased risks of acute side effects. Dosing can be complex due to pharmacokinetic factors, including patient weight, age, sex, concomitant medications, plasma binding protein stats, the presence of uremia or hyperbilirubinemia, and specific pharmacogenetic variants. As such, therapeutic drug monitoring is often used to adjust dose and maintain serum concentrations within the therapeutic range (10–20 µg/mL).

CYP2C9 is one of the main enzymes involved in the metabolism of phenytoin, and variant *CYP2C9* alleles are known to influence phenytoin drug levels. Individuals who carry decreased activity *CYP2C9* variants may have reduced clearance rates of phenytoin and be at greater risk for dose-related side effects (2).

An individual's human leukocyte antigen B (*HLA-B*) genotype is a known risk factor for drug-induced hypersensitivity reactions. HLA-B has an important immunological role in pathogen recognition and response, as well as to non-pathogens such as drugs. Carriers of the variant *HLA-B*15:02* allele are at high risk of developing potentially life-threatening phenytoin-induced Stevens-Johnson syndrome (SJS) and the related toxic epidermal necrolysis (TEN).

The *HLA-B*15:02* variant is most commonly found among individuals of Southeast Asian descent, where there is a strong association between SJS/TEN and exposure to carbamazepine. Carbamazepine is an antiseizure medication used to treat the same types of seizures as phenytoin, as well as trigeminal neuralgia and bipolar disorder.

The FDA-approved drug label for phenytoin states that consideration should be given to avoiding phenytoin as an alternative for carbamazepine in patients positive for *HLA-B*15:02*. The label also mentions that variant *CYP2C9* alleles may contribute to unusually high levels of phenytoin (1).

The Clinical Pharmacogenetics Implementation Consortium (CPIC) recommends the use of an antiseizure medication other than carbamazepine, phenytoin (or its prodrug fosphenytoin) for any *HLA-B*15:02* carrier regardless of *CYP2C9* genotype, patient ancestry or age. CPIC also recommends consideration of at least a 25% reduction in the starting maintenance dose for patients who are *CYP2C9* intermediate metabolizers and *HLA-B*15:02* negative, and at least a 50% reduction for *CYP2C9* poor metabolizers and *HLA-B*15:02* negative, with subsequent maintenance doses adjusted based on therapeutic drug monitoring and response (Table 1) (2).

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Table 1. 2014 Therapeutic recommendations for phenytoin therapy based on *HLA-B* and *CYP2C9* genotypes, adapted from Clinical Pharmacogenetics Implementation Consortium (CPIC)

Phenotype	*HLA-B*15:02* positive		*HLA-B*15:02* negative	
	Implication	Therapeutic recommendation	Implication	Therapeutic recommendation
CYP2C9 normal metabolizer	Increased risk of phenytoin- induced SJS/ TEN	If patient is phenytoin naive,[A] do not use phenytoin/ fosphenytoin[B]	Normal phenytoin metabolism	Initiate therapy with recommended maintenance dose [C]
CYP2C9 intermediate metabolizer	Increased risk of phenytoin- induced SJS/ TEN	If patient is phenytoin naive,[A] do not use phenytoin/ fosphenytoin[B]	Reduced phenytoin metabolism. Higher plasma concentrations will increase probability of toxicities	Consider 25% reduction of recommended starting maintenance dose.[C] Subsequent maintenance doses should be adjusted according to therapeutic drug monitoring and response
CYP2C9 poor metabolizer	Increased risk of phenytoin- induced SJS/ TEN	If patient is phenytoin naive,[A] do not use phenytoin/ fosphenytoin[B]	Reduced phenytoin metabolism. Higher plasma concentrations will increase probability of toxicities	Consider 50% reduction of recommended starting maintenance dose.[C] Subsequent maintenance doses should be adjusted according to therapeutic drug monitoring and response

SJS/TEN: Stevens–Johnson syndrome/toxic epidermal necrolysis.
The strength of the therapeutic recommendations is classified as "strong" for all recommendations, with the exception of the recommendation for *CYP2C9* intermediate metabolizers who are *HLA-B*15:02* non carriers, which is classified as "moderate".
[A] If the patient has previously used phenytoin for longer than 3 months without incidence of cutaneous adverse reactions, reinitiate phenytoin with caution. Adjust dose based on *CYP2C9* genotype if known.
[B] Carbamazepine should not be used as an alternative. Alternative medications such as oxcarbazepine, eslicarbazepine acetate, and lamotrigine have some evidence linking SJS/TEN with the *HLA-B*15:02* allele, and thus caution should be used in choosing alternatives to phenytoin).
[C] Recommended maintenance dose based on patient's clinical characteristics.
Table is adapted from Caudle KE, Rettie AE, Whirl-Carrillo M, Smith LH, Mintzer S, Lee MT, Klein TE, Callaghan JT. Clinical pharmacogenetics implementation consortium guidelines for CYP2C9 and HLA-B genotypes and phenytoin dosing. Clinical pharmacology and therapeutics. 2014:96(5):542-8 (2).
Note: The nomenclature used in this table reflects the standardized pharmacogenetic terms proposed by CPIC (3).

Drug: Phenytoin

Phenytoin is a generic antiseizure drug that is rarely prescribed to newly diagnosed patients due to its propensity for long-term side effects. Nevertheless, it continues to be used by many patients who initiated treatment prior to the availability of newer medications that have fewer side effects and drug-drug interactions. Phenytoin is used for the control of partial seizures and generalized tonic-clonic convulsions. It is also used in the treatment of status epilepticus and may be used to prevent or treat seizures that occur during and following neurosurgery (1).

Phenytoin belongs to the sodium channel blockers class of antiseizure drugs, which are thought to suppress seizure activity by blocking voltage-gated sodium channels that are responsible for the upstroke of action potentials (4, 5). The block by phenytoin and other members of this class of antiseizure drugs occurs in a state-dependent fashion, with preferential binding and block of the inactivated state of the channel. This results in voltage- and frequency-dependent block in which high frequency action potential firing, which occurs during epileptic activity, is preferentially inhibited (1, 6)

The dosing of phenytoin can be complex, as treatment is typically initiated at a low starting dose, which considers patient age, weight, and the presence of concomitant medications that may influence phenytoin metabolism or protein binding. The dose is then carefully escalated to obtain the desired therapeutic effect. There is a wide variation in how individuals respond to phenytoin (2). Therapeutic drug monitoring is often used to adjust the dose to ensure that plasma levels are within therapeutic range (10–20 μg/dl in adults). Measurement of plasma levels is useful when adding or discontinuing concomitant medications that effect phenytoin levels. Periodic measurement of plasma phenytoin concentrations may also be valuable in pregnancy, because altered phenytoin pharmokinetics increases the risk of seizures.

Phenytoin use during pregnancy has been associated with an 11% risk in the offspring of the fetal hydantoin syndrome, in which there is dysmorphism, hypoplasia and irregular ossification of the distal phalanges. Facial dysmorphism includes epicanthal folds, hypertelorism, broad flat nasal bridges, an upturned nasal tip, wide prominent lips, and, in addition, distal digital hypoplasia, intrauterine growth retardation, and mental retardation. An additional 30% of the *in utero*-exposed children express fetal hydantoin effects, in which there is a more limited pattern of dysmorphic characteristics. Some studies have found significant associations between in utero exposure to phenytoin and major congenital abnormalities (mainly, cardiac malformations and cleft palate) whereas others have failed to find such associations (7, 8).

The adverse effects of phenytoin fall into two categories, types A and B. Type A adverse drug reactions account for up to 90% of reactions. They are predictable and can occur in any individual if their drug exposure is high enough. Some of these reactions occur rapidly and are reversible when the dose is reduced. These include acute central nervous system adverse effects such as sedation, nystagmus, and ataxia. Other common side effects occur with long-term exposure and include changes to the physical appearance, such as gingival hyperplasia, coarsening of the facial features, hirsuitism, and acne.

Type B adverse drug reactions include idiosyncratic hypersensitivity reactions. Such reactions can occur at any dose and develop through a mechanism that is unrelated to the mechanism of action of the drug.

A rare but life-threatening hypersensitivity reaction associated with phenytoin treatment is Stevens-Johnson syndrome (SJS) and the related toxic epidermal necrolysis (TEN). Both are severe cutaneous reactions to specific drugs, and are characterized by fever and lesions of the skin and mucous membranes, with a mortality rate of up to 30% (9).

It is difficult to predict in whom a drug-induced hypersensitivity reaction is likely to occur. For phenytoin, however, carriers of a specific *HLA* variant are known to be susceptible to phenytoin-induced SJS/TEN. *HLA* testing of patients can identify those at-risk individuals so that an alternative drug can be used.

HLA gene family

The human leukocyte antigen (*HLA*) genes are members of the Major Histocompatability Complex (*MHC)* gene family, which includes more than 200 genes. The *MHC* family has been subdivided into three subgroups based on the structure and function of the encoded proteins: Class I, Class II, and Class III. The class I region contains the genes encoding the HLA molecules HLA-A, HLA-B, and HLA-C. These molecules are expressed on the surfaces of almost all cells and play an important role in processing and presenting antigens. The class I gene region also contains a variety of other genes, many of which are not known to be involved in immune function.

An important role of HLA class I molecules is to present peptide fragments to immune cells (CD8+ T cells). Most of these peptides originate from the breakdown of normal cellular proteins ("self"). However, if foreign peptide fragments are presented, e.g., from a pathogen, CD8+T cells will recognize the peptides as "non-self" and will be activated to release inflammatory cytokines and launch an immune response to dispose of the pathogen (or foreign body).

Because HLA molecules need to present such a wide variety of "self" and "non-self" peptides, the HLA genes are both numerous and highly polymorphic. More than 1,500 HLA-B alleles have been identified (10). HLA allele nomenclature includes the HLA prefix, followed by the gene, an asterisk and a two digit number that corresponds to antigen specificity, and the assigned allele number (11). For example, the *HLA-B*15:02* allele is composed of:

- HLA: the HLA prefix (the HLA region on chromosome 6)
- B: the B gene (a particular HLA gene in this region)
- 15: the allele group (historically determined by serotyping, i.e., a group of alleles that share the same serotype)
- 02: the specific HLA allele (a specific protein sequence; determined by genetic analysis).

Additional digits have recently been added to the nomenclature to discriminate alleles that do not differ in the protein amino acid sequence, but differ in their genetic sequence (i.e., due to synonymous and noncoding genetic variants).

Variation in *HLA* genes plays an important role in the susceptibility to autoimmune disease and infections and they are also critical in the context of transplant surgery where better outcomes are observed if the donor and recipient are HLA-compatible.

More recently, *HLA* variants have been associated with susceptibility to Type B adverse drug reactions. For example, *HLA-B* variants have been associated with severe hypersensitivity reactions to abacavir (used to treat HIV), allopurinol (used to treat gout), and the antiepileptic drugs, carbamazepine and phenytoin.

Gene: *HLA-B*15:02*

Individuals who carry one or two copies of the high risk *HLA-B*15:02* allele are known as *HLA-B*15:02* positive (Table 2).

Table 2. 2014 Assignment of likely HLA-B phenotype based on genotype (CPIC)

Likely phenotype[a]	Genotype	Examples of diplotypes
Negative High-risk *HLA-B*15:02* allele not detected (constitutes ~98.6% of patients)	No copies of high-risk *HLA-B*15:02* allele	*X/*X[b]
Positive Detection of high-risk *HLA-B*15:02* allele (constitutes ~1.4% of patients)	Homozygous or heterozygous for high-risk *HLA-B*15:02* allele	*15:02/*X[b], *15:02/*15:02

[a] Global frequencies presented in parentheses. Haplotype frequencies vary among populations; please see (2) for individual population frequencies

[b] Where *X = any genotype other than *15:02.

Table is adapted from Caudle KE, Rettie AE, Whirl-Carrillo M, Smith LH, Mintzer S, Lee MT, Klein TE, Callaghan JT. Clinical pharmacogenetics implementation consortium guidelines for CYP2C9 and HLA-B genotypes and phenytoin dosing. Clinical pharmacology and therapeutics. 2014:96(5):542-8 (2).

Note: The nomenclature used in this table reflect the standardized pharmacogenetic terms proposed by CPIC (3).

The association between the *HLA-B*15:02* allele and SJS/TEN was first reported with the use of carbamazepine in the Han Chinese population. In the initial study, all patients who had carbamazepine-induced SJS/TEN were found to be a carrier of the *HLA-B*15:02* allele (44/44, 100%), whereas the allele was much less common among carbamazepine-tolerant patients (3/101, 3%)(12). In subsequent studies, this association was replicated, with a *HLA-B*15:02* carrier frequency of 70100% among cases of carbamazepine-induced SJS/TEN (13).

The *HLA-B*15:02* allele was later associated with phenytoin-induced hypersensitivity reactions, including phenytoin-induced SJS in a Thai population and phenytoin-induced SJS/TEN in Chinese Asians (14, 15).

There are fewer studies on phenytoin-induced hypersensitivity then carbamazepine, and the strength of association between phenytoin and SJS/TEN is weaker than that of carbamazepine and SJS/TEN. However, from the evidence available, the FDA recommends consideration of avoiding phenytoin as an alternative treatment to carbamazepine in individuals who are carriers of *HLA-B*15:02* (2).

The prevalence of carbamazepine-induced SJS/TEN is higher in populations where *HLA-B*15:02* is more common. Of note, the *HLA-B*15:02* allele frequency is highest in Southeast Asia, as populations from Hong Kong, Thailand, Malaysia, Vietnam, and parts of the Philippines have an allele frequency > 15%. It is slightly lower (~ 10-13%) in Taiwan and Singapore, and around 4% in North China. South Asians, including Indians, appear to have a *HLA-B*15:02* allele frequency of ~2 to 4%, with higher frequencies in some subpopulations (12-14, 16-27).

The *HLA-B*15:02* allele is rare (< 1%) in East Asia (Japan and Korea) and among individuals who are not of Asian descent. For example, the variant is very rare in Europeans, Hispanics, Africans, African Americans, and Native Americans (13, 18).

Gene: *CYP2C9*

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs in the liver. The CYP450 genes are very polymorphic and can result in reduced, absent, or increased enzyme activity.

The *CYP2C9* gene is highly polymorphic, with more than 50 known alleles. Variation in *CYP2C9* is thought to contribute to the pharmacogenetic variability in phenytoin metabolism.

*CYP2C9*1* is the wild-type allele and is associated with normal enzyme activity (2). Individuals who have two normal-function alleles (e.g., *CYP2C9 *1/*1*) are classified as "normal metabolizers" (Table 3). For individuals who are CYP2C9 normal metabolizers, the recommended starting maintenance dose of phenytoin does not need to be adjusted based on genotype (2).

Table 3. 2014 Assignment of likely *CYP2C9* phenotype based on genotype (CPIC)

Likely phenotype[a]	Genotype	Examples of diplotypes
Normal metabolizer (normal activity) (constitutes ~91% of patients)	An individual carrying two normal-function alleles	**1/*1*
Intermediate metabolizer (heterozygote or intermediate activity) (constitutes ~8% of patients)[b]	An individual carrying one normal-function allele plus one decreased-function allele	**1/*3, *1/*2*
Poor metabolizer (homozygous variant, low or deficient activity) (constitutes ~1% of patients)	An individual carrying two decreased function alleles	**2/*2, *3/*3, *2/*3*

[a] Global frequencies presented in parentheses. Haplotype frequencies vary among populations; please see (2) for individual population frequencies

[b] The enzyme activity in this grouping varies widely. Please see (2) for activity ranges.

Table is adapted from Caudle KE, Rettie AE, Whirl-Carrillo M, Smith LH, Mintzer S, Lee MT, Klein TE, Callaghan JT. Clinical pharmacogenetics implementation consortium guidelines for CYP2C9 and HLA-B genotypes and phenytoin dosing. Clinical pharmacology and therapeutics. 2014:96(5):542-8 (2).

Note: The nomenclature used in this table reflect the standardized pharmacogenetic terms proposed by CPIC (3).

Two allelic variants associated with reduced enzyme activity are *CYP2C9*2* and **3*. The **2* allele is more common in Caucasian (10-20%) than Asian (1-3%) or African (0-6%) populations, whereas the **3* allele is less common (<10% in most populations) and is extremely rare in African populations (24, 25, 28-30).

Individuals with one decreased function allele (e.g., *CYP2C9*1/*2* and **1/*3*) have mild to moderately reduced clearance of phenytoin; these individuals are classified as CYP2C9 intermediate metabolizers. The CPIC recommendations for CYP2C9 intermediate metabolizers include "to consider at least a 25% reduction of the recommended starting maintenance dose" (2).

Individuals with two decreased function alleles (e.g., *CYP2C9*2/*2*, **3/*3*) have reduced clearance of phenytoin and are classified as CYP2C9 poor metabolizers. CPIC recommendations for CYP2C9 poor metabolizers include "to consider at least a 50% reduction of the starting maintenance dose" (2).

In African Americans, the *CYP2C9*5*, **6*, **8* and **11* variants are more common, and these variants are also associated with a decrease in phenytoin metabolism (31).

Genetic Testing

The NIH's Genetic Testing Registry provides examples of the genetic tests that are currently available for the phenytoin drug response, the HLA-B gene, and the CYP2C9 gene.

The genotype results for an *HLA* allele such as *HLA-B*15:02* can either be "positive" or "negative." There are no intermediate phenotypes because the HLA genes are expressed in a codominant manner.

A positive result indicates the individual is either "heterozygous" or "homozygous" for the variant, depending upon whether they are carrying one or two copies of the **15:02* allele, respectively.

A negative result indicates that the individual does not carry the *HLA-B*15:02* allele. However, a negative result does not rule out the possibility of a patient developing phenytoin-induced SJS/TEN. Therefore, clinicians should carefully monitor all patients according to standard practices.

For *CYP2C9*, the variants that are routinely tested for include *CYP2C9*2* and **3*. Results are typically reported as a diplotype, such as *CYP2C9 *1/*2*.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1, 2] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2015 Statement from the US Food and Drug Administration (FDA)

Regarding HLA-B:

Studies in patients of Chinese ancestry have found a strong association between the risk of developing SJS/TEN and the presence of *HLA-B*1502*, an inherited allelic variant of the *HLA B* gene, in patients using carbamazepine. Limited evidence suggests that *HLA-B*1502* may be a risk factor for the development of SJS/TEN in patients of Asian ancestry taking other antiepileptic drugs associated with SJS/TEN, including phenytoin. Consideration should be given to avoiding phenytoin as an alternative for carbamazepine in patients positive for *HLA-B*1502*.

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

[2] Square brackets indicate insertions by the author to reflect the standardized nomenclature for pharmacokinetic terms proposed by CPIC in 2016 3. Caudle, K.E., H.M. Dunnenberger, R.R. Freimuth, J.F. Peterson, et al., Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC). Genet Med, 2016.

The use of *HLA-B*1502* genotyping has important limitations and must never substitute for appropriate clinical vigilance and patient management. The role of other possible factors in the development of, and morbidity from, SJS/TEN, such as antiepileptic drug (AED) dose, compliance, concomitant medications, comorbidities, and the level of dermatologic monitoring have not been studied.

Regarding CYP2C9:

In most patients maintained at a steady dosage, stable phenytoin serum levels are achieved. There may be wide interpatient variability in phenytoin serum levels with equivalent dosages. Patients with unusually low levels may be noncompliant or hypermetabolizers of phenytoin. Unusually high levels result from liver disease, variant *CYP2C9* and *CYP2C19* alleles, or drug interactions which result in metabolic interference. The patient with large variations in phenytoin plasma levels, despite standard doses, presents a difficult clinical problem. Serum level determinations in such patients may be particularly helpful. As phenytoin is highly protein bound, free phenytoin levels may be altered in patients whose protein binding characteristics differ from normal.

Please review the complete therapeutic recommendations that are located here: (1).

2014 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC)

Regarding HLA-B: […] Therefore, regardless of the *CYP2C9* genotype and the individual's ancestry or age, if the *HLA-B*15:02* test result is positive, the recommendation is to consider using an anticonvulsant other than carbamazepine and phenytoin, unless the benefits of treating the underlying disease clearly outweigh the risks. Some evidence exists linking SJS/TEN with the *HLA-B*15:02* allele in association with the use of alternative medications such as oxcarbazepine, eslicarbazepine acetate, and lamotrigine, and thus caution should be used in choosing alternatives to phenytoin.

Regarding CYP2C9: The recommended phenytoin maintenance dose does not need adjustment based on genotype for CYP2C9 extensive ["normal"] metabolizers. Available evidence does not clearly indicate the amount of dose reduction needed to prevent phenytoin-related toxicities in CYP2C9 intermediate and poor metabolizers; thus, our recommendations should be considered conservative estimates, given the variability surrounding phenytoin dosing in an individual. On the basis of the doses reported in the pharmacokinetic and pharmacogenetic studies mentioned above and in Supplementary Table S9 online, at least a 25% reduction of the recommended starting maintenance dose may be considered for CYP2C9 intermediate metabolizers, with subsequent maintenance doses adjusted based on therapeutic drug monitoring and response. For CYP2C9 poor metabolizers, consider at least a 50% reduction of starting maintenance dose, with subsequent maintenance doses adjusted based on therapeutic drug monitoring or response.

Please review the complete therapeutic recommendations that are located here: (2).

Nomenclature of selected *HLA-B* alleles

Allele name	dbSNP reference identifier for allele location
*HLA-B*15:02*	rs2844682 and rs3909184

For the *MHC* region, variations in genes such as *HLA-B* occur across the whole sequence of the gene, not a single locus. Therefore, the *HLA-B*15:02* allele is defined by its sequence rather than single coding or protein variations. If there is strong linkage disequilibrium between one or more SNPs and a specific *HLA* allele, the presence of these SNPs (tag SNPs) may be used for *HLA* typing in some populations; however, genotyping tag SNPs should not be considered diagnostic or equivalent to actual HLA testing. For *HLA-B*15:02*, rs2844682 and rs3909184 are the tag SNPs (32).
Guidelines on nomenclature of the HLA system are available from HLA Nomenclature: http://hla.alleles.org/

Nomenclature of selected *CYP2C9* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C9*2*	430C>T Arg144Cys	NM_000771.3:c.430C>T	NP_000762.2:p.Arg144Cys	rs1799853
*CYP2C9*3*	1075A>C Ile359Leu	NM_000771.3:c.1075A>C	NP_000762.2:p.Ile359Leu	rs1057910
*CYP2C9*5*	1080C>G Asp360Glu	NM_000771.3:c.1080C>G	NP_000762.2:p.Asp360Glu	rs28371686
*CYP2C9*6*	817delA Lys273Argfs	NM_000771.3:c.817delA	NP_000762.2:p.Lys273Argfs	rs9332131
*CYP2C9*8*	449G>A Arg150His	NM_000771.3:c.449G>A	NP_000762.2:p.Arg150His	rs7900194
*CYP2C9*11*	1003C>T Arg335Trp	NM_000771.3:c.1003C>T	NP_000762.2:p.Arg335Trp	rs28371685

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines
Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: http://www.cypalleles.ki.se/

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Piroxicam Therapy and *CYP2C9* Genotype

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Introduction

Piroxicam (brand name Feldene) is a nonsteroidal anti-inflammatory drug (NSAID) used to treat osteoarthritis and rheumatoid arthritis. Piroxicam provides pain relief and reduces inflammation.

Piroxicam is primarily metabolized by CYP2C9. Individuals who lack CYP2C9 activity ("CYP2C9 poor metabolizers") have an increased exposure to piroxicam, and an increased risk of side effects.

Like all NSAIDs, piroxicam increases the risk of serious cardiovascular events, including myocardial infarction and stroke, and serious gastrointestinal (GI) adverse events such as bleeding, ulceration, and perforation.

The standard dose of piroxicam for osteoarthritis and rheumatoid arthritis in adults is 20 mg once daily. But for all patients, the lowest effective dose of piroxicam should be used for the shortest length of time, consistent with the treatment goals of each individual (1).

The FDA-approved drug label for piroxicam states that a dose reduction should be considered in "patients who are known or suspected to be poor CYP2C9 metabolizers based on genotype or previous history/experience with other CYP2C9 substrates (such as warfarin and phenytoin)". Dose reductions should be considered because these patients may have abnormally high plasma levels of piroxicam caused by reduced metabolic clearance. However, specific dose reductions based on CYP2C9 phenotype are not provided (Table 1) (1).

As for all NSAIDs, piroxicam is contraindicated in patients with a known hypersensitivity, a history of asthma, urticaria, or other allergic-type reactions after taking aspirin or another NSAID, and following coronary artery bypass graft (CABG) surgery. Piroxicam should also be avoided by pregnant women starting at 30 weeks gestation.

Table 1. The FDA (2018) Drug Label for Piroxicam. Recommendations for CYP2C9 Phenotype. Pharmacogenomics.

Phenotype	Recommendations
CYP2C19 poor metabolizers	In patients who are known or suspected to be poor CYP2C9 metabolizers based on genotype or previous history/experience with other CYP2C9 substrates (such as warfarin and phenytoin) consider dose reduction as they may have abnormally high plasma levels due to reduced metabolic clearance.

This table is adapted from (1).

Drug Class: NSAIDs

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used to treat inflammation, fever, and pain. They are one of the most commonly used classes of medicine. Worldwide, it is estimated that more than 30 million people receive NSAIDs daily (2).

Currently, more than 20 NSAIDs are licensed for use. Several NSAIDs (e.g., aspirin, ibuprofen, and naproxen) are available over-the-counter, but stronger doses and other types of NSAIDs, such as celecoxib and piroxicam, are only available via prescription.

The main action of NSAIDs is to inhibit cyclooxygenase (COX). Cyclooxygenase is the central enzyme in the synthesis of prostaglandins, prostacyclin, and thromboxanes from arachidonic acid. Prostaglandins can be

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protective (e.g., protect the gastric mucosal lining and aid platelet aggregation) or inflammatory (e.g., recruiting inflammatory white blood cells).

There are 2 main isoforms of COX, and the safety, and effectiveness of NSAIDs may be influenced by the degree they inhibit the 2 different forms. Cyclooxygenase-1 (COX-1) is a "housekeeping enzyme" which is expressed in most tissues. It protects the GI tract and induces platelet aggregation in response to injury. In contrast, COX-2 is often undetectable in tissues. However, the expression of COX-2 is increased during inflammation.

Most NSAIDs are non-selective COX inhibitors that inhibit both COX-1 and COX-2. There are exceptions, such as celecoxib, which is a selective COX-2 inhibitor that appears to be associated with less adverse GI events. However, GI adverse events still occur.

Approximately 25% of the exposed US population has experienced NSAID-related side effects that required medical care (3). All NSAIDs carry a boxed warning regarding the risk of serious GI and cardiovascular adverse events; e.g.,

"NSAIDs cause an increased risk of serious cardiovascular thrombotic events, including myocardial infarction and stroke, which can be fatal. This risk may occur early in treatment and may increase with duration of use.

NSAIDs cause an increased risk of serious gastrointestinal adverse events including bleeding, ulceration, and perforation of the stomach or intestines, which can be fatal. These events can occur at any time during use and without warning symptoms. Elderly patients and patients with a prior history of peptic ulcer disease and/or GI bleeding are at greater risk for serious GI events" (1).

Drug: Piroxicam

Piroxicam is an NSAID used for the relief of osteoarthritis and rheumatoid arthritis. The recommended dose in adults is 20 mg daily, and although therapeutic effects are seen early, it takes up to 12 days for steady-state levels to be reached. Therefore, the effect of therapy should not be assessed for the first 2 weeks.

Because of the adverse events associated with any type of NSAID, the lowest effective dose of piroxicam should be used for the shortest duration. And, as for all NSAIDs, piroxicam is contraindicated in patients with a known hypersensitivity, or a history of asthma, urticaria, or other allergic-type reactions after taking aspirin or another NSAID. Piroxicam is also contraindicated to treat pain in the days following CABG surgery (NSAIDs cause an increased risk of myocardial infarction and stroke post-operatively), and piroxicam should be avoided by pregnant women starting at 30 weeks gestation (NSAID use in the third trimester causes an increased risk of premature closure of the fetal ductus arteriosus).

A subset of NSAIDs, known as oxicams, are highly potent and share a similar structure with a new binding fold that is different to typical NSAIDs. Piroxicam was the first oxicam to be licensed, other oxicams include isoxicam, meloxicam, tenoxicam, and lornoxicam (4, 5).

One study found that oxicams (piroxicam and tenoxicam) had a higher risk of Stevens -Johnson Syndrome (SJS), and toxic epidermal necrolysis (TEN) (relative risk [RR] of 34) than diclofenac (RR 4.1) and ibuprofen (RR 5.3). However, the absolute risk of SJS or TEN during piroxicam is still thought to be low –– the incidence of SJS or TEN during the first 8 weeks of piroxicam or tenoxicam therapy is one per 100,000 patients (6).

CYP2C9 is the main enzyme involved in the metabolism of piroxicam to its major inactive metabolite: 5′-hydroxy-piroxicam. Individuals with low CYP2C9 activity ("CYP2C9 poor metabolizers") have a higher exposure to piroxicam (1).

Gene: *CYP2C9*

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs in the liver. The CYP450 genes are very polymorphic and can result in reduced, absent, or increased enzyme activity (7).

The *CYP2C9* gene is highly polymorphic, with approximately 60 known alleles. *CYP2C9*1* is considered the wild-type allele when no variants are detected, and is categorized by normal enzyme activity (8). Individuals who have 2 normal-function alleles (e.g., *CYP2C9 *1/*1*) are classified as "normal metabolizers" (Table 2).

Table 2. Assignment of likely *CYP2C9* Phenotype based on Genotype (CPIC, 2014)

Likely phenotype[a]	Genotype	Examples of diplotypes
Ultrarapid metabolizer (increased activity) (frequency unknown)	Unknown – currently there are no known increased activity alleles	Unknown
Normal metabolizer (normal activity) (approximately 91% of individuals)	An individual with 2 normal-function alleles	*1/*1
Intermediate metabolizer (heterozygote or intermediate activity) (approximately 8% of individuals)[b]	An individual with one normal-function allele plus one decreased-function allele	*1/*3, *1/*2
Poor metabolizer (homozygous variant, low or deficient activity) (approximately 1% of individuals)	An individual with 2 decreased function alleles	*2/*2, *3/*3, *2/*3

Note: There are no known cases of CYP2C9 ultrarapid metabolizers
[a] Global frequencies are approximate. Because haplotype frequencies vary considerably among popualations, please see (8) for individual population frequencies.
[b] The enzyme activity in this grouping varies widely. Please see (8) for activity ranges.
This table is adapted from (8). Note: The nomenclature used in this table reflects the standardized pharmacogenetic terms proposed by CPIC (9).

Two allelic variants associated with reduced enzyme activity are *CYP2C9*2* and **3*. The **2* allele is more common in Caucasian (10-20%), than Asian (1-3%) or African (0-6%) populations. The **3* allele is less common (<10% in most populations) and is extremely rare in African populations. In African-Americans, the *CYP2C9*5*, **6*, **8* and **11* alleles are more common (10-12).

Linking Gene Variation with Treatment Response

Studies have shown that CYP2C9 poor metabolizers have increased exposure and reduced clearance when taking standard doses of piroxicam (1, 13). A gene-dose effect was proposed recently to explain the gradual increase in piroxicam exposure in an individual with a *CYP2C9* *3/*3 genotype compared with those with the *1/*1 and *1/*3 genotypes. And although data are lacking, overall it appears that the decreased function alleles *CYP2C9*2*, *CYP2C9*3* are associated with an increased risk of acute GI bleeding in patients receiving NSAID therapy. The *CYP2C8* variant, *CYP2C8*3*, may also contribute to this increased risk (3, 14, 15).

A recent small study (n=102 volunteers heterozygous for *CYP2C8*3* and *CYP2C9*3*) reported that the administration of 20 mg oral piroxicam for 4 days was effective in the control of pain following molar surgery regardless of the CYP haplotype (16). However, this study was not specifically designed to address the risk of adverse events across genotype groups, and the study used a low dose for a short duration (20 mg once daily for 4 days).

In addition to increased exposure of piroxicam by decreased CYP2C9 activity in poor metabolizers, CYP2C9 may also impact cardiovascular morbidity by altering the metabolism of fatty acids, prostanoids, and steroid hormones, especially in poor metabolizers of CYP2C9 (7).

Genetic Testing

Clinical genotyping tests are available for several *CYP2C9* alleles. The NIH Genetic Testing Registry (GTR) displays genetic tests that are currently available for the *CYP2C9* gene.

The *CYP2C9* variants that are routinely tested for include *CYP2C9*2* and **3*. Usually, the results are reported as a diplotype, such as *CYP2C9 *1/*1*, and may also include an interpretation of the patient's predicted metabolizer phenotype (normal, intermediate, or poor). Table 2 summarizes common CYP2C9 phenotypes.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2018 Statement from the US Food and Drug Administration (FDA)

Higher systemic exposure of piroxicam has been noted in subjects with *CYP2C9* polymorphisms compared to normal metabolizer type subjects.

[...]

CYP2C9 activity is reduced in individuals with genetic polymorphisms, such as the *CYP2C9*2* and *CYP2C9*3* polymorphisms. Limited data from two published reports showed that subjects with heterozygous *CYP2C9*1/*2* (n=9), heterozygous *CYP2C9*1/*3* (n=9), and homozygous *CYP2C9*3/*3* (n=1) genotypes showed 1.7-, 1.7-, and 5.3-fold higher piroxicam systemic levels, respectively, than the subjects with *CYP2C9*1/*1* (n=17, normal metabolizer genotype) following administration of a single oral dose. The mean elimination half-life values of piroxicam for subjects with *CYP2C9*1/*3* (n=9) and *CYP2C9*3/*3* (n=1) genotypes were 1.7- and 8.8-fold higher than subjects with *CYP2C9*1/*1* (n=17). It is estimated that the frequency of the homozygous **3/*3* genotype is 0% to 1% in the population at large; however, frequencies as high as 5.7% have been reported in certain ethnic groups.

Poor Metabolizers of CYP2C9 Substrates: In patients who are known or suspected to be poor CYP2C9 metabolizers based on genotype or previous history/experience with other CYP2C9 substrates (such as warfarin and phenytoin) consider dose reduction as they may have abnormally high plasma levels due to reduced metabolic clearance.

Please review the complete therapeutic recommendations that are located here: (1).

Nomenclature for selected *CYP2C9* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C9*2*	430C>T Arg144Cys	NM_000771.3:c.430C>T	NP_000762.2:p.Arg144Cys	rs1799853

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

Table continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C9*3*	1075A>C Ile359Leu	NM_000771.3:c.1075A>C	NP_000762.2:p.Ile359Leu	rs1057910
*CYP2C9*5*	1080C>G Asp360Glu	NM_000771.3:c.1080C>G	NP_000762.2:p.Asp360Glu	rs28371686
*CYP2C9*6*	818delA Lys273Argfs	NM_000771.3:c.817delA	NP_000762.2:p.Lys273Argfs	rs9332131
*CYP2C9*8*	449G>A Arg150His	NM_000771.3:c.449G>A	NP_000762.2:p.Arg150His	rs7900194
*CYP2C9*11*	1003C>T Arg335Trp	NM_000771.3:c.1003C>T	NP_000762.2:p.Arg335Trp	rs28371685

Note: the normal "wild-type" allele is *CYP2C9*1* and is reported when no variant is detected.
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (17).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for cytochrome P450 enzymes is available from Pharmacogene Variation (PharmVar) Consortium.

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Prasugrel Therapy and *CYP* Genotype

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Introduction

Prasugrel is a third-generation thienopyridine platelet inhibitor used in the management of patients with acute coronary syndrome (ACS) undergoing percutaneous coronary intervention (PCI). Prasugrel is used to reduce thrombotic cardiovascular events, such as stent thrombosis, myocardial infarction, and stroke in these patients. Prasugrel, along with other antiplatelet agents such as clopidogrel and ticagrelor, inhibits platelet activation by irreversibly binding to the platelet receptor, P2RY12.

Prasugrel is metabolized to its active metabolite primarily by CYP3A5 and CYP2B6, and to a lesser extent by CYP2C9 and CYP2C19. The FDA-approved label for prasugrel states that genetic variations in *CYP2B6*, *CYP2C9*, *CYP2C19*, or *CYP3A5* genes do not have a relevant effect on prasugrel pharmacokinetics and the generation of its active metabolite or its inhibition of platelet aggregation in healthy subjects, patients with stable atherosclerosis, or ACS (1).

Another commonly prescribed antiplatelet is the second-generation thienopyridine clopidogrel, which is bioactivated primarily by CYP2C19. Consequently, clopidogrel is less effective among patients with decreased or no function variant alleles in the *CYP2C19* gene. In contrast, *CYP2C19* variants are not associated with a decrease in effectiveness of prasugrel, which is a more potent antiplatelet agent than clopidogrel, but has a higher risk of bleeding (2-5).

Drug: Prasugrel

Prasugrel is a third-generation thienopyridine antiplatelet agent that binds irreversibly to the P2RY12 receptor and inhibits ADP-mediated platelet activation and aggregation. Other P2RY12 receptor blockers include clopidogrel and ticagrelor.

As an antiplatelet agent, prasugrel inhibits the formation of blood clots in the coronary, peripheral, and cerebrovascular arteries among patients with acute coronary syndrome (ACS).

ACS reflects a decreased blood flow in the coronary arteries, and includes unstable angina, which occurs suddenly, often at rest or with minimal exertion. Unstable angina may be new in onset or it may occur with less exertion than previously. Another form of ACS is a myocardial infarction (MI), which may be classified as "STEMI" or "NSTEMI" based on EKG findings. EKG findings that include ST-segment elevation is termed "ST segment elevation MI" (STEMI). If no ST segment elevation is present but myocardial biomarkers such as troponin I or T are increased, the term "non-ST segment elevation MI" (NSTEMI) is applied.

Patients with ACS are usually treated with a P2Y12 receptor blocker and aspirin (called dual antiplatelet therapy, DAPT) to reduce the risk of developing a coronary artery thrombus. Platelet adhesion and aggregation are early stages in the formation of a thrombus, which may occlude the coronary artery. Patients who undergo PCI are at risk of stent occlusion via this mechanism.

A large trial, TRITON-TMI 38, compared prasugrel with clopidogrel in 13,608 patients with ACS who were undergoing PCI. Prasugrel was found to provide more potent platelet inhibition than clopidogrel: after 15 months, the patients treated with prasugrel had a lower incidence of the combined endpoint of cardiovascular

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death, nonfatal myocardial infarction, or nonfatal stroke as compared with patients treated with clopidogrel (9.9% vs. 12.1%) (2, 3). However, prasugrel was associated with a higher risk of bleeding, leading to the FDA warning that prasugrel use is contraindicated in patients with active pathological bleeding, or a history of stroke or transient ischemic attack (TIA) (4, 5).

Prasugrel inhibits ADP-induced platelet aggregation by selectively binding to the platelet purinergic receptor, P2RY12. Because prasugrel is a pro-drug, it requires conversion into an active metabolite before it can act as an antiplatelet agent. Prasugrel is rapidly metabolized to thiolactone, which is then converted to an active metabolite by CYP3A5 and CYP2B6, and to a lesser extent by CYP2C9 and CYP2C19.

The active prasugrel metabolite (R-138727) contains a reactive thiol group, which forms a disulfide bridge with a free cysteine residue on the P2RY12 receptor. Once irreversibly bound to prasugrel, the receptor is unable to bind ADP, and platelet activation via this pathway is prevented for the rest of the platelet's lifespan of about 10 days (6).

Despite the general efficacy of clopidogrel as an antiplatelet agent, interindividual variability in metabolite levels, platelet inhibition, and clinical response has been reported. It has been estimated that between 16–50% of patients treated with clopidogrel have high on-treatment platelet reactivity (HTPR), indicating that despite clopidogrel treatment, a portion of P2RY12 receptors are not blocked (7). This is due, in part, to genetic variants in the *CYP2C19* gene, which encodes the principal hepatic enzyme involved in converting clopidogrel to its active metabolite. Patients that carry no function *CYP2C19* alleles (e.g., *CYP2C19*2*) have reduced plasma active clopidogrel metabolites and an increased risk for HTPR.

In contrast, there is no relevant effect of genetic variation in *CYP3A5*, *CYP2B6*, *CYP2C9*, or *CYP2C19* on the prasugrel pharmacokinetics and generation of active metabolites, or its inhibition of platelet aggregation (8-12). Therefore, although both clopidogrel and prasugrel form active metabolites with similar potency, prasugrel is a more potent antiplatelet agent than clopidogrel due to the more efficient formation of the active metabolite from the prodrug (13).

Although prasugrel is more effective than standard-dose clopidogrel, DAPT with clopidogrel and aspirin remains the standard of care at some institutions for some patients with ACS undergoing PCI (14). This is mainly because clopidogrel has a lower bleeding risk and is less expensive (15). However, the availability of *CYP2C19* genetic testing can facilitate personalized antiplatelet therapy, as individuals with impaired CYP2C19 activity could be identified and offered an alternative antiplatelet agent, such as prasugrel (16-19). Recent studies have found that *CYP2C19*-genotype guided antiplatelet therapy results in a higher likelihood of achieving a therapeutic level of on-treatment platelet reactivity (20-22), which may also be cost effective among ACS patients undergoing PCI (23).

The Cytochrome P450 Superfamily

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP450 genes are highly polymorphic and can result in no, decreased, normal, or increased enzyme activity.

CYP2C19, CYP2C9, CYP3A5, and CYP2B6 are involved in the metabolism of prasugrel, but genetic variations in these genes do not appear to influence the pharmokinetics of prasugrel. In contrast, genetic variation in the *CYP2C19* gene may lead to decreased effectiveness of the related drug, clopidogrel. To read more about CYP variants and the clopidogrel drug response, please see "Clopidogrel Therapy and *CYP2C19* Genotype".

Genetic Testing

The NIH Genetic Testing Registry, GTR, displays genetic tests that are currently available for the genes *CYP2C19*, *CYP2C9*, *CYP3A5*, and *CYP2B6*. Given that the formation of the active metabolite of prasugrel is not known to be affected by CYP variants, genetic testing prior to the use of prasugrel is not currently recommended.

For clopidogrel, its effectiveness is dependant on its activation to an active metabolite, principally by CYP2C19. Therefore, the FDA states that tests that identify a patient's CYP2C19 genotype can be used as an aid to determining therapeutic strategy.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

Statement from the US Food and Drug Administration (FDA): In healthy subjects, patients with stable atherosclerosis, and patients with ACS receiving prasugrel, there was no relevant effect of genetic variation in CYP2B6, CYP2C9, CYP2C19, or CYP3A5 on the pharmacokinetics of prasugrel's active metabolite or its inhibition of platelet aggregation.

[...]

In TRITON-TIMI 38, prasugrel reduced ischemic events (mainly nonfatal MIs) and increased bleeding events relative to clopidogrel. The findings are consistent with the intended greater inhibition of platelet aggregation by prasugrel at the doses used in the study. There is, however, an alternative explanation: both prasugrel and clopidogrel are pro-drugs that must be metabolized to their active moieties. Whereas the pharmacokinetics of prasugrel's active metabolite are not known to be affected by genetic variations in CYP2B6, CYP2C9, CYP2C19, or CYP3A5, the pharmacokinetics of clopidogrel's active metabolite are affected by CYP2C19 genotype, and approximately 30% of Caucasians are reduced-metabolizers. Moreover, certain proton pump inhibitors, widely used in the ACS patient population and used in TRITON-TIMI 38, inhibit CYP2C19, thereby decreasing formation of clopidogrel's active metabolite. Thus, reduced-metabolizer status and use of proton pump inhibitors may diminish clopidogrel's activity in a fraction of the population, and may have contributed to prasugrel's greater treatment effect and greater bleeding rate in TRITON-TIMI 38. The extent to which these factors were operational, however, is unknown.

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[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

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Propafenone Therapy and *CYP2D6* Genotype

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Introduction

Propafenone is an antiarrhythmic medication. It is used to prevent the reoccurrence of atrial fibrillation in patients with episodic atrial fibrillation who do not have underlying structural heart disease (propafenone may provoke proarrhythmic events in patients with structural heart disease).

Propafenone belongs to class IC of antiarrhythmic agents and acts on cardiac sodium channels to inhibit action potentials. In general, because of the lack of evidence that antiarrhythmic agents improve survival, they should only be used to treat arrhythmias that are thought to be life-threatening.

Propafenone is metabolized by CYP2D6, CYP3A4, and CYP1A2 enzymes. Approximately 6% of Caucasians in the US lack CYP2D6 activity, and are known as "CYP2D6 poor metabolizers" (Table 1) (1). Standard doses of propafenone will lead to higher plasma drug concentrations in poor metabolizers, compared to normal metabolizers. In addition, drugs that inhibit CYP2D6, CYP3A4, and CYP1A2 may also increase propafenone levels, which may lead to cardiac arrhythmia episodes.

The FDA-approved drug label for propafenone states that the recommended dosing regimen of propafenone is the same for all patients (CYP2D6 poor metabolizers and normal metabolizers). However, the label also cautions that the simultaneous use of propafenone with both a CYP2D6 inhibitor (or in patients with CYP2D6 deficiency) and a CYP3A4 inhibitor should be avoided, because of the increased risk of causing arrhythmias and other adverse events (1).

A guideline from The Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Pharmacists Association (KNMP) provides dosing recommendations for propafenone, based on *CYP2D6* genotype. For CYP2D6 poor metabolizers, the guideline recommends reducing the initial dose of propafenone by 70%, ECG monitoring, and monitoring plasma concentrations. For intermediate and ultrarapid metabolizers, the guideline states there is insufficient data to allow for a calculation of dose adjustment. Therefore, it is recommended to adjust the dose in response to plasma concentration and to monitor with ECG, or select an alternative drug (e.g., sotalol, disopyramide, quinidine, amiodarone) (2, 3) (Table 2).

Drug class: Antiarrhythmics

Antiarrhythmic agents suppress abnormal heart rhythms (cardiac arrhythmias), which can originate from the atria (e.g., atrial fibrillation, atrial flutter) or the ventricles (e.g., ventricular tachycardia, ventricular fibrillation).

There are five main classes of antiarrhythmic agents, based on their primary site of action:

- Class I: block sodium (Na+) channels e.g., quinidine (class IA), lidocaine (class IB), propafenone (class IC)
- Class II: block beta adrenoreceptors e.g., carvedilol, metoprolol, propranolol
- Class III: block potassium (K+) channels e.g., amiodarone, sotalol
- Class IV: block calcium (Ca2+) channels e.g., verapamil, diltiazem
- Class V: work by other or unknown mechanisms e.g., adenosine, digoxin

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Drug: Propafenone

Propafenone is an antiarrhythmic used to prevent the recurrence of atrial fibrillation in patients who have episodic atrial fibrillation and no underlying structural heart disease. Propafenone is also used in the management of paroxysmal supraventricular tachycardia and atrial flutter (1).

Because there are no well-controlled studies in pregnant women, the FDA-approved drug label states that propafenone should only be used during pregnancy if the benefit justifies the potential risk to the fetus. The label also states that the safety and effectiveness of propafenone in pediatric patients have not been established.

Atrial fibrillation is the most common type of harmful cardiac arrhythmias. It is more common in men than women, and the risk of developing atrial fibrillation increases with age. Atrial fibrillation may be paroxysmal (intermittent), persistent (persists for at least 7 days), long-standing (more than 12 months), or permanent.

The symptoms of atrial fibrillation range from no symptoms, to feeling dizzy, short of breath, and experiencing palpitations. The pulse feels irregular, and an ECG will show an absence of P waves and an irregular QRS complex. Atrial fibrillation can lead to reduced cardiac output, increase the risk of thrombosis and stroke, and affected patients may be at an increased risk for mortality (4). Management typically includes antithrombotic therapy and rhythm control.

Propafenone is a class IC *antiarrhythmic* agent. All class I agents have a "membrane stabilizing effect"—by reducing the fast influx of sodium ions into the cardiac muscle cells, they inhibit the propagation of action potentials. Propafenone also has some Class II activity—it can act as a beta blocker. Side effects of this action include bradycardia and bronchospasm (5, 6).

The class IC agents encainide and flecainide have been associated with increasing the risk of cardiac arrest or death, compared to placebo. Consequently all class IC agents, including propafenone, are considered to have a significant risk of provoking proarrhythmic events in patients with structural heart disease. Therefore, propafenone should not be used in patients with underlying structural heart disease. Its use is contraindicated in a number of conditions, including heart failure, conduction disorders, bradycardia, and recent myocardial infarction (within the last 3 months) (1, 7-9).

Propafenone is metabolized into two active metabolites: 5-hydroxypropafenone, which is formed by CYP2D6, and norpropafenone, which is formed by both CYP3A4 and CYP1A2. Multiple studies have found that genetic variants in the *CYP2D6* gene influence the plasma drug levels of propafenone (10-13).

In patients who lack CYP2D6 activity, metabolism of propafenone is slower, so the 5-hydroxy metabolite is not formed or is formed at very slow rates. In these patients, high doses of propafenone (850mg daily) lead to plasma concentrations of propafenone that are about twice those of patients who have normal CYP2D6 activity. At lower initial doses, the difference between propafenone and 5-hydroxy metabolite concentrations is even greater (1, 14).

However, the FDA recommends that the dosing regimen of propafenone should be the same for all patients, regardless of their CYP2D6 activity levels. This is because even at high doses, the effects of high propafenone levels are mitigated by the lack of the active 5-hydroxy metabolite in the slow metabolizers, and also because steady-state conditions are achieved after 4 to 5 days of titrating the dose in all patients. But the FDA also recommends that because of the large variation in plasma drug levels between individuals, the dose of propafenone should be individually titrated on the basis of response and tolerance, with close attention paid to clinical and ECG evidence of toxicity (1).

The FDA-approved drug label for propafenone cautions against the simultaneous use of propafenone with both a CYP2D6 inhibitor and a CYP3A4 inhibitor. This is because the combination of CYP3A4 inhibition and either

CYP2D6 inhibition or deficiency may increase propafenone exposure, which may trigger new cardiac arrhythmias and exaggerate beta adrenoreceptor blockage (1).

The Cytochrome P450 Superfamily

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The *CYP450* genes are very polymorphic and can result in reduced, absent, or increased enzyme activity.

Gene: *CYP2D6*

CYP2D6 is highly polymorphic, with over 100 star (*) alleles described (15). *CYP2D6*1* is the reference (or wild-type) allele encoding enzyme with normal activity. The *CYP2D6*2*, **33*, and **35* alleles are also considered to confer normal activity (Table 1).

Table 1. Activity status of selected *CYP2D6* alleles

Allele type	*CYP2D6* Alleles
Normal function	**1, *2, *33, *35*
Decreased function	**9, *10, *17, *29, *36, *41*
No function	**3-*8, *11-*16, *19-*21, *38, *40, *42*

For a detailed list of *CYP2D6* alleles, please see (15).

Individuals who have more than two normal function copies of the *CYP2D6* (*CYP2D6*xN*) gene are "ultrarapid metabolizers," whereas individuals who carry two normal or one normal and one decreased function allele are classified as "normal metabolizers."

Individuals with one normal and one no function allele or two decreased function alleles are categorized as "normal metabolizers" by recent nomenclature guidelines (16), but have also been categorized as "intermediate metabolizers" in the literature. Subjects with one decreased and one no function allele are predicted to be intermediate metabolizers and those with two no function alleles are classified as poor metabolizers.

The most common no function alleles include *CYP2D6*3*, **4*, **5*, and **6* (17-20), and the most common decreased function alleles include *CYP2D6*9, *10, *17, *29* and **41* (5, 6, 18, 20, 21) (Table 1).

There are large inter-ethnic differences in the frequency of these alleles. For example, *CYP2D6*4* is the most common no function allele in Caucasians, but is less abundant in subjects with African ancestry, and is rare in Asians. In contrast, the decreased function allele *CYP2D6*10* is the most common allele in Asians, and *CYP2D6*17* is almost exclusively found in individuals with African ancestry (22).

Consequently, the phenotype frequencies vary substantially among the major ethnicities and may vary among populations. Approximately 6-10% of European Caucasians and their descendants are poor metabolizers, mainly due to the prevalent no function *CYP2D6*4* and **5* alleles (17, 23).

Genetic Testing

The NIH's Genetic Testing Registry (GTR) lists genetic tests currently available for propafenone response and the CYP2D6 gene.

Results are typically reported as a diplotype, such as *CYP2D6 *1/*1* (wild type). A result for copy number, if available, is also important when interpreting *CYP2D6* results (19).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2016 Statement from the US Food and Drug Administration (FDA): Propafenone is metabolized by CYP2D6, CYP3A4, and CYP1A2 isoenzymes. Approximately 6% of Caucasians in the US population are naturally deficient in CYP2D6 activity and other demographic groups are deficient to a somewhat lesser extent. Drugs that inhibit these CYP pathways (such as desipramine, paroxetine, ritonavir, sertraline for CYP2D6; ketoconazole, erythromycin, saquinavir, and grapefruit juice for CYP3A4; and amiodarone and tobacco smoke for CYP1A2) can be expected to cause increased plasma levels of propafenone.

Increased exposure to propafenone may lead to cardiac arrhythmias and exaggerated beta-adrenergic blocking activity. Because of its metabolism, the combination of CYP3A4 inhibition and either CYP2D6 deficiency or CYP2D6 inhibition in users of propafenone is potentially hazardous. Therefore, avoid simultaneous use of propafenone with both a CYP2D6 inhibitor and a CYP3A4 inhibitor.

Please review the complete therapeutic recommendations that are located here: (1).

2016 Summary of recommendations from The Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Pharmacists Association (KNMP): For CYP2D6 poor metabolizers (PMs), defined as patients carrying two defective alleles, dose reductions are recommended for clomipramine, flecainide, haloperidol, zuclopenthixol (all 50%); doxepin, nortriptyline (both 60%); imipramine, propafenone (both 70%); and metoprolol (75%).

[…].

For CYP2D6 intermediate metabolizers (IMs), defined as patients carrying two decreased-activity alleles or one active/decreased-activity allele and one inactive allele, dose reductions ranging from 20 to 50% are advised for doxepin, amitriptyline, zuclopenthixol, imipramine, nortriptyline, and metoprolol. There were insufficient data to calculate dose adjustments for clomipramine, oxycodone, propafenone, risperidone, and venlafaxine (Table 2).

Please review the complete therapeutic recommendations that are located here: (2, 3).

Table 2. *CYP2D6* phenotypes and the therapeutic recommendations for propafenone therapy, from The Dutch Pharmacogenetics Working Group (2016)

CYP2D6 Phenotype	Recommendations for propafenone therapy
Ultrarapid metabolizer	Insufficient data to allow calculation of dose adjustment. Adjust dose in response to plasma concentration and record ECG or select alternative drug (e.g., sotalol, disopyramide, quinidine, amiodarone).
Intermediate metabolizer	Insufficient data to allow calculation of dose adjustment. Adjust dose in response to plasma concentration and record ECG or select alternative drug (e.g., sotalol, disopyramide, quinidine, amiodarone).
Poor metabolizer	Reduce dose by 70%, record ECG, monitor plasma concentration

The level of evidence for the therapeutic (dose) recommendations is 4/4 ("good quality") for poor metabolizers, and 3/4 ("moderate quality") for intermediate and ultrarapid metabolizer types. The Table is adapted from Swen J.J., Nijenhuis M., de Boer A., Grandia L. et al. Pharmacogenetics: from bench to byte - an update of guidelines. Clinical pharmacology and therapeutics. 2011;89(5):662–73 (2, 3).

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

Nomenclature of selected *CYP2D6* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*4*	1846G>A	NM_000106.5:c.506-1G>A	Variant occurs in a non-coding region (splice variant causes a frameshift)	rs3892097
*CYP2D6*5*	Variant results in a whole gene deletion			
*CYP2D6*6*	1707 del T Trp152Gly CYP2D6T	NM_000106.5:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655
*CYP2D6*10*	100C>T (Pro34Ser)	NM_000106.5:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
*CYP2D6*17*	1023C>T[1] (Thr107Ile)	NM_000106.5:c.320C>T	NP_000097.3:p.Thr107Ile	rs28371706
	2850C>T[2] (Cys296Arg)	NM_000106.5:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
*CYP2D6*41*	2850C>T[2] (Cys296Arg)	NM_000106.5:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
	2988G>A	NM_000106.5:c.985+39 G>A	Variant occurs in a non-coding region (impacts slicing).	rs28371725

[1] In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.
[2] In the literature, 2850C>T is also referred to as 2938C>T.

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: http://www.cypalleles.ki.se/

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Risperidone Therapy and *CYP2D6* Genotype

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Introduction

Risperidone is the most commonly prescribed antipsychotic medication in the US. It is an atypical (second generation) antipsychotic used in the treatment of schizophrenia, bipolar disorder, severe dementia, and irritability associated with autism.

Risperidone is metabolized to the active metabolite 9-hydroxyrisperidone by the enzyme CYP2D6 and to a lesser extent by CYP3A4. Individuals who carry two inactive copies of the *CYP2D6* gene are termed "poor metabolizers" and may have a decreased capacity to metabolize risperidone. These individuals may be at a higher risk of adverse effects because of increased exposure to plasma risperidone, compared to normal metabolizers, who carry two active copies of *CYP2D6*. Individuals who are CYP2D6 ultrarapid metabolizers (who carry more than two functional copies of *CYP2D6*) may have a decreased response to therapy, resulting from lower steady-state risperidone concentrations.

The FDA-approved drug label states that analysis of clinical studies involving a modest number of poor metabolizers (n=70) does not suggest that poor and extensive (normal) metabolizers have different rates of adverse effects (1). In addition, the Dutch Pharmacogenetics Working Group (DPWG) recently changed its dosing recommendations to "no action is needed" for CYP2D6 poor metabolizers taking risperidone (2).

Drug: Risperidone

Risperidone is an atypical antipsychotic primarily used in the treatment of schizophrenia and manic or mixed episodes in bipolar disorder. Risperidone may also be used as part of the management of aggression and/or psychosis in severe dementia and irritability associated with autistic disorder in children and adolescents (1).

The first antipsychotics to be discovered in the 1950s were haloperidol and chlorpromazine. Known as "first-generation" or "typical" antipsychotics, these drugs are used to treat psychosis (regardless of the underlying cause), chronic psychotic disorders (e.g., schizophrenia), and other psychiatric conditions.

All antipsychotics, with the exception of aripiprazole, are dopamine receptor antagonists. Blockade of the D2 dopamine receptor in the brain's limbic system is thought to improve the "positive" symptoms of schizophrenia, such as delusions and hallucinations, which are signs of psychosis.

However, typical antipsychotics also block dopamine receptors in the nigrostriatal pathway. This can cause movement disorders known as extrapyramidal side effects. These disorders include akathisia (motor restlessness), dystonia (abnormal muscle tone), and tardive dyskinesia (involuntary and repetitive movements).

Newer antipsychotics, known as "second generation" or "atypical" antipsychotics, have a lower risk of extrapyramidal side effects. Risperidone is an atypical antipsychotic. The most common side effects of risperidone therapy are sedation and dry mouth, but the rates of both appear to be low, at around 5% (3). Other atypical antipsychotics approved by the FDA include aripiprazole, asenapine, brexpiprazole, cariprazine, clozapine, lurasidone, olanzapine, quetiapine, and ziprasidone.

Atypical antipsychotics, such as risperidone, are thought to transiently occupy D2 receptors and then rapidly dissociate, to allow for normal dopamine neurotransmission (4). Because risperidone has high affinity for the D2

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receptor but binds it "loosely", it does not block dopamine receptors in the nigrostriatal pathway and extrapyramidal side effects are less likely (5).

Risperidone also blocks serotonin receptors, alpha 1 adrenergic receptors, and, to a lesser extent, histamine H1 and alpha 2 adrenergic receptors.

The main route of risperidone metabolism is in the liver by the enzyme CYP2D6. The major active metabolite, 9-hydroxyrisperidone, contributes to the pharmacological effects of this drug (5). While risperidone and 9-hydroxyrisperidone are often regarded as equipotent, they display different affinities towards the two target receptors (D2 and 5HT2A), where risperidone appears to be approximately 2-fold more potent than 9-hydroxyrisperidone. There is also a difference in brain distribution; risperidone is distributed more to the CNS (6).

Genetic variations in the *CYP2D6* gene may contribute to an increased risk of adverse events associated with risperidone therapy (7). Individuals who are "CYP2D6 poor metabolizers" carry two no function copies of the *CYP2D6* gene. In these individuals, standard doses of risperidone may lead to increased plasma levels of risperidone and decreased levels of 9-hydoxyrisperidone.

However, it is unclear to the extent to which *CYP2D6* genotype influences the efficacy and safety of risperidone therapy. One small study of 76 patients with schizophrenia reported that CYP2D6 poor metabolism was associated with greater clinical improvement in the total Positive and Negative Syndrome Scale (PANSS) (8). Other studies have reported a higher rate of adverse reactions and drug discontinuations in CYP2D6 poor metabolizers compared to normal metabolizers (5, 9, 10).

The ratio of risperidone to 9-hdroxyrisperidone, which largely reflects CYP2D6 phenotype, may be a risk factor for different side effects (11). Because prolactin levels mainly correlate with 9-hydroxyrisperidone levels, CYP2D6 ultrarapid metabolizers may experience different side effects than normal metabolizers (12). In addition, because elderly patients accumulate 9-hydroxyrisperidone due to reduced renal function, older patients who are CYP2D6 poor metabolizers (and others with reduced renal function) are at particular risk of side effects during risperidone treatment (5).

Individuals who are "CYP2D6 ultrarapid metabolizers" may have decreased plasma levels of risperidone, due to increased CYP2D6 activity—these individuals carry more than two functional copies of the *CYP2D6* gene. A small study of 85 patients taking long-lasting risperidone showed that the plasma concentrations of risperidone and its active metabolite were subtherapeutic in three individuals who were CYP2D6 ultrarapid metabolizers. The study, however, did not report whether these changes affected the effectiveness or tolerability of the drug in these patients (13).

Overall, it remains unclear whether the accurate determination of an individual's *CYP2D6* genotype, together with therapeutic drug monitoring, has the potential to optimize the response of CYP2D6 poor metabolizers and ultrarapid metabolizers to antipsychotic therapy (9, 14).

The Cytochrome P450 Superfamily

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing or detoxifying lipids, hormones, toxins, and drugs. The *CYP450* genes are very polymorphic and can result in reduced, absent, or increased enzyme activity.

Gene: *CYP2D6*

CYP2D6 is highly polymorphic, with over 100 star (*) alleles described (15). *CYP2D6*1* is the reference (or wild-type) allele encoding an enzyme with normal activity. The *CYP2D6*2*, **33*, and **35* alleles are also considered to confer normal enzyme activity (Table 1).

Table 1. Activity status of selected *CYP2D6* alleles

Allele type	*CYP2D6* Alleles
Normal function	**1, *2, *33, *35*
Decreased function	**9, *10, *17, *29, *36, *41*
No function	**3, *4, *5, *6, *7, *8, *11, *12, *13, *14, *15, *16, *19, *20, *21, *38, *40, *42*

For a detailed list of *CYP2D6* alleles, please see (15).

Individuals who have more than two normal function copies of the *CYP2D6* gene are classified as "ultrarapid metabolizers," whereas individuals who carry two normal or one normal and one decreased function allele are classified as "normal metabolizers" (also referred to as "extensive metabolizers").

Individuals with one normal and one no function allele or two decreased function alleles are also categorized as "normal metabolizers" by recent nomenclature guidelines (16), but have also been categorized as "intermediate metabolizers" elsewhere in the literature. Subjects with one decreased and one no function allele are predicted to be "intermediate metabolizers" and those with two no function alleles are considered to be "poor metabolizers" (Table 2).

Table 2: 2016 Assignment of CYP2D6 phenotypes by CPIC

Phenotype	Activity Score	Genotypes	Examples of diplotypes
CYP2D6 ultrarapid metabolizer (approximately 1–20% of patients)[a]	Greater than 2.0	An individual carrying duplications of functional alleles	*(*1/*1)xN* *(*1/*2)xN* *(*2/*2)xN*[b]
CYP2D6 normal metabolizer (approximately 72–88% of patients)	1.0 – 2.0[c]	An individual carrying two normal function alleles or two decreased function alleles or one normal and no function allele or one normal function and decreased function allele or combinations of duplicated alleles that result in an activity score of 1.0 to 2.0	**1/*1* **1/*2* **2/*2* **1/*9* **1/*41* **41/*41* **1/*5* **1/*4*
CYP2D6 intermediate metabolizer (approximately 1–13% of patients)	0.5	An individual carrying one decreased function and one no function allele	**4/*41* **5/*9* **4/*10*

Table 2 continued from previous page.

Phenotype	Activity Score	Genotypes	Examples of diplotypes
CYP2D6 poor metabolizer (approximately 1–10% of patients)	0	An individual carrying two no function alleles	**4/*4* **4/*4xN* **3/*4* **5/*5* **5/*6*

[a] For population-specific allele and phenotype frequencies, please see (17).
[b] Where *xN* represents the number of *CYP2D6* gene copies (N is 2 or more).
[c] Patients with an activity core of 1.0 may be classified as intermediate metabolizers by some reference laboratories.
For more information about activity scores, please see the Genetic Testing section.
This table has been adapted from Hicks J.K., Sangkuhl K., Swen J.J., Ellingrod V.L., Müller D.J., Shimoda K., Bishop J.R., Kharasch E.D., Skaar T.C., Gaedigk A., Dunnenberger H.M., Klein T.E., Caudle K.E. Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC®) for *CYP2D6* and *CYP2C19* Genotypes and Dosing of Tricyclic Antidepressants: 2016 Update. Clinical pharmacology and therapeutics. 2016 Dec 20 [Epub ahead of print] (17).

The most common no function alleles include *CYP2D6*3*, **4*, **5*, and **6* (18-21), and the most common decreased function alleles include *CYP2D6*9, *10, *17, *29* and **41* (19, 21-24). There are large inter-ethnic differences in the frequency of these alleles. For example, *CYP2D6*4* is the most common no function allele in Caucasians, but is less abundant in subjects with African ancestry and is rare in Asians. In contrast, the decreased function allele *CYP2D6*10* is the most common allele in Asians, and *CYP2D6*17* is almost exclusively found in individuals with African ancestry (25).

Consequently, the phenotype frequencies also vary substantially among the major ethnicities and may vary among populations. Approximately 6–10% of European Caucasians and their descendants are poor metabolizers, mainly due to the prevalent no function *CYP2D6*4* and **5* alleles (26, 27).

Genetic Testing

The NIH's Genetic Testing Registry provides examples of the genetic tests that are currently available for risperidone response and for the *CYP2D6* gene.

Results are typically reported as a diplotype, such as *CYP2D6 *1/*1*. A result for copy number, if available, is also important when interpreting *CYP2D6* genotyping results (28). However, it is important to note that the number of variants tested can vary among laboratories, which can result in diplotype result discrepancies between testing platforms and laboratories (29).

If the test results include an interpretation of the patient's predicted metabolizer phenotype, this should be confirmed by checking the diplotype and assigning an activity score to each allele (e.g., 0 for no function, 0.5 for decreased function, and 1 for each copy of a normal function allele). The phenotype is defined by the sum of the two scores:

- A normal (previously referred to as "extensive") metabolizer phenotype has an activity score of 1 to 2
- An intermediate metabolizer has an activity score of 0.5
- A poor metabolizer has an activity score of 0
- An ultrarapid metabolizer has an activity score greater than 2 (17, 30)

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2016 Statement from the US Food and Drug Administration (FDA): Risperidone is extensively metabolized in the liver. The main metabolic pathway is through hydroxylation of risperidone to 9-hydroxyrisperidone by the enzyme, CYP 2D6. A minor metabolic pathway is through N-dealkylation. The main metabolite, 9-hydroxyrisperidone, has similar pharmacological activity as risperidone. Consequently, the clinical effect of the drug results from the combined concentrations of risperidone plus 9-hydroxyrisperidone.

CYP 2D6, also called debrisoquin hydroxylase, is the enzyme responsible for metabolism of many neuroleptics, antidepressants, antiarrhythmics, and other drugs. CYP 2D6 is subject to genetic polymorphism (about 6%–8% of Caucasians, and a very low percentage of Asians, have little or no activity and are "poor metabolizers") and to inhibition by a variety of substrates and some non-substrates, notably quinidine. Extensive[2] CYP 2D6 metabolizers convert risperidone rapidly into 9-hydroxyrisperidone, whereas poor CYP 2D6 metabolizers convert it much more slowly. Although extensive metabolizers have lower risperidone and higher 9-hydroxyrisperidone concentrations than poor metabolizers, the pharmacokinetics of risperidone and 9-hydroxyrisperidone combined, after single and multiple doses, are similar in extensive and poor metabolizers.

Please review the complete therapeutic recommendations that are located here: (1).

2017 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP):

CYP2D6 Poor metabolizers:

No action is needed for this gene-drug interaction.

The genetic variation can result in both an increase in side effects and a stronger decrease in schizophrenia symptoms. In addition to this, the genetic variation may lead to a decrease in the required maintenance dose. However, as the effect on the dose is smaller than that of the normal biological variation, action is not useful.

CYP2D6 intermediate metabolizers:

No action is needed for this gene-drug interaction.

There is little evidence to support an increase in side effects caused by the genetic variation. The genetic variation may lead to a decrease in the required maintenance dose. However, as the effect on the dose is smaller than that of the normal biological variation, action is not useful.

CYP2D6 ultrarapid metabolizers:

No action is needed for this gene-drug interaction.

Genetic variation may lead to an increase in the required maintenance dose. However, as the effect is smaller than that of the normal biological variation, action is not useful.

Please review the complete therapeutic recommendations that are located here: (2).

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

[2] The FDA statement uses the term "extensive metabolizer." CPIC recently introduced standardized nomenclature for pharmacogenetic terms, which included replacing the term "extensive metabolizer" with the term "normal metabolizer." More details can be found in the 2016 paper, "Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC)" 16. Caudle, K.E., H.M. Dunnenberger, R.R. Freimuth, J.F. Peterson, et al., *Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC).* Genet Med, 2016.

Nomenclature

Nomenclature for selected *CYP2D6* alleles

Common allele name	Alternative names / Major SNP	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*4*	1846G>A	NM_000106.5:c.506-1G>A	Not applicable - variant occurs in a non-coding region	rs3892097
*CYP2D6*5*	Not applicable - variant results in a whole gene deletion			
*CYP2D6*6*	1707 del T Trp152Gly	NM_000106.5:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655
*CYP2D6*10*	100C>T Pro34Ser	NM_000106.5:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
*CYP2D6*17*	Includes at least two functional variants*: 1023C>T (Thr107Ile) 2850C>T (Cys296Arg)	NM_000106.5:c.320C>T NM_000106.5:c.886T>C	NP_000097.3:p.Thr107Ile NP_000097.3:p.Cys296Arg	rs28371706 rs16947
*CYP2D6*41*	2988G>A	NM_000106.5:c.985+39 G>A	Not applicable – variant occurs in a non-coding region	rs28371725

SNP= Single Nucleotide Polymorphism
*In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: http://www.cypalleles.ki.se/

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Simeprevir Therapy and *IFNL3* Genotype

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Introduction

Simeprevir is a hepatitis C virus (HCV) protease inhibitor used in combination with other drugs to treat chronic hepatitis genotype 1 or 4 infection (1).

Previously, the standard care of patients with HCV infection was peginterferon alfa and ribavirin, but ~40-50% of patients with HCV genotype 1 infection had a suboptimal sustained virological response (SVR) (2).

A SVR is defined as undetectable HCV RNA by the end of treatment and at a specific number of weeks after the end of treatment. The addition of simeprevir increased the SVR in patients with HCV genotype 1 infection who were previously untreated. However, there were reports of treatment failure, most commonly in adults, who failed to respond to previous peginterferon and ribavirin treatment (3).

The FDA-approved drug label for simeprevir contains information regarding a genetic variant near the *IFNL3* gene (a C to T change; rs12979860), which is a strong predictor of response to peginterferon alfa and ribavirin treatment. The label states that in phase 3 clinical trials, SVR rates were lower in patients with CT and TT genotypes, compared to patients with the CC genotype. However, patients of all *IFNL3* genotypes had highest SVR rates when being treated with regimens that included simeprevir.

In addition, the label strongly recommends patients with HCV genotype 1a infection should be screened for the presence of virus with the S3 Q80K polymorphism. If Q80K is detected, the label strongly recommends that alternative therapy be considered (4).

Drug class: HCV Protease Inhibitors

The treatment of hepatitis C virus (HCV) has evolved over the years. Initially, interferon (IFN) was used as monotherapy. This was followed by the addition of the antiviral agent ribavirin (a nucleoside analogue) to peginterferon (PEG-IFN), and more recently, the addition of antiviral protease inhibitors such as simeprevir.

Protease inhibitors are the first direct-acting antivirals to be approved for the treatment of HCV, and simeprevir is the first second-generation agent to become available. Simeprevir has largely replaced the use of the first-generation protease inhibitors, boceprevir and telaprevir, which have less favorable side effect profiles.

Successful treatment of hepatitis C is confirmed when no trace of HCV can be found after treatment has finished. This is referred to as the SVR, which is defined as undetectable HCV RNA by a quantification assay at the end of treatment, and typically 12 (SVR12) or 24 weeks (SVR24) after the end of treatment.

The addition of simeprevir to a PEG-IFN and ribavirin treatment regimen increases the SVR in patients with chronic hepatitis caused by genotype type 1 or 4 hepatitis C virus, and the response to treatment is influenced by the patient's *IFNL3* genotype.

The FDA-approved drug label for simeprevir states that simeprevir should only be used in combination with other antiviral drugs, such as in combination with PEG-IFN and ribavirin; or in combination with sofosbuvir (HCV nucleotide-analogue NS5B polymerase inhibitor) (1). However, because IFN-free regimes are fast

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becoming the current standard of care for hepatitis C, simeprevir tends to be prescribed with sofosbuvir rather than IFNs.

Drug: Simeprevir

Acute infection with HCV is usually asymptomatic, and about 15-45% of people who are infected clear the virus within 6 months of infection without any treatment. The remaining 55-85% of people will develop chronic HCV infection, which may also be asymptomatic for many years. It is thought that over 180 million people are infected with HCV worldwide (5).

The HCV is classified by genotype, based on the RNA viral strands. There are 6 classes of genotype, numbered 1-6, with multiple subtypes e.g., 1a, 1b, 2a, 2b. In the US, approximately 70% of people with HCV infection have genotype 1, with genotype 1a more common than 1b (6). Genotype 1 is the most difficult to treat, as it is less likely than genotypes 2 and 3 to respond to therapy.

Simeprevir has been FDA-approved for use in combination with other drugs, for the treatment of adults with chronic hepatitis C, caused by an infection with genotype 1 or 4 HCV.

During the natural course of HCV infection, patients develop liver fibrosis, which, without treatment, can progress to liver cancer (hepatocellular carcinoma). Approximately 45% of patients with chronic hepatitis C will develop liver cancer within 20 years from the initial infection.

Until recently, the standard of care for hepatitis C infection was based on therapy with peginterferon and ribavirin. Approximately half of the patients cleared the HCV infection, as shown by a SVR, but adverse effects were common and sometimes life-threatening (2). Treatment was expensive and inconvenient, lasting up to 48 weeks.

Protease inhibitors such as simeprevir were specifically developed to improve the effectiveness of peginterferon and ribavirin therapy. Teleprevir was the first drug to be developed, but severe dermatological adverse effects and liver toxicity limited its use. Simeprevir belongs to the second generation of drugs, and has an improved therapeutic index.

Simeprevir prevents maturation of the HCV by blocking viral protein synthesis. Specifically, simeprevir inhibits the viral protease NS3/4A which is responsible for cleaving and processing the HCV polyprotein precursor (7). Several mutations in this viral NS3/4A protease are associated with a reduced susceptibility to simeprevir. One of the most common and clinically significant mutations is the Q80K polymorphism. The FDA-approved drug label states that patients with HCV genotype 1a infection should be screened for the presence of virus with the Q80K polymorphism. If Q80K is detected, the label strongly recommends that alternative therapy be considered (1).

The combination of protease inhibitors such as simeprevir with peginterferon and ribavirin therapy has led to a much more effective treatment of hepatitis C in patients who were "treatment naïve" (no history of HCV treatment) and among "relapsers" (patients who had relapsed after previous HCV therapy). This was evidenced by improvement in the SVR and reduction of treatment from 48 to 24 weeks, without any increase in peginterferon and ribavirin adverse effects (3, 8).

The treatment options for hepatitis C continue to evolve. Currently, IFN-free treatment regimes for hepatitis C are considered to be the standard of care. The IFN-free combination of simeprevir plus sofosbuvir has been found to be a highly effective treatment, with studies reporting high SVR12 rates for the majority of patients with chronic HCV infection (from about 84% to 94%) (9-11).

Genetic variants in the *IFNL3* gene have been shown to strongly influence treatment response to PEG interferon-alpha-based regimens (including regimens with simeprevir) in previously untreated patients with

HCV genotype 1 infection (4). However, data are currently lacking on how *IFNL3* variants influence an individual's response to simeprevir when used with sofosbuvir in an IFN-free regimen.

Gene: *IFNL3*

The *IFNL3* gene, previously known as *IL28B*, encodes interferon lambda-3 (IFN-λ3) and is involved in the immune response to hepatitis C.

When a person is infected by a virus, their immune response includes the production of interferons. These signaling proteins induce changes in infected and uninfected cells to block viral replication and stop the spread of virus. Interferons are given as part of treatment for HCV to strengthen this innate response.

There are three classes of IFNs: type I (IFN-α/β), type II (IFN-γ) and type III (IFN-λ). The IFNL3 is a type III interferon, and as such, induces a strong antiviral state in responsive cells with a higher risk of viral infection, such as mucosal cells (12).

IFNL3 is only highly expressed in hepatocytes and epithelial cells, in contrast to other similar interferons, such as IFN-α, which are expressed in most cell types. IFNL3 exerts its actions by interacting with a cytokine receptor complex, which is composed of the IL10RB and IL28RA receptor chains (4).

The first two *IFNL3* variants to be commonly tested for are rs12979860 and rs8099917. These variants are in close proximity to each other near the *IFNL3* gene, and are in strong linkage disequilibrium. HCV genotype 1 patients with the "favorable" genotypes (CC for rs12979860 and TT for rs8099917) respond better to treatment as they are associated with an approximate 2-fold increase in SVR. However, the exact mechanism how these variants influence treatment outcome is not yet known (4).

In a US cohort of mixed ethnicity, variants in rs12979860 predicted treatment response in HCV genotype 1 infection patients: CC genotype individuals were more likely to spontaneously clear acute HCV infection and TT genotype individuals had the poorest response to treatment. Accordingly, CT genotype individuals had an intermediate response that was between those of the CC and TT genotype patients (4).

The response to HCV treatment varies across different populations, which can be largely explained by differences in allele frequencies. The rs12979860 'C' allele is commonly found in East Asians (allele frequency nearly 0.9), followed by Caucasians (0.63) and Hispanics (0.55), and is the least common among individuals of African origin (0.39) (4).

Among Asians and individuals of European descent, the rs8099917 variant best predicts treatment response (13-15). Moreover, recently a variant in the *IFNL4* gene (rs368234815), was found to be superior to rs12979860 in predicting treatment outcome in individuals of African ancestry. Together with another *IFNL4* variant (rs117648444), the combination of these two variants was found to have greater treatment response prediction compared to testing for single variants (12).

Genetic Testing

Genetic testing for *IFNL3* is available, and is used to predict response to peg-IFN and RBV in HCV genotype 1 patients. The results can help clinicians and patients make informed decisions on how to best manage their HCV infection.

The rs12979860 variant is most commonly tested, and the results are typically reported in the following format:

rs12979860 CC, favorable genotype

rs12979860 CT, unfavorable genotype

rs12979860 TT, unfavorable genotype (4).

Before starting a treatment regimen with simeprevir in patients with HCV genotype 1a infection, the FDA strongly recommends screening patients for the presence of virus with the "NS3 Q80K" polymorphism. The FDA states that an alternative therapy to simeprevir should be considered if Q80K is detected (1).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2015 Statement from the US Food and Drug Administration (FDA): A genetic variant near the gene encoding interferon-lambda-3 (IL28B rs12979860, a C [cytosine] to T [thymine] substitution) is a strong predictor of response to Peg-IFN-alfa and RBV (PR). In the Phase 3 trials, IL28B genotype was a stratification factor.

Overall, SVR rates were lower in subjects with the CT and TT genotypes compared to those with the CC genotype. Among both treatment-naïve subjects and those who experienced previous treatment failures, subjects of all IL28B genotypes had the highest SVR rates with simeprevir-containing regimens.
Please review the complete therapeutic recommendations that are located here: (1)

Nomenclature

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
rs12979860	/	NM_001276254.2:c.151-152G>A	N/A	rs12979860
rs8099917	/	N/A	N/A	rs8099917

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

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[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

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Sofosbuvir Therapy and *IFNL4* Genotype

Laura Dean, MD[1]

Created: January 25, 2017.

Introduction

Sofosbuvir is an antiviral agent used in the treatment of chronic hepatitis C virus (HCV) infection. Sofosbuvir is FDA-approved to treat patients infected with HCV genotypes 1, 2, 3, and 4, as part of a combination antiviral treatment regimen (1). HCV genotype 1 is the most prevalent worldwide and HCV genotype 3 is the next most prevalent (2). Sofosbuvir may also be used as part of the treatment regimen of HCV genotypes 5 or 6 (3).

About 180 million people worldwide are infected with chronic hepatitis C, which is a major cause of chronic liver disease, cirrhosis, and liver cancer. Viral eradication is suboptimal with peginterferon plus ribavirin-based therapy, with only about half of patients with HCV genotype 1 infection achieving a sustained virological response (SVR) after 24 weeks (4). A SVR is defined as undetectable HCV RNA by the end of treatment or at a specific number of weeks after the initiation of treatment, e.g., undetectable HCV RNA at 12 weeks is annotated (SVR12).

Direct-acting antivirals (DAAs), such as sofosbuvir, were developed to improve viral eradication rates. They target HCV-encoded proteins involved in viral replication and infection. Sofosbuvir, the first and thus far only DAA, targets NS5B polymerase, the viral enzyme required for HCV RNA replication.

Sofosbuvir may be used in combination with peginterferon. The genetic variant rs12979860, located in the *INFL4* gene, is a strong predictor of response to peginterferon-based therapies. The variant is a C to T change—individuals with the favorable "C/C" genotype have about a 2-fold higher likelihood of achieving SVR compared to individuals with CT or TT genotypes (5). (Note, because the association of rs12979860 with treatment response was reported several years before the discovery of *IFNL4*, the variant is commonly, but mistakenly, referred to as *IL28B*, which is the previous name for the *IFNL3* gene.)

For specific treatment regimens that include sofosbuvir, although the *IFNL4* variant still influences treatment outcomes, the SVR remains relatively high for all *IFNL4* genotypes. For example in the NEUTRINO study, which is referred to in the FDA-approved drug label for sofosbuvir, the SVR12 rate was 99% in individuals with baseline C/C alleles and 87% in individuals with baseline non-C/C alleles. The individuals in this study had HCV genotype 1 or 4 infection, and were receiving sofosbuvir plus peginterferon plus ribavirin therapy (1, 6).

The drug label for sofosbuvir also discusses viral resistance. In cell culture, the amino acid substitution S282T in the viral NS5B polymerase is associated with reduced susceptibility to sofosbuvir (7). During the ELECTRON trial, this substitution was transiently detected in one individual who relapsed during sofosbuvir monotherapy. However, the clinical significance of such substitutions remains unknown (1).

Drug Class: Direct Acting Antivirals for HCV

The treatment of hepatitis C virus (HCV) has evolved over the years. Initially, interferon (IFN) was used as monotherapy. This was followed by the addition of the antiviral agent ribavirin (a nucleoside analogue) to peginterferon. However, only about half of the HCV genotype 1-infected patients cleared their infection, and adverse effects were common and sometimes life-threatening (4). Treatment was also expensive and inconvenient, lasting up to 48 weeks.

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Direct-acting antivirals (DDAs) improved the effectiveness of peginterferon and ribavirin therapy. These agents target specific viral proteins required for viral replication and infection.

HCV is a single-stranded RNA virus that encodes structural proteins (to encode the viral capsid and envelope) and non-structural proteins (required for viral replication). The DDAs target several of the non-structural proteins, the viral protease (NS3/NS4A), the viral RNA polymerase (NS5B), and a viral protein thought to regulate replication and viral assembly (NS5A).

Currently, there are four classes of drugs in clinical use or in development, which are classified by their therapeutic target:

- Protease inhibitors e.g., simeprevir, grazoprevir, paritaprevir
- Nucleoside polymerase inhibitors e.g., sofosbuvir
- Non-nucleoside polymerase inhibitors
- NS5A inhibitors e.g., ledipasvir

Successful treatment of hepatitis C is confirmed when no trace of HCV can be found after treatment has finished. This is referred to as the SVR, which is defined as undetectable HCV RNA by a quantification assay at the end of treatment, and typically 12 (SVR12) or 24 weeks (SVR24) after the end of treatment.

Drug: Sofosbuvir

Sofosbuvir is a nucleotide analogue used in the treatment of chronic HCV infection as part of a combination antiviral treatment regimen.

The early stages of infection with HCV are usually asymptomatic—about 15-45% of people spontaneously clear the virus within 6 months of infection without any treatment. The remaining 55-85% of people will develop chronic HCV infection, which may also be asymptomatic for many years (8).

However, during the natural course of HCV infection, patients develop liver fibrosis, which, without treatment, can progress to liver cirrhosis and liver cancer (hepatocellular carcinoma). The risk of developing liver cancer for a patient with HCV-related cirrhosis is approximately 2-6% per year (9).

HCV is classified by genotype, based on the nucleotide sequence of the viral RNA. There are six major classes of genotype, numbered 1-6, with multiple subtypes e.g., 1a, 1b, 2a, 2b. In the US, approximately 70% of people with HCV infection have genotype 1, with genotype 1a more common than 1b (8). Genotype 1 was formerly the most difficult to cure with interferon-based therapies, as it was less likely than genotypes 2 and 3 to respond to therapy. With the introduction of DAA-based, interferon-free treatments, this is no longer the case.

Sofosbuvir is indicated for the treatment of genotype 1, 2, 3 or 4 chronic HCV infection and is generally considered to have moderate to high efficacy for all six genotypes (10). For the treatment of genotype 1 or 4 infections, the drug label recommends a combination therapy of sofosbuvir plus peginterferon alfa plus ribavirin. For the treatment of genotype 2 or 3 infections, the combination therapy of sofosbuvir plus ribavirin is recommended (1).

Sofosbuvir is a NS5B nucleotide analogue and a prodrug. Once inside a liver cell, sofosbuvir is activated by phosphorylation to a nucleoside triphosphate that competes with nucleotides during viral replication. Binding of the analogue to the viral NS5B polymerase results in RNA chain termination, thus inhibiting the virus from replicating its genome (11).

The safety and efficacy of sofosbuvir has been established in several clinical trials. The usual dose of sofosbuvir is a 400mg tablet, taken once a day for 12 weeks, in combination with other antiviral agents. Sofosbuvir is generally well tolerated, with no side effects beyond those associated with placebo therapy (10, 12).

Sofosbuvir forms the backbone of a several treatment regimens including DAA such as sofosbuvir/velpatasvir and sofosbuvir/velpatasvir/voxilaprevir. The regimen sofosbuvir/ ledipasvir has been found to result in high SVR rates in shorter periods of time, but costs may be prohibitive (7).

Genetic variants in the *IFNL4* gene have been shown to strongly influence treatment response to peginterferon-based regimens in previously untreated patients with HCV genotype 1 infection (5, 13). Such variants also appear to influence the outcomes of treatment regimens that include sofosbuvir. For example, the rs12979860 genotype predicts the response to 8 weeks of treatment with sofosbuvir/ledipasvir (14).

In addition, several substitutions that occur with the viral NS5B polymerase have been reported. Most notably, a S282T polymorphism has been associated with sofosbuvir resistance (15). In cell cultures, the S282T substitution is associated with a reduced susceptibility to sofosbuvir. However, the clinical significance of such substitutions is not yet known, as they appear to be detrimental to viral fitness. So far, the S282T substitution has only been detected in one patient who experienced a relapse while being treated with sofosbuvir monotherapy in a trial, and the substitution was no longer detectable at week 12 post-treatment (1).

Gene: *IFNL4*

The *IFNL4* gene encodes interferon lambda-4 (IFN-λ4) and is involved in the immune response to hepatitis C.

When a person is infected by viruses, including HCV, their immune response includes the production of interferons. These signaling proteins induce changes in infected and uninfected cells to block the viral replication cycle and stop the spread of virus. Interferons are given as part of treatment for HCV to strengthen this innate response.

Three classes of IFNs exist: type I (IFN-α/β), type II (IFN-γ), and type III (IFN-λ). The most recent interferon to be discovered, *IFNL4*, belongs to the type III class. It is located upstream of *IFNL3* and is a functional gene in the majority (>95%) of the African population. But in about 50% of the European population and in most of the east Asian population, IFNL4 is a pseudogene, created by a frameshift-causing deletion polymorphism (rs368234815) (16-18).

As a type III interferon, IFNL4, induces an antiviral state in responsive cells with a higher risk of viral infection, such as mucosal cells (17). IFNL4 exerts its actions by interacting with a cytokine receptor complex, which is composed of the IL10RB and IFNLR1 receptor chains (5). Expression of IFNLR1 is largely restricted to cells of epithelial origin, which includes hepatocytes. In contrast, receptors for type I interferons, such as IFN-α, are expressed in most cell types.

The first two variants to be commonly tested for are rs12979860 (located in *IFNL4*) and rs8099917, which lies proximate to *IFNL4*. These variants are in close proximity to each other and are in strong linkage disequilibrium (5). Linkage disequilibrium means that the variants are linked to treatment response more than would be expected in the general population.

HCV genotype 1 patients with the "favorable" genotypes (CC for rs12979860 and TT for rs8099917) respond better to interferon-based treatment—favorable genotypes are associated with an approximate 2-fold increase in SVR (5). However, for specific treatment regimens which include sofosbuvir, although an individual's *IFNL4* genotype still influences treatment outcomes, the SVR for non-favorable genotypes remains relatively high (1).

In the NETURINO study, patients with HCV genotype 1 or 4 who had not received previous treatments for HCV infection were treated with a regimen of sofosbuvir plus peginterferon plus ribavirin for 12 weeks. The SVR12 rate was 99% (89/90) in subjects with baseline rs12979860 C/C alleles and 87% (200/230) in subjects with baseline rs12979860 non-C/C alleles (6).

Similarly, in the PHOTON trial, patients with HCV genotype 1 infection and co-infection with HIV were treated with a combination of sofosbuvir and ribavarin. The SVR12 rates were 80% (24/30) in subjects with baseline rs12979860 C/C allele and 75% (62/83) in subjects with baseline rs12979860 non-C/C alleles (1).

The frequency of the rs12979860 'C' allele varies globally across different populations—it is commonly found in East Asians (allele frequency nearly 0.9), followed by Caucasians (0.63) and Hispanics (0.55), and is the least common among individuals of African origin (0.39) (5).

In individuals of African ancestry, the rs368234815 variant is superior to rs12979860, and together with another *IFNL4* variant (rs117648444), the combination of testing these two variants gives a greater treatment response prediction compared to testing for single variants (16, 17).

Genetic Testing

Genetic testing for *IFNL4* is used to predict response to peginterferon and ribavarin in HCV genotype 1 patients. The results can help clinicians and patients make informed decisions on how to manage HCV infection.

The rs12979860 variant is most commonly tested, and the results are typically reported in the following format:

rs12979860 CC, favorable genotype

rs12979860 CT, unfavorable genotype

rs12979860 TT, unfavorable genotype (5).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

Statement from the US Food and Drug Administration (FDA): NEUTRINO was an open-label, single-arm trial that evaluated 12 weeks of treatment with sofosbuvir in combination with peginterferon alfa 2a and ribavirin in treatment-naïve subjects with genotype 1, 4, 5 or 6 HCV infection compared to pre-specified historical control. [...] SVR12 rates were 99% (89/90) in subjects with genotype 1 or 4 HCV and baseline IL28B C/C allele and 87% (200/230) in subjects with genotype 1 or 4 HCV and baseline IL28B non-C/C alleles[2].

It is estimated that the SVR12 in patients who previously failed pegylated interferon and ribavirin therapy will approximate the observed SVR12 in NEUTRINO subjects with multiple baseline factors traditionally associated with a lower response to interferon-based treatment. The SVR12 rate in the NEUTRINO trial in genotype 1 subjects with IL28B non-C/C alleles, HCV RNA greater than 800,000 IU/mL and Metavir F3/F4 fibrosis was 71% (37/52).

[…]

In a pooled analysis of 982 subjects who received sofosbuvir in Phase 3 trials, 224 subjects had post- baseline NS5B genotypic data from next generation nucleotide sequencing (assay cutoff of 1%).

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

[2] Note: Recent studies report that the rs12979860 variant is in the IFNL4 gene, and not the IFNL3 gene (previously called IL28B). Therefore, a more accurate term for describing an individual's genotype would be "rs12979860 C/C", instead of "IL28B C/C".

Treatment-emergent substitutions L159F (n=6) and V321A (n=5) were detected in post-baseline samples from GT3a-infected subjects across the Phase 3 trials. No detectable shift in the phenotypic susceptibility to sofosbuvir of subject isolates with L159F or V321A substitutions was seen. The sofosbuvir-associated resistance substitution S282T was not detected at baseline or in the failure isolates from Phase 3 trials. However, an S282T substitution was detected in one genotype 2b subject who relapsed at Week 4 post-treatment after 12 weeks of sofosbuvir monotherapy in the Phase 2 trial P7977-0523 [ELECTRON]. The isolate from this subject displayed a mean 13.5-fold reduced susceptibility to sofosbuvir. For this subject, the S282T substitution was no longer detectable at Week 12 post-treatment by next generation sequencing with an assay cutoff of 1%.

Please review the complete therapeutic recommendations that are located here: (1).

Nomenclature

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
rs12979860	/	NM_001276254.2:c.151-152G>A	N/A	rs12979860
rs8099917	/	N/A	N/A	rs8099917

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

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Tamoxifen Therapy and *CYP2D6* Genotype

Laura Dean, MD[1]

Created: October 7, 2014; Updated: May 1, 2019.

Introduction

Tamoxifen (brand name Nolvadex) is a selective estrogen receptor modulator (SERM) that is commonly used in both the treatment and prevention of breast cancer. When taken for 5 years, tamoxifen almost halves the rate of breast cancer recurrence in individuals who have had surgery for estrogen-receptor–positive (ER+) breast cancer.

Tamoxifen is the endocrine therapy of choice for treatment of premenopausal women with ER+ breast cancer, and an important alternative, or sequential treatment for postmenopausal women with ER+ breast cancer. In addition, tamoxifen is the only hormonal agent approved by the FDA for the prevention of premenopausal breast cancer in women who are at high risk, and the treatment of premenopausal invasive breast cancer and ductal carcinoma *in situ* (DCIS).

The CYP2D6 enzyme metabolizes a quarter of all prescribed drugs and is one of the main enzymes involved in converting tamoxifen into its major active metabolite, endoxifen. Genetic variation in the *CYP2D6* gene may lead to increased ("ultrarapid metabolizer"), decreased ("intermediate metabolizer"), or absent ("poor metabolizer") enzyme activity. Individuals who are intermediate or poor metabolizers may have reduced plasma concentrations of endoxifen and benefit less from tamoxifen therapy.

At this time, the FDA-approved drug label for tamoxifen does not discuss genetic testing for *CYP2D6* (Table 1) (1). The National Comprehensive Cancer Network (NCCN) Breast Cancer Panel does not recommend CYP2D6 testing as a tool to determine the optimal adjuvant endocrine strategy (Table 2), and this recommendation is consistent with the 2010 update of the American Society of Clinical Oncology (ASCO) Guidelines (the most recent update, 2014, does not discuss pharmacogenetic testing) (2, 3).

The Clinical Pharmacogenetics Implementation Consortium (CPIC) recently published updated guidelines for the dosing of tamoxifen based on CYP2D6 phenotype, with therapeutic recommendations for each metabolizer phenotype (Table 3). For CYP2D6 poor metabolizers, CPIC recommends using an alternative hormonal therapy, such as an aromatase inhibitor for postmenopausal women; or an aromatase inhibitor along with ovarian function suppression in premenopausal women. This recommendation is based on these approaches being superior to tamoxifen regardless of *CYP2D6* genotype, and the knowledge that CYP2D6 poor metabolizers who switched from tamoxifen to anastrozole do not have an increased risk of recurrence. The CPIC recommendation also states that higher dose tamoxifen (40 mg/day) can be considered if there are contraindications to aromatase inhibitor therapy; however, the increased endoxifen concentration among CYP2D6 poor metabolizers treated with a higher tamoxifen dose does not typically reach the level as in normal metabolizers (4).

Recommendations from the Dutch Pharmacogenetics Working Group (DWPG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP) also discuss using an alternative drug to tamoxifen in CYP2D6 poor metabolizers (Table 4) (5).

Table 1. The FDA (2018) Drug Label for Tamoxifen: Metabolism

Recommendations
Tamoxifen is a substrate of CYP3A, CYP2C9 and CYP2D6, and an inhibitor of P-glycoprotein.

This FDA table is adapted from (1)

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Table 2. NCCN (2018). *CYP2D6* Phenotypes and Therapeutic Recommendations for Tamoxifen

Genetic test	Recommendation
CYP2D6	Given the limited and conflicting evidence at this time, the NCCN Breast Cancer Panel does not recommend CYP2D6 testing as a tool to determine the optimal adjuvant endocrine strategy. This recommendation is consistent with the ASCO Guidelines. When prescribing a selective serotonin reuptake inhibitor (SSRI), it is reasonable to avoid potent and intermediate CYP2D6 inhibiting agents, particularly paroxetine and fluoxetine, if an appropriate alternative exists.

This National Comprehensive Cancer Network (NCCN) table is adapted from (2). ASCO - American Society of Clinical Oncology

Table 3. CPIC (2018). Dosing Recommendations for Tamoxifen based on CYP2D6 Phenotype

Phenotype		Implications	Therapeutic recommendation[b]	Classification of recommendation[a]
Metabolizer status	Activity score			
CYP2D6 ultrarapid metabolizer	>2.0	Therapeutic endoxifen concentrations	Avoid moderate and strong CYP2D6 inhibitors. Initiate therapy with recommended standard of care dosing (tamoxifen 20 mg/day).	Strong
CYP2D6 normal metabolizer	1.5–2.0	Therapeutic endoxifen concentrations	Avoid moderate and strong CYP2D6 inhibitors. Initiate therapy with recommended standard of care dosing (tamoxifen 20 mg/day).	Strong
CYP2D6 normal metabolizer or intermediate metabolizer (controversy remains)[b]	1.0 (no *10 allele present)[b]	Lower endoxifen concentrations compared with normal metabolizers; higher risk of breast cancer recurrence, event-free and recurrence-free survival compared with normal metabolizers.	Consider hormonal therapy such as an aromatase inhibitor for post-menopausal women or aromatase inhibitor along with ovarian function suppression in premenopausal women, given that these approaches are superior to tamoxifen regardless of *CYP2D6* genotype. If aromatase inhibitor use is contraindicated, consideration should be given to use a higher but FDA approved tamoxifen dose (40 mg/day). Avoid CYP2D6 strong to weak inhibitors.	Optional[b]
CYP2D6 normal metabolizer or intermediate metabolizer (controversy remains)[b]	1.0 (*10 allele present)[b]	Lower endoxifen concentrations compared with normal metabolizers; higher risk of breast cancer recurrence, event-free and recurrence-free survival compared with normal metabolizers.	Consider hormonal therapy such as an aromatase inhibitor for post-menopausal women or aromatase inhibitor along with ovarian function suppression in premenopausal women, given that these approaches are superior to tamoxifen regardless of *CYP2D6* genotype. If aromatase inhibitor use is contraindicated, consideration should be given to use a higher but FDA approved tamoxifen dose (40 mg/day). Avoid CYP2D6 strong to weak inhibitors.	Moderate[b]

Table 3. continued from previous page.

Phenotype		Implications	Therapeutic recommendation[b]	Classification of recommendation[a]
Metabolizer status	Activity score			
CYP2D6 intermediate metabolizer	0.5	Lower endoxifen concentrations compared with normal metabolizers; higher risk of breast cancer recurrence, event-free and recurrence-free survival compared with normal metabolizers.	Consider hormonal therapy such as an aromatase inhibitor for post-menopausal women or aromatase inhibitor along with ovarian function suppression in premenopausal women, given that these approaches are superior to tamoxifen regardless of *CYP2D6* genotype. If aromatase inhibitor use is contraindicated, consideration should be given to use a higher but FDA approved tamoxifen dose (40 mg/day). Avoid CYP2D6 strong to weak inhibitors.	Moderate
CYP2D6 poor metabolizer	0	Lower endoxifen concentrations compared with normal metabolizers; higher risk of breast cancer recurrence, event-free and recurrence-free survival compared with normal metabolizers.	Recommend alternative hormonal therapy such as an aromatase inhibitor for postmenopausal women or aromatase inhibitor along with ovarian function suppression in premenopausal women given that these approaches are superior to tamoxifen regardless of *CYP2D6* genotype and based on knowledge that CYP2D6 poor metabolizers switched from tamoxifen to anastrozole do not have an increased risk of recurrence. Note, higher dose tamoxifen (40 mg/day) increases but does not normalize endoxifen concentrations and can be considered if there are contraindications to aromatase inhibitor therapy.	Strong

Activity score – for a description of how scores are calculated, please see the "Genetic Testing" section below.
[a]Rating scheme described in the CPIC Supplement (4).
[b] CPIC has generally classified individuals with an activity score of 1 as a "normal metabolizer." However, in the case of tamoxifen, prescribing recommendations for those with an activity score (AS) of 1.0 are allele dependent, based on the presence of the *10 allele. Those individuals with an AS of 1.0 on the basis of a *10 allele are provided a "moderate" recommendation. In contrast, prescribing recommendations for those with an activity score of one based on the presence of CYP2D6 alleles other than *10 are graded as "optional" because the recommendations are primarily extrapolated from evidence generated from *10 individuals (i.e., limited data for clinical outcomes and pharmacokinetics for this group).
This Clinical Pharmacogenetics Implementation Consortium (CPIC) table is adapted from (4)

Table 4. DPWG (2015). *CYP2D6* Phenotypes and Therapeutic Recommendations for Tamoxifen

CYP2D6 phenotype	Recommendation
Ultrarapid metabolizer	No action is needed for this gene-drug interaction.
Intermediate metabolizer	1 Select an alternative or measure the endoxifen concentration and increase the dose if necessary by a factor of 1.5–2. Aromatase inhibitors are a possible alternative for post-menopausal women. 2. If tamoxifen is selected: avoid co-medication with CYP2D6 inhibitors such as paroxetine and fluoxetine.

Table 4. continued from previous page.

CYP2D6 phenotype	Recommendation
Poor metabolizer	Select an alternative or increase the dose to 40 mg/day and monitor the endoxifen concentration. Studies have demonstrated that poor metabolizers can achieve an adequate endoxifen concentration when the dose is increased to 40-60 mg/day. Aromatase inhibitors are a possible alternative for post-menopausal women.

This Dutch Pharmacogenetics Working Group (DWPG) table is adapted from (5).

Drug: Tamoxifen

Tamoxifen is a SERM that is used in both the treatment and prevention of breast cancer.

For treatment, tamoxifen is used in both men and women with metastatic breast cancer, particularly among individuals with ER+ tumors. Tamoxifen is also used as adjuvant treatment among women who have undergone surgery and radiation, as this almost halves the rate of reoccurrence of breast cancer in woman with ER+ tumors. Tamoxifen reduces the risk of progression to invasive breast cancer in women with DCIS.

For prevention, tamoxifen has been shown to reduce the occurrence of contralateral breast cancer. And in women who do not have breast cancer, tamoxifen has been shown to reduce the incidence of breast cancer in women at high risk. Risk factors for breast cancer include increasing age, Caucasian race, the number of first-degree relatives with breast cancer, obesity (for postmenopausal women), and an increased exposure to estrogen (e.g., early menarche, later age of first pregnancy or no children, absence of breastfeeding, later menopause) (1, 4).

Tamoxifen acts on the estrogen receptor and has both estrogenic and anti-estrogenic actions, depending on the target tissue. In the breast tissue, it acts as an anti-estrogen (inhibitory effect) and competitively inhibits cancerous ER+ cells from receiving the estrogen they need to proliferate.

In other tissues, such as the endometrium, tamoxifen acts as an estrogen agonist (stimulatory effect) leading to some of the adverse effects associated with tamoxifen therapy. These include endometrial hyperplasia, endometrial polyps, and around a 2.5 times higher risk of developing endometrial cancer. Hot flashes are the most common side effect associated with tamoxifen use, which affect up to 80% of women, and there is also an increased risk of depression (6-8).

The antiestrogenic properties of tamoxifen are expected to affect fetal reproductive functions and increase the risk of fetal harm. Therefore, women may be advised not to become pregnant while taking tamoxifen or within 2 months of discontinuing tamoxifen, and to use barrier or nonhormonal contraception (1).

Tamoxifen also increases the risk of thromboembolic events, such as deep vein thrombosis and pulmonary embolism. The risk of tamoxifen-associated thromboembolic events is further increased when tamoxifen is coadministered with chemotherapy. The drug label for tamoxifen states that the risks and benefits of tamoxifen therapy should be carefully considered in women with a history of thromboembolic events (1).

Some studies suggest that clinicians should consider screening breast cancer individuals before prescribing adjuvant tamoxifen to identify women who are at risk of thrombotic embolic disease as a result of having the Factor V Leiden (p.R506Q) variant, or a variant in the estrogen receptor gene (*ESR1*) (9-12). However, a small substudy (N=81) of the national surgical adjuvant breast and bowel project breast cancer prevention (NSABP P-1) trial found no benefit in screening women for Factor V Leiden or *F2* prothrombin (c.*97G>A) thrombophilia to identify women who may not be appropriate for tamoxifen therapy due to an increased risk for thromboembolic side effects (13).

Tamoxifen is inactive, and its active metabolite endoxifen (4-hydroxy-N-desmethyl tamoxifen) is thought to mediate most of its therapeutic effects. Both endoxifen and another metabolite, 4-hydroxytamoxifen, have around a 100-fold higher affinity for the ER compared with tamoxifen, but endoxifen is thought to be the major metabolite because plasma levels of endoxifen tend to be several-fold higher.

The mechanism of action of tamoxifen involves binding to the ER and inducing a conformational change that blocks or changes the expression of estrogen-dependent genes. It is also likely that tamoxifen interacts with other protein cofactors (both activators and repressors) and binds with different estrogen receptors (ER-alpha or ER-beta) to produce estrogenic and anti-estrogenic effects in different tissues (14).

The tamoxifen metabolite, norendoxifen, has also been found to act as an aromatase inhibitor *in vitro* (albeit at high concentrations). Aromatase inhibitors are a class of drug used to treat breast cancer and gynecomastia. They decrease the amount of estrogen available by inhibiting the conversion of steroids such as androgen into estradiol (15).

The pharmacokinetics of tamoxifen are complex, involving many enzymes (including several cytochrome P450 enzymes) and transporter proteins (including ATP-binding cassette transporters (ABC) transporters). However, CYP2D6 is thought to be important because it mediates the formation of endoxifen via the conversion of the inactive primary metabolite N-desmethyl tamoxifen.

The response to tamoxifen therapy (i.e., clinical efficacy and side effects) varies widely between individuals. This is due to a number of variables, including drug interactions (e.g., coadministration of a drug that inhibits or induces *CYP2D6*) and interindividual differences in drug metabolism driven by polymorphic germline *CYP2D6* variant alleles (16-18).

Gene: *CYP2D6*

The cytochrome P450 superfamily (CYP450) is a large and diverse superfamily of enzymes that form the major system for metabolizing or detoxifying lipids, hormones, toxins, and drugs. The *CYP450* genes are often very polymorphic and can result in reduced, absent, or increased enzyme activity.

The CYP2D6 enzyme is responsible for the metabolism of many commonly prescribed drugs, including antidepressants, antipsychotics, analgesics, and beta-blockers. And, CYP2D6 is the main enzyme that catalyzes the rate-limiting step in the metabolism of tamoxifen to its potent metabolite, endoxifen. Other CYP enzymes involved in tamoxifen metabolism include CYP2C9, CYP2C19, CYP2B6, CYP3A4, and CYP3A5.

CYP2D6 Alleles

The CYP2D6 enzyme catalyzes the main pathway for converting tamoxifen into its most potent metabolite, endoxifen, and together with other CYP enzymes, catalyzes the formation of 4-hydroxytamoxifen. Therefore, genetic variations in the *CYP2D6* gene can influence tamoxifen metabolism (19).

The *CYP2D6* gene is highly polymorphic, as over 100 star (*) alleles have been described and cataloged at the Pharmacogene Variation (PharmVar) Consortium, and each allele is associated with either normal, decreased, or absent enzyme function (Table 5).

The combination of *CYP2D6* alleles that a person has is used to determine their diplotype (e.g., CYP2D6 *4/*4). Based on function, each allele can be assigned an activity score from 0 to 1, which in turn is often used to assign a phenotype (e.g., CYP2D6 poor metabolizer). However, the activity score system is not standardized across clinical laboratories or *CYP2D6* genotyping platforms.

Table 5. Activity Status of Selected *CYP2D6* Alleles

Allele type	*CYP2D6* alleles
Normal function	**1, *2, *33, *35*
Decreased function	**9, *10, *14B, *17, *29, *41*
No function	**3, *4, *5, *6, *7, *8, *11, *12, *13, *15, *19, *20, *21, *36, *38, *40, *42*

For a comprehensive list of *CYP2D6* alleles, please see PharmVar.

*CYP2D6*1* is assigned when no variant is detected and is assumed to have normal enzyme activity (CYP2D6 normal metabolizer phenotype). The *CYP2D6 *2*, **33*, and **35* alleles are also considered to have near-normal activity.

Alleles that encode an enzyme with decreased activity include **10*, **17*, and **41*, and alleles that encode a nonfunctioning enzyme include **3*, **4*, **5,* and **6*. There are large inter-ethnic differences in the frequency of these alleles, with **3*, **4*, **6,* and **41* being more common in Caucasians, **10* more common in Asians, and **17* more common in Africans (20).

Additional variant alleles and their multi-ethnic population frequencies have previously been reported (21). Moreover, given the structural variability of the *CYP2D6* region at chromosome 22q13.2, full gene deletion and duplication alleles, as well as complex tandem alleles with *CYP2D6*'s pseudogene, *CYP2D7*, also occur in some individuals and populations (22).

CYP2D6 Phenotypes

In the US and globally, most individuals, around 70-80%, are classified as "normal metabolizers" (also referred to as "extensive metabolizers"). They either have 2 normal function alleles (e.g., *1/*1) or one normal and one decreased function allele (e.g., *1/*41).

Individuals who have one normal function and one no function allele (e.g., *1/*4) or 2 decreased function alleles (e.g., *41/*41) are also categorized as "normal metabolizers" by recent nomenclature guidelines (23), but have also been categorized as "intermediate metabolizers" (24).

Individuals who have more than 2 normal function copies of the *CYP2D6* gene are classified as "ultrarapid metabolizers," which accounts for 1–10% of Caucasian individuals. For individuals of North African, Ethiopian and Saudi ancestry, the frequency is 16–28% (Table 6) (4).

Individuals who do not have any fully functional alleles are either intermediate metabolizers (one decreased function and one no function allele, e.g., *4/*41) or poor metabolizers (2 no function alleles e.g., *4/*4).

Approximately 6–10% of European Caucasians are poor metabolizers, mainly due to the prevalent nonfunctional **3*, **4* and **5* alleles. Compared with Europeans, individuals of Asian descent are likelier to be intermediate metabolizers due to high population frequencies of the *CYP2D6*10* decreased function allele. Approximately 30% of Asians and individuals of Asian descent are intermediate metabolizers. Similarly, Africans and African Americans are likelier than Europeans to be intermediate metabolizers because of the prevalence of a wide range of decreased function variants. (20, 25-27)

Table 6. CPIC (2018). Assignment of likely CYP2D6 Phenotype based on Genotype

Phenotype[a]		Genotype	Examples of *CYP2D6* diplotypes[b]
Metabolizer status	Activity score		
CYP2D6 ultrarapid metabolizer	>2.0	An individual with duplications of functional alleles	*1/*1xN, *1/*2xN, *2/*2xN[c]

Table 6. continued from previous page.

Phenotype[a]		Genotype	Examples of *CYP2D6* diplotypes[b]
Metabolizer status	Activity score		
CYP2D6 normal metabolizer	1.5–2.0	An individual with 2 normal function alleles or one normal function and one decreased function allele	*1/*1, *1/*2, *1/*9, *1/*41, *2/*2
CYP2D6 normal metabolizer or intermediate metabolizer (controversy remains)[b]	1.0	An individual with 2 decreased function alleles or one normal function and one no function allele. *An activity score (AS) of 1.0 is associated with decreased tamoxifen metabolism to endoxifen compared with an AS of 1.5 or 2.*	*1/*4, *1/*5, *41/*41
CYP2D6 intermediate metabolizer	0.5	An individual with one decreased function and one no function allele	*4/*10, *4/*41, *5/*9
CYP2D6 poor metabolizer	0	An individual with only no functional alleles	*3/*4, *4/*4, *5/*5, *5/*6

[a] See the CYP2D6 frequency table 1 in (4) for race-specific allele and phenotype frequencies.
[b]For a complete list of CYP2D6 diplotypes and resulting phenotypes, see the CYP2D6 genotype to phenotype table in (4). Note that genotypes with an activity score of 1 are classified as normal metabolizers in the online CPIC CYP2D6 genotype to phenotype table (4).
[c]Where xN represents the number of CYP2D6 gene copies. For individuals with CYP2D6 duplications or multiplications, see supplemental data for additional information on how to translate diplotypes into phenotypes.
[d]Individuals with an activity score of 1.0 may be classified as intermediate metabolizers by some reference laboratories. A group of CYP2D6 experts are currently working to standardize the CYP2D6 genotype to phenotype translation system. CPIC will update the CPIC website accordingly (CYP2D6 genotype to phenotype table).
This Clinical Pharmacogenetics Implementation Consortium (CPIC) table is adapted from (4).

Linking Gene Variation with Treatment Response

Genetic variation in the *CYP2D6* gene is associated with variation in plasma concentrations of endoxifen and is thought to account for up to approximately 50% of the variability in endoxifen concentrations (28).

- Individuals who are CYP2D6 poor metabolizers (activity score 0) have lower plasma endoxifen concentrations compared with normal metabolizers (with an activity score of 1.5-2.0).
- Individuals with reduced CYP2D6 activity (activity score 0.5-1) have lower plasma endoxifen concentrations compared with normal metabolizers (with an activity score of 1.5-2.0) (4).

However, while it is clear that tamoxifen biotransformation to endoxifen is highly dependent on CYP2D6 activity, the association between tamoxifen efficacy and *CYP2D6* genotype or endoxifen concentration is less clear. Because the role of *CYP2D6* in tamoxifen response has yet to be fully determined, *CYP2D6* testing remains controversial (29-40).

Some studies conclude that the *CYP2D6* genotype has minimal or no effect on tamoxifen therapy outcomes (41-45). A 2019 prospective clinical study (n=667) found no association between *CYP2D6* genotype or endoxifen concentration and clinical outcome in individuals with early-stage breast cancer receiving adjuvant tamoxifen (46).

In contrast, other studies suggest that *CYP2D6* variant alleles may be important predictors of tamoxifen clinical outcomes (28, 40, 47-52). In particular, in Asians, studies of populations with a high frequency of the decreased function *CYP2D6*10* allele (e.g., Han Chinese), found that individuals with *CYP2D6**10/*10 received less benefit from tamoxifen and poorer disease-free survival (53-55).

However, the high degree of inter-individual variability of tamoxifen metabolism and treatment outcomes is not fully accounted for by *CYP2D6* variation. Additional contributors may include genetic variation in other metabolic pathways and the sequestration of lipophilic tamoxifen metabolites into fat tissues (17, 30, 48, 56).

Genetic Testing

The NIH Genetic Testing Registry (GTR) provides examples of the genetic tests that are currently available for tamoxifen response and for the *CYP2D6* gene.

The *CYP2D6* gene is a particularly complex gene that is difficult to genotype because of the large number of variants and the presence of gene deletions, duplications, multiplications, pseudogenes, and tandem alleles. The complexity of genetic variation complicates the correct determination of *CYP2D6* diplotype.

Genetic testing is currently available for approximately 30 variant *CYP2D6* alleles (over 100 alleles have been identified so far). Test results are typically reported as a diplotype, such as *CYP2D6 *1/*1*. However, it is important to note that the number of variants tested can vary among laboratories, which can result in diplotype result discrepancies between testing platforms and laboratories (4).

A result for copy number, if available, is also important when interpreting CYP2D6 genotyping results. Gene duplications and multiplications are denoted by "xN" e.g., *CYP2D6*1xN with xN representing the number of *CYP2D6* gene copies.

If the test results include an interpretation of the individual's predicted metabolizer phenotype, such as "*CYP2D6* *1/*1, normal metabolizer", this should be confirmed by checking the diplotype and assigning an activity score to each allele (e.g., 0 for no function, 0.5 for decreased function, and 1.0 for each copy of a normal function allele, Table 6).

The CYP2D6 phenotype can be defined by the sum of the 2 activity scores, which is usually in the range of 0–3.0:

- An ultrarapid metabolizer has an activity score greater than 2
- A normal metabolizer phenotype has an activity score of 1.5–2.0
- A normal metabolizer or intermediate metabolizer has a score of 1.0
- An intermediate metabolizer has an activity score of 0.5
- A poor metabolizer has an activity score of 0 (4)

A standardized *CYP2D6* genotype to phenotype assignment logic is currently being developed by an international working group of CYP2D6 experts and both the CPIC and DPWG.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2018 Statement from the US Food and Drug Administration (FDA)

Tamoxifen is extensively metabolized after oral administration. N-desmethyl tamoxifen is the major metabolite found in patients' plasma. The biological activity of N-desmethyl tamoxifen appears to be similar to that of tamoxifen. 4-Hydroxytamoxifen and a side chain primary alcohol derivative of tamoxifen have been identified as

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations where necessary, other author insertions are shown in square brackets.

minor metabolites in plasma. Tamoxifen is a substrate of cytochrome P-450 3A, 2C9 and 2D6, and an inhibitor of P-glycoprotein.

Please review the complete therapeutic recommendations that are located here: (1).

2018 Statement from the National Comprehensive Cancer Network (NCCN)

The cytochrome P-450 (CYP450) enzyme, CYP2D6, is involved in the conversion of tamoxifen to endoxifen. Over 100 allelic variants of *CYP2D6* have been reported in the literature. Individuals with wild-type *CYP2D6* alleles are classified as extensive metabolizers of tamoxifen. Those with one or two variant alleles with either reduced or no activity are designated as intermediate metabolizers and poor metabolizers, respectively. A large retrospective study of 1325 patients found that time to disease recurrence was significantly shortened in poor metabolizers of tamoxifen. However, the Breast International Group (BIG) 1-98 trial reported on the outcome based on *CYP2D6* genotype in a subset of postmenopausal patients with endocrine-responsive, early invasive breast cancer. The study found no correlation between *CYP2D6* allelic status and disease outcome or between *CYP2D6* allelic status and tamoxifen-related adverse effects. A genetic analysis of the ATAC trial found no association between *CYP2D6* genotype and clinical outcomes. Given the limited and conflicting evidence at this time, the NCCN Breast Cancer Panel does not recommend CYP2D6 testing as a tool to determine the optimal adjuvant endocrine strategy. This recommendation is consistent with the ASCO Guidelines. When prescribing a selective serotonin reuptake inhibitor (SSRI), it is reasonable to avoid potent and intermediate CYP2D6 inhibiting agents, particularly paroxetine and fluoxetine, if an appropriate alternative exists.

Please review the complete therapeutic recommendations that are located here: (2).

2018 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC)

Table 3 summarizes the therapeutic recommendations for tamoxifen prescribing based on the CYP2D6 phenotype. Based on current evidence, CYP2D6 UMs and NMs are expected to achieve therapeutic endoxifen concentrations after administration of tamoxifen and should receive the recommended standard of care doses of tamoxifen. CYP2D6 PMs and IMs (including patients with an AS of 1.0, see Supplement) are expected to have lower endoxifen concentrations compared to NMs and have a higher risk of breast cancer recurrence, and worse event-free survival compared to NMs. For CYP2D6 PMs, a "strong" therapeutic recommendation was provided to recommend alternative hormonal therapy such as an aromatase inhibitor (AI) for postmenopausal women or AI along with ovarian function suppression in premenopausal women, given that these approaches are superior to tamoxifen regardless of *CYP2D6* genotype and based on knowledge that CYP2D6 PMs patients who switch from tamoxifen to anastrozole do not exhibit an increased risk of recurrence. Given that escalation of tamoxifen dose from 20–40 mg/day in CYP2D6 PM significantly increases endoxifen concentrations (but not to concentrations achieved in CYP2D6 NMs), the use of an AI (± ovarian function suppression) is recommended in this setting. Tamoxifen 40 mg/day can be considered for CYP2D6 PM if there are contraindications to AI use. There are no clinical data that toremifene, another selective estrogen receptor modulator that also undergoes bioactivation, should be substituted for tamoxifen based on *CYP2D6* genotype.

For CYP2D6 IMs and *CYP2D6*10/*10* or *CYP2D6*10*/decreased function allele, a "moderate" recommendation was made to consider use of an alternative hormonal therapy (i.e., aromatase inhibitor) for postmenopausal women or AI plus ovarian function suppression in premenopausal women is recommended. In CYP2D6 IMs, if AIs are contraindicated, consideration can be given to the use of a higher FDA-approved dose of tamoxifen (40 mg/day), which is known to result in significantly higher endoxifen concentrations without an increase in toxicity. Based on extrapolation from evidence in *10 individuals, a similar recommendation applies to

individuals who carry other decreased function alleles resulting in an AS of 1.0 but with an "optional" recommendation, given the paucity of data for this group.

In general, prolonged overlap of tamoxifen with strong and moderate CYP2D6 inhibitors should be avoided in tamoxifen-treated patients, whereas weak inhibitors are also contraindicated in CYP2D6 IMs.

Please review the complete therapeutic recommendations that are located here: (4)

2015 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

CYP2D6 IM: TAMOXIFEN

This gene variation reduces the conversion of tamoxifen to the active metabolite endoxifen. This can result in reduced effectiveness.

Recommendation:

1 select an alternative or measure the endoxifen concentration and increase the dose if

necessary by a factor of 1.5-2

Aromatase inhibitors are a possible alternative for post-menopausal women.

2. if TAMOXIFEN is selected: avoid co-medication with CYP2D6 inhibitors such as paroxetine

and fluoxetine

CYP2D6 PM: TAMOXIFEN

This gene variation reduces the conversion of tamoxifen to the active metabolite endoxifen. This can result in reduced effectiveness.

Recommendation:

1 select an alternative or increase the dose to 40 mg/day and monitor the endoxifen

concentration

Studies have demonstrated that PM can achieve an adequate endoxifen concentration when the dose is increased to 40-60 mg/day.

Aromatase inhibitors are a possible alternative for post-menopausal women.

CYP2D6 UM: TAMOXIFEN

No action is needed for this gene-drug interaction.

As a result of the genetic variation, the plasma concentration of the active metabolites 4- hydroxytamoxifen and endoxifen can increase. However, there is no evidence that this results in an increase in the side effects.

Background information

Mechanism: The main conversion route of tamoxifen is by CYP3A4/5 to the relatively inactive N-desmethyltamoxifen. This is converted by CYP2D6 to endoxifen (4-hydroxy-N-desmethyltamoxifen), which has an anti-oestrogenic effect that is 30-100x stronger than tamoxifen. Tamoxifen is further converted by CYP2D6 to

the active metabolite 4-hydroxytamoxifen. This metabolite is as potent as endoxifen, but occurs at much lower concentrations. CYP3A4/5 converts 4-hydroxytamoxifen further to endoxifen.

Please review the complete therapeutic recommendations that are located here: (5).

2010 Excerpt from the American Society of Clinical Oncology (ASCO) guideline[2]

"Are There Specific Patient Populations That Derive Differing Degrees of Benefit from an AI Compared With Tamoxifen?"

Recommendation: Direct evidence from randomized trials does not identify a specific marker or clinical subset that predicted which adjuvant treatment strategy—tamoxifen, AI monotherapy, or sequential therapy—would maximally improve outcomes for a given patient. Among men with breast cancer, tamoxifen remains the standard adjuvant endocrine treatment. The Update Committee recommends against using *CYP2D6* genotype to select adjuvant endocrine therapy. The Committee encouraged caution with concurrent use of CYP2D6 inhibitors (such as bupropion, paroxetine, fluoxetine; see Table 11 in the full guideline for a complete list of inhibitors) and tamoxifen because of the known drug-drug interactions.

Comment: The adjuvant endocrine therapy recommendations in this update are for all women, irrespective of any specific clinical subset or prognostic marker. AI therapy has not been evaluated in men, thus the continued recommendation that men with breast cancer receive adjuvant tamoxifen.

Data suggest that variability in tamoxifen metabolism affects the likelihood of cancer recurrence in patients treated with tamoxifen. Factors that contribute to this variability include concurrent use of other drugs that inhibit the CYP2D6 isoenzyme and pharmacogenetic variation (polymorphisms) in *CYP2D6* alleles. It is not yet known whether these variations account for differences in outcomes among patients treated with tamoxifen.

Available data on CYP2D6 pharmacogenetics are insufficient to recommend testing as a tool to determine an adjuvant endocrine strategy. Patients who clearly benefit from known CYP2D6 inhibitors might consider avoiding tamoxifen because of potential pharmacologic interactions. Conversely, patients who receive tamoxifen may prefer to avoid concurrent use of known CYP2D6 inhibitors if suitable alternatives are available."

Please review the complete therapeutic recommendations that are located here: (3).

Nomenclature

Nomenclature for Selected *CYP2D6* Alleles

Common allele name	Alternative names / major SNP	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*4*	1846G>A	NM_000106.5:c.506-1G>A	Not applicable - variant occurs in a non-coding region and results in a splicing defect	rs3892097
*CYP2D6*5*	Not applicable - variant results in a whole gene deletion			
*CYP2D6*6*	1707delT Trp152Gly	NM_000106.5:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655
*CYP2D6*10*	100C>T Pro34Ser	NM_000106.6:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852

[2] The 2014 ASCO practice guideline focused update does not address pharmacogenetic testing (JCO July 20, 2014 vol. 32 no. 212255-2269).

Nomenclature for Selected continued from previous page.

Common allele name	Alternative names / major SNP	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*17*	Includes at least two functional variants: 1023C>T (Thr107Ile) 2850C>T (Cys296Arg)	NM_000106.5:c.320C>T NM_000106.6:c.886C>T	NP_000097.3:p.Thr107Ile NP_000097.3:p.Arg296Cys	rs28371706 rs16947
*CYP2D6*41*	2988G>A	NM_000106.5:c.985+39 G>A	Not applicable – variant occurs in a non-coding region and is linked to aberrant splicing	rs28371725

SNP= Single Nucleotide Polymorphism
Note: In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.
Note: The variant 1846G>A often occurs with both 4180G>C and 100C>T; and 2988G>A occurs with 2850C>T.
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (57).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for Cytochrome P450 enzymes is available from Pharmacogene Variation (PharmVar) Consortium.

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Version History

To view an earlier version of this summary, please see 2014 and 2016 editions.

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Thioguanine Therapy and *TPMT* Genotype

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Introduction

Thioguanine is an antineoplastic agent that belongs to the drug class of thiopurines. It is used in the treatment of acute myeloid leukemia (1).

Thioguanine is a prodrug that must first be activated to form thioguanine nucleotides (TGNs), the major active metabolites. Thiopurine S-methyltransferase (TPMT) inactivates thioguanine, leaving less parent drug available to form TGNs.

An adverse effect of thioguanine therapy is bone marrow suppression, which can occur in any patient, is dose-dependent, and may be reversed by reducing the dose of thioguanine. However, patients who carry two nonfunctional *TPMT* alleles universally experience life-threatening myelosuppression when treated with thioguanine, due to high levels of TGNs. Patients who carry one nonfunctional *TPMT* allele may also be unable to tolerate conventional doses of thioguanine (2, 3).

The FDA-approved drug label for thioguanine states that there are individuals with an inherited deficiency of the thiopurine methyltransferase (TPMT) enzyme who may be unusually sensitive to the myelosuppressive effects of thioguanine and prone to developing rapid bone marrow suppression following treatment initiation. Substantial dosage reductions may be required to avoid the development of life-threatening bone marrow suppression in these patients. Prescribers should be aware that some laboratories offer testing for TPMT deficiency.

The Clinical Pharmacogenetics Implementation Consortium (CPIC) has published recommendations for *TPMT* genotype-based thioguanine dosing. These recommendations include:

Start with reduced doses of thioguanine for patients with one nonfunctional *TPMT* allele, or drastically reduced doses for patients with malignancy and two nonfunctional alleles; adjust dose based on degree of myelosuppression and disease-specific guidelines. Consider alternative nonthiopurine immunosuppressant therapy for patients with nonmalignant conditions and two nonfunctional alleles (see Table 1) (2-4).

Table 1. *TPMT* phenotypes and the therapeutic recommendations for thioguanine therapy, adapted from CPIC

Phenotype	Phenotype details	*TPMT* Genotype	Examples of diplotypes	Therapeutic recommendations for thioguanine (TG)
Homozygous wild-type ("normal")	High enzyme activity. Found in approximately 86–97% of patients.	Two or more functional *TPMT* alleles	**1/*1*	Start with normal starting dose. Adjust doses of TG and of other myelosuppressive therapy without any special emphasis on TG. Allow 2 weeks to reach steady state after each dose adjustment.

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Table 1. continued from previous page.

Phenotype	Phenotype details	*TPMT* Genotype	Examples of diplotypes	Therapeutic recommendations for thioguanine (TG)
Heterozygous	Intermediate enzyme activity. Found in approximately 3–14% of patients.	One functional *TPMT* allele plus one nonfunctional *TPMT* allele	**1/*2* **1/*3A* **1/*3B* **1/*3C* **1/*4*	Start with reduced doses (reduce by 30–50%) and adjust doses of TG based on degree of myelosuppression and disease-specific guidelines. Allow 2–4 weeks to reach steady state after each dose adjustment. In setting of myelosuppression, and depending on other therapy, emphasis should be on reducing TG over other agents.
Homozygous variant	Low or deficient enzyme activity. Found in approximately 1 in 178 to 1~3736 patients.	Two nonfunctional *TPMT* alleles	**3A/*3A* **2/*3A* **3C/*3A* **3C/*4* **3C/*2* **3A/*4*	Start with drastically reduced doses (reduce daily dose by 10-fold and dose thrice weekly instead of daily) and adjust doses of TG based on degree of myelosuppression and disease-specific guidelines. Allow 4–6 weeks to reach steady state after each dose adjustment. In setting of myelosuppression, emphasis should be on reducing TG over other agents. For nonmalignant conditions, consider alternative nonthiopurine immunosuppressant therapy.

The strength of therapeutic recommendations is "moderate" for heterozygous individuals, and "strong" for the other phenotypes. Table is adapted from Relling M.V. et al. Clinical Pharmacogenetics Implementation Consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing. Clinical pharmacology and therapeutics.2011;89(3):387–91 (2, 3).

Drug Class: Thiopurines

Thiopurines are used as anticancer agents and as immunosuppressants in inflammatory bowel disease, rheumatoid arthritis, and other autoimmune conditions. Three thiopurines are used clinically: thioguanine, mercaptopurine, and azathioprine (a prodrug for mercaptopurine). All three agents have similar effects but are typically used for different indications. Thioguanine is most commonly used in the treatment of myeloid leukemias, mercaptopurine is used for lymphoid malignancies, and mercaptopurine and azathioprine are used for immune conditions.

Thiopurines are either activated to form TGNs (the major active metabolite) or deactivated by TPMT. Individuals who carry two non-functional *TPMT* alleles ("*TPMT* homozygotes") universally experience life-threatening bone marrow suppression because of high levels of TGNs when treated with conventional doses. Individuals who carry one non-functional *TPMT* allele ("*TPMT* heterozygotes") may also be unable to tolerate conventional doses of thiopurines due to increased levels of TGNs.

Drug: Thioguanine

Thioguanine is a neoplastic agent used in the treatment of acute myeloid leukemia (AML). AML is the most common acute leukemia in adults, accounting for approximately 80% of cases, and the incidence increases with age. It is a less common cause of acute leukemia in children accounting for less than 10% of cases.

AML is characterized by a proliferation of the myeloid lineage of blood cells, causing an accumulation of abnormal and immature cells in the blood, bone marrow, and sometimes other tissues. This causes a disruption in the production of normal red blood cells, platelets, and mature granulocytes, leading to anemia, bleeding, and an increased risk of infection.

Combination chemotherapy for AML, which includes thioguanine, more frequently induces remission and a longer duration of remission than using thioguanine alone, but because of the high risk of liver toxicity, thioguanine is not recommended for long-term use. Younger patients with AML tend to have a better response to thioguanine than older patients (1).

Like all thiopurines, thioguanine is a purine analogue, and acts as an antimetabolite. Thioguanine is metabolized by two main pathways—it is either activated by HPRT1 (hypoxanthine phosphoribosyltransferase) and metabolized to form TGNs, or deactivated by TPMT. The cytotoxicity of thioguanine is due, in part, to the incorporation of TGNs into DNA. In addition to inhibiting de novo purine synthesis, thioguanine may also inhibit purine nucleotide interconversions (1).

The most frequent adverse reaction to thioguanine is myelosuppression, which can occur in any patient, and can usually be reversed by decreasing the dose of thioguanine. However, all patients who carry two nonfunctional *TPMT* alleles (approximately 0.3%) experience life-threatening myelosuppression after starting treatment with conventional doses of thioguanine.

Individuals who are heterozygous for nonfunctional *TPMT* alleles (approximately 3–14%) are at an increased risk of moderate to severe bone marrow suppression, whereas individuals who are homozygous for wild-type *TPMT* alleles have a lower risk of bone marrow suppression. However, all individuals receiving thioguanine require close monitoring (2, 3, 5, 6).

The FDA-approved drug label for thioguanine states that substantial dosage reductions may be required to avoid the development of life-threatening bone marrow suppression in patients with an inherited deficiency of TPMT. A concern among oncologists may be that a reduced dose of thioguanine will have less anti-tumor efficacy. However, CPIC recommendations (table 1) state that "dose adjustments based on *TPMT* genotype have reduced thiopurine-induced adverse effects without compromising desired antitumor and immunosuppressive therapeutic effects in several clinical settings".

Another adverse effect of thioguanine treatment is hyperuricemia, which frequently occurs because of the rapid lysis of tumor cells. In addition, liver toxicity associated with vascular endothelial damage has been reported when thioguanine is used for maintenance, or for similar long-term continuous therapy. Liver toxicity usually presents as the clinical syndrome of hepatic veno-occlusive disease (hyperbilirubinemia, tender hepatomegaly, weight gain due to fluid retention, and ascites) or with signs of portal hypertension (splenomegaly, thrombocytopenia, and oesophageal varices). For this reason, the long-term use of thioguanine is not recommended (1).

Gene: *TPMT*

The *TPMT* gene encodes one of the important enzymes of phase II metabolism, thiopurine S-methyltransferase. TPMT is one of the main enzymes involved in the metabolism of thiopurines, such as thioguanine. TPMT activity is inherited as a co-dominant trait, as the *TPMT* gene is highly polymorphic with over 40 reported variant alleles (7-10).

The wild-type *TPMT*1* allele is associated with normal enzyme activity. Individuals who are homozygous for *TPMT*1* (TPMT normal metabolizers) are more likely to have a typical response to thioguanine and a lower risk of myelosuppression. This accounts for the majority of patients (~86–97%) (2, 3).

Individuals who are TPMT poor (approximately 0.3%) or intermediate (approximately 3–14%) metabolizers carry variant *TPMT* alleles that encode reduced or absent enzyme activity. Three variant *TPMT* alleles account for over 90% of the reduced or absent activity *TPMT* alleles (11, 12):

- *TPMT*2* (c.238G>C)
- *TPMT*3A* (c.460G>A and c.719A>G)
- *TPMT*3B* (c.460G>A)
- *TPMT*3C* (c.719A>G)

The frequency of *TPMT* alleles varies among different populations. In the United States, the most common low-activity allele in the Caucasian population is *TPMT*3A* (~5%). This allele is also found in individuals who originate from India and Pakistan, but less frequently (7, 13).

In East Asian, African-American, and some African populations, the most common variant is *TPMT*3C* (~2%), although *TPMT*8* may be more common in African populations than previously thought (~2%). In general, *TPMT*2* occurs much less commonly, and *TPMT*3B* occurs rarely (7, 14).

Genetic Testing

Genetic testing is available for several *TPMT* variant alleles, which most commonly includes *TPMT*2*, **3A*, and **3C* as they account for >90% of inactivating alleles. Of note, rare and/or previously undiscovered variants will not be detected by variant-specific genotyping methods (2, 3, 15-18).

TPMT phenotype enzyme activity testing is also available by measuring TPMT activity in red blood cells directly (5). In adult patients taking thioguanine as an immunosuppressive agent, there is strong evidence of a near 100% concordance between phenotype and genotype testing. Inflammatory disease processes do not interfere with the accuracy of TPMT activity measurements if the blood sample is taken under standard conditions (e.g., not within two months of a blood transfusion).

However in patients with leukemia, the concordance between TPMT phenotype and genotype is poor (19). By the time of diagnosis, red cell TPMT activity is typically greatly reduced because of atypical hematopoiesis. Therefore, phenotype testing may wrongly identify an individual as having a TPMT deficiency, e.g., a patient who has two functional copies of the *TPMT* gene (homozygous wild-type) may be determined as having only one functional copy and one nonfunctional variant (*TPMT* heterozygous); and a patient who is *TPMT* heterozygous may be wrongly determined to be *TPMT* homozygous (two copies of nonfunctional *TPMT* variants). In addition, during the course of chemotherapy, *TPMT* phenotype testing may reveal excessively high TPMT activity. This is thought to be due to an excess of young red blood cells with their associated higher level of TPMT enzyme activity. Therefore, to avoid an incorrect TPMT status, genotype testing is recommended for patients with leukemia (19).

Finally, one study reported that *TPMT* genotyping was more reliable than phenotyping in identifying patients at risk of adverse reactions from thiopurine treatment (20), and several studies reported that the *TPMT* genotype is a better indicator than TPMT activity for predicting TGN accumulation or treatment outcome (6, 21-23).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

2013 Statement from the US Food and Drug Administration (FDA): There are individuals with an inherited deficiency of the enzyme thiopurine methyltransferase (TPMT) who may be unusually sensitive to the myelosuppressive effects of thioguanine and prone to developing rapid bone marrow suppression following the initiation of treatment. Substantial dosage reductions may be required to avoid the development of life-threatening bone marrow suppression in these patients. Prescribers should be aware that some laboratories offer testing for TPMT deficiency. Since bone marrow suppression may be associated with factors other than TPMT deficiency, TPMT testing may not identify all patients at risk for severe toxicity. Therefore, close monitoring of clinical and hematologic parameters is important. Bone marrow suppression could be exacerbated by coadministration with drugs that inhibit TPMT, such as olsalazine, mesalazine, or sulphasalazine.

Please review the complete therapeutic recommendations that are located here: (1).

2013 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC): Testing for *TPMT* status is recommended prior to starting thioguanine therapy so that the starting dosages can be adjusted accordingly—see Table 1 for dosing recommendations. In homozygous variant individuals, consider an alternative agent for nonmalignant conditions and drastically reduce doses in malignant conditions. In heterozygous individuals, the starting doses should be reduced. In both patient groups, a longer period of time should be left after each dose adjustment to allow for a steady state to be reached.

Please review the complete therapeutic recommendations that are located here: (2, 3).

Nomenclature

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*TPMT*2*	238G>C Ala80Pro	NM_000367.2:c.238G>C	NP_000358.1:p.Ala80Pro	rs1800462
*TPMT*3A*	This allele contains two variants in cis: c.460G>A and c.719A>G			
*TPMT*3B*	460G>A Ala154Thr	NM_000367.2:c.460G>A	NP_000358.1:p.Ala154Thr	rs1800460
*TPMT*3C*	719A>G Tyr240Cys	NM_000367.2:c.719A>G	NP_000358.1:p.Tyr240Cys	rs1142345

The TPMT Nomenclature Committee defines the nomenclature and numbering of novel TPMT variants: http://www.imh.liu.se/tpmtalleles

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

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The Pharmacogenomics Knowledgebase: http://www.pharmgkb.org

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Version History

To view an earlier version of this summary (Update: March 18, 2013), please click here.

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Thioridazine Therapy and *CYP2D6* Genotypes

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Introduction

Thioridazine is an antipsychotic used in the treatment of schizophrenia and psychosis. Its use is reserved for patients who have failed to respond to or cannot tolerate other antipsychotics.

Thioridazine has been shown to prolong the QT interval (the time taken for the heart ventricles to depolarize and repolarize) in a dose related manner. Drugs with this potential have been associated with the life-threatening ventricular tachycardia, "torsades de pointes".

The CYP2D6 enzyme is involved in metabolizing thioridazine. About 7% of the population has reduced enzyme activity because of variants in the *CYP2D6* gene. In individuals with low CYP2D6 activity, standard doses of thioridazine may lead to higher drug levels in the plasma, and increase the risk of cardiac arrhythmias.

The FDA-approved drug label for thioridazine states that thioridazine is contraindicated in individuals who are known to have reduced levels of CYP2D6 activity. The label also states it is contraindicated to coadminister thioridazine with drugs that inhibit CYP2D6 (e.g., fluoxetine, paroxetine) or inhibit the metabolism of thioridazine (e.g., fluvoxamine, propranolol, and pindolol) (1).

Drug Class: Antipsychotics

The first antipsychotics to be discovered in the 1950s were haloperidol and chlorpromazine, followed by other agents, including fluphenazine, loxapine, pherphenazine, pimozide, thioridazine, thiothixene, and trifluoperazine.

Known as "first generation" or "typical" antipsychotics, these drugs were used to treat psychosis (regardless of the cause), chronic psychotic disorders (e.g., schizophrenia), and other psychiatric conditions. However, prominent adverse effects included extrapyramidal side effects such as tardive dyskinesia, muscle rigidity, and tremors, i.e., Parkinsonian-like symptoms.

Newer antipsychotics, known as "second generation" or "atypical" antipsychotics, have a lower risk of extrapyramidal side effects. However, many have serious metabolic effects. These antipsychotics include aripiprazole, clozapine, iloperidone, olanzapine, and risperidone.

Drug: Thioridazine

Thioridazine is a first generation "typical" antipsychotic used in the treatment of schizophrenia. Schizophrenia is a severe neurodevelopmental disorder with a worldwide prevalence of around 0.3–0.7% (2). The etiology of schizophrenia is unknown, but it is thought to result from a combination of complex genetic and environmental factors. Before the discovery of the first antipsychotics in the 1950s, the management of schizophrenia relied heavily upon sedation, electroconvulsive therapy, and institutionalization.

The symptoms of schizophrenia fall into three main categories: positive, negative, and cognitive. Positive symptoms are generally not found in healthy individuals, but may come and go or persist in individuals with schizophrenia. Positive symptoms include reality distortion (e.g., delusions, hallucinations), and thought disorders. These symptoms often respond well to treatment.

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Negative symptoms are deficits in normal emotions and behavior, and may be mistaken for depression. Symptoms divide into reduced expression of emotion (e.g., speaking without moving or with a monotonous voice) and avolition (a lack of motivation to start or continue with a task). No treatment has established efficacy for these pathologies.

Cognitive symptoms may also be difficult to recognize. They include poor executive functioning (understanding information and using it to make decisions) and trouble focusing or paying attention. And again, no treatment has established efficacy.

The use of thioridazine is reserved for patients who have failed to respond to or cannot tolerate the side effects of other antipsychotics. The FDA-approved drug label for thioridazine strongly recommends that prior to starting thioridazine, a patient should be given at least two trials, each with a different antipsychotic drug product, at an adequate dose, for an adequate duration of time. The label also states that for patients who do require chronic treatment with thioridazine, the smallest dose and the shortest duration of treatment should be sought and the need for continued treatment should be reassessed periodically; and cautions that the efficacy of thioridazine in treating patients with refractory schizophrenia is unknown (1).

The main action of both first-generation and second-generation antipsychotics appears to be the post-synaptic blockade of D2 dopamine receptors in the brain. (An exception is aripiprazole, which is a D2 partial agonist.) Blockade of the D2 receptor in the brain's limbic system is thought to improve the "positive" symptoms of schizophrenia (3).

However, because the first-generation antipsychotics also block dopamine receptors in the nigrostriatal pathway, they cause movement disorders known as extrapyramidal side effects. These disorders include akathisia (motor restlessness), dystonia (abnormal muscle tone), and tardive dyskinesia (involuntary and repetitive movements).

Compared to other first generation antipsychotics, thioridazine shares a similar efficacy, but has a lower risk of extrapyramidal side effects (4-6). However, a higher level of EKG changes is associated with thioridazine therapy (6).

Antipsychotics, and thioridazine in particular, can inhibit cardiac ion channels. Most first generation antipsychotics block the cardiac potassium channel KCNH2, previously known as the human ether-a-go-go-related gene (hERG) (7, 8). Blockade of this channel reduces inward potassium current, resulting in longer cardiac repolarization times. On the EKG, this manifests as a prolonged QT interval. In extreme cases, this can lead to a life-threatening ventricular tachycardia known as torsades de pointes ("twisting of the points") (9-11).

At one point, thioridazine was one of the most commonly used medications for major mental health disorders. However, numerous case reports of sudden, unexpected death led to label changes in 2000, which recommended that thioridazine be used as a last resort (12). In 2005, the manufacturer Novartis discontinued the branded form of thioridazine because of its association with QT prolongation, but generic forms are still available in the US (13, 14).

Thioridazine is metabolized by CYP2D6 to the active metabolite mesoridazine, which is further metabolized to sulforidazine, both of which are more potent than thioridazine. In addition, both thioridazine and mesoridazine have similar effects on the QT interval (15, 16).

Recent research has found that thioridazine is active against multidrug resistant tuberculosis, when used in combination with other antituberculosis drugs. Thioridazine increases the permeability of the cell-envelope, enabling the enhanced uptake of antibiotics (17).

The FDA drug label states that no teratogenic effect has been shown with thioridazine to date. However, all drugs should be kept to a minimum during pregnancy, so thioridazine should be given only when the benefits exceed the possible risks to mother and fetus. Of note, neonates exposed to antipsychotic drugs during the third

trimester are at risk for extrapyramidal and/or withdrawal symptoms following delivery which vary in severity; while in some cases symptoms have been self-limited, in other cases neonates have required intensive care unit support and prolonged hospitalization.

The Cytochrome P450 Superfamily

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The *CYP450* genes are very polymorphic and can result in reduced, absent, or increased enzyme activity.

Gene: *CYP2D6*

CYP2D6 is highly polymorphic; over 100 star (*) alleles are described and currently catalogued at the Human Cytochrome P450 (CYP) Allele Nomenclature Database (18). *CYP2D6*1* is the reference (or wild-type) allele encoding enzyme with normal activity. The *CYP2D6*2*, **33*, and **35* alleles are also considered to confer normal activity (Table 1).

Table 1. Activity status of selected *CYP2D6* alleles

Allele type	*CYP2D6* Alleles
Normal function	**1, *2, *33, *35*
Decreased function	**9, *10, *17, *29, *41*
No function	**3-*8, *11-*16, *19-*21, *38, *40, *42*

For a detailed list of *CYP2D6* alleles, please see (18).

An activity score can be assigned to each *CYP2D6* allele, e.g., 1 for each functional allele, 0.5 for a decreased function allele, and 0 for a no function allele. Individuals who carry more than two normal function copies (e.g., multiple copies) of the *CYP2D6* gene are "ultrarapid metabolizers", whereas individuals who are "normal metabolizers" either carry two normal function copies of *CYP2D6*, or a combination of normal/decreased/no function alleles that result in an activity score between 1.0 and 2.0. Individuals who are intermediate or poor metabolizers carry copies of decreased or no function *CYP2D6* alleles, respectively (Table 2).

Table 2. 2016 Assignment of *CYP2D6* phenotypes by CPIC

Phenotype	Activity Score	Genotypes	Examples of diplotypes
CYP2D6 Ultrarapid metabolizer (approximately 1-20% of patients)[a]	Greater than 2.0	An individual carrying duplications of functional alleles	*(*1/*1)xN* *(*1/*2)xN* *(*2/*2)xN*[b]
CYP2D6 Normal metabolizer (approximately 72-88% of patients)	1.0 – 2.0[c]	An individual carrying two normal function alleles or two decreased function alleles or one normal and no function allele or one normal function and decreased function allele or combinations of duplicated alleles that result in an activity score of 1.0 to 2.0	**1/*1* **1/*2* **2/*2* **1/*9* **1/*41* **41/*41* **1/*5* **1/*4*
CYP2D6 Intermediate metabolizer (approximately 1-13% of patients)	0.5	An individual carrying one decreased function and one no function allele	**4/*41* **5/*9* **4/*10*

Table 2. continued from previous page.

Phenotype	Activity Score	Genotypes	Examples of diplotypes
CYP2D6 Poor metabolizer (approximately 1-10% of patients)	0	An individual carrying two no function alleles	**4/*4* **4/*4xN* **3/*4* **5/*5* **5/*6*

[a] For population-specific allele and phenotype frequencies, please see
[b] Where *xN* represents the number of *CYP2D6* gene copies (N is 2 or more).
[c] Patients with an activity core of 1.0 may be classified as intermediate metabolizers by some reference laboratories.
For more information about activity scores, please see the Genetic Testing section.
This table has been adapted from Hicks J.K., Sangkuhl K., Swen J.J., Ellingrod V.L., Müller D.J., Shimoda K., Bishop J.R., Kharasch E.D., Skaar T.C., Gaedigk A., Dunnenberger H.M., Klein T.E., Caudle K.E. Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC®) for *CYP2D6* and *CYP2C19* Genotypes and Dosing of Tricyclic Antidepressants: 2016 Update. Clinical pharmacology and therapeutics. 2016 Dec 20 [Epub ahead of print] (19).

The most common no function alleles include *CYP2D6*3*, **4*, **5*, and **6* (20-23), and the most common decreased function alleles include *CYP2D6*9, *10, *17, *29* and **41* (24-28) .There are large inter-ethnic differences in the frequency of these alleles. For example, *CYP2D6*4* is the most common no function allele in Caucasians, but is less abundant in subjects with African ancestry, and is rare in Asians. In contrast, the decreased function allele *CYP2D6*10* is the most common allele in Asians, and *CYP2D6*17* is almost exclusively found in individuals with African ancestry (29).

Consequently, the phenotype frequencies also vary substantially among the major ethnicities and may vary among populations. Approximately 6-10% of European Caucasians and their descendants are poor metabolizers, mainly due to the prevalent no function *CYP2D6*4* and **5* alleles (30, 31).

In individuals who are *CYP2D6* poor metabolizers, standard doses of thioridazine may lead to the drug accumulating in the plasma. Since a dose-related side effect of thioridazine is prolongation of the QTc interval, which is a potentially life threatening event, the FDA has stated that the use of thioridazine is contraindicated in individuals who are known to have reduced CYP2D6 activity (1, 32). In addition, the label also states it is contraindicated to coadminister thioridazine with other drugs that inhibit CYP2D6 activity (e.g., the antidepressants fluoxetine and paroxetine) or inhibit the metabolism of thioridazine (e.g., the beta-blockers propranolol and pindolol, and the antidepressant fluvoxamine) (1).

Genetic Testing

The NIH's Genetic Testing Registry, GTR, provides examples of the genetic tests that are currently available for the thioridazine response and the CYP2D6 gene.

Results are typically reported as a diplotype, such as *CYP2D6 *1/*1*. A result for copy number, if available, is also important when interpreting *CYP2D6* results (33). However, it needs to be noted that the number of variants tested varies substantially among laboratories and there is no standardized way to report results (34).

If the test results include an interpretation of the patient's predicted metabolizer phenotype, this should be confirmed by checking the diplotype and assigning an activity score to each allele (e.g., 0 for no function, 0.5 for decreased function, and 1 for each copy of a normal function allele). The phenotype is defined by the sum of the two scores:

- An extensive (normal) metabolizer phenotype has an activity score of 1 to 2
- An intermediate metabolizer has an activity score of 0.5
- A poor metabolizer has an activity score of 0
- An ultrarapid metabolizer has an activity score of greater than 2 (19, 35)

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2016 Statement from the US Food and Drug Administration (FDA): Reduced cytochrome P450 2D6 isozyme activity drugs that inhibit this isozyme (e.g., fluoxetine and paroxetine) and certain other drugs (e.g., fluvoxamine, propranolol, and pindolol) appear to appreciably inhibit the metabolism of thioridazine. The resulting elevated levels of thioridazine would be expected to augment the prolongation of the QTc interval associated with thioridazine and may increase the risk of serious, potentially fatal, cardiac arrhythmias, such as Torsades de pointes type arrhythmias. Such an increased risk may result also from the additive effect of coadministering thioridazine with other agents that prolong the QTc interval. Therefore, thioridazine is contraindicated with these drugs as well as in patients, comprising about 7% of the normal population, who are known to have a genetic defect leading to reduced levels of activity of P450 2D6.

Please review the complete therapeutic recommendations that are located here: (1).

Nomenclature

Nomenclature of selected *CYP2D6* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*4*	1846G>A	NM_000106.5:c.506-1G>A	Variant occurs in a non-coding region (splice variant causes a frameshift)	rs3892097
*CYP2D6*5*	Variant results in a whole gene deletion			
*CYP2D6*6*	1707 del T Trp152Gly • CYP2D6T	NM_000106.5:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655
*CYP2D6*10*	100C>T (Pro34Ser)	NM_000106.5:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
*CYP2D6*17*	1023C>T[1] (Thr107Ile)	NM_000106.5:c.320C>T	NP_000097.3:p.Thr107Ile	rs28371706
	2850C>T[2] (Cys296Arg)	NM_000106.5:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
*CYP2D6*41*	2850C>T[2] (Cys296Arg)	NM_000106.5:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
	2988G>A	NM_000106.5:c.985+39G>A	Variant occurs in a non-coding region (impacts slicing).	rs28371725

[1] In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.
[2] In the literature, 2850C>T is also referred to as 2938C>T.

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: http://www.cypalleles.ki.se/

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

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Tramadol Therapy and *CYP2D6* Genotype

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Introduction

Tramadol is an analgesic used to treat moderate to moderately severe pain. It is a synthetic opioid, related to codeine, and is used to treat both acute and chronic pain. Tramadol is often prescribed for post-operative pain, and pain caused by cancer, osteoarthritis, and other musculoskeletal diseases (1).

The CYP2D6 enzyme metabolizes a quarter of all prescribed drugs, including tramadol. Individuals who carry two inactive copies of *CYP2D6* are known as poor metabolizers and have higher plasma concentrations of tramadol compared with individuals who have two copies of normal activity alleles (1). Individuals who carry one or more reduced or inactive copies of *CYP2D6* are known as intermediate metabolizers, and individuals who carry more than two active copies of *CYP2D6* are known as ultrarapid metabolizers.

The FDA states that the levels of tramadol are approximately 20% higher in poor metabolizers compared to extensive ("normal") metabolizers, while concentrations of the tramadol metabolite, M1, are 40% lower. Inhibitors of CYP2D6, such as fluoxetine and amitriptyline, also inhibit the metabolism of tramadol, and the full pharmacological impact of these alterations of tramadol dose in terms of either efficacy or safety is unknown (1).

A guideline from the Dutch Pharmacogenetics Working Group includes dose recommendations for poor metabolizers (either select an alternative drug—not oxycodone or codeine—or be alert to the symptoms of insufficient pain relief). It also contains dose recommendations for intermediate metabolizers (be alert to decreased efficacy of tramadol, consider increasing the dose and if the response is still inadequate, either select an alternative drug—not oxycodone or codeine, or be alert to the symptoms of insufficient pain relief) and ultrarapid metabolizers (either reduce the dose of tramadol by 30% and be alert to adverse drug events, or select an alternative drug e.g., acetaminophen, NSAID, morphine—not oxycodone or codeine) (see Table 1) (2).

Table 1. *CYP2D6* phenotypes and the therapeutic recommendations for tramadol therapy

Phenotype	Genotype	Therapeutic recommendation for tramadol
Ultrarapid metabolizer	More than two copies of functional alleles	Reduce dose by 30% and be alert to ADEs (e.g., nausea, vomiting, constipation, respiratory depression, confusion, urinary retention) or select alternative drug (e.g., acetaminophen, NSAID, morphine—not oxycodone or codeine)
Intermediate metabolizer	One active allele and one inactive allele, or two decreased activity alleles, or one decreased activity allele and one inactive allele	Be alert to decreased efficacy. Consider dose increase. If response is still inadequate, select alternative drug—not oxycodone or codeine—or be alert to symptoms of insufficient pain relief
Poor metabolizer	Two inactive alleles	Select alternative drug—not oxycodone or codeine—or be alert to symptoms of insufficient pain relief

ADE: Adverse Drug Event

The strength of the tramadol therapeutic recommendations scored a maximum of 4/4 (the highest quality of evidence) for poor and intermediate metabolizers, and a score of 3/4 for ultrarapid metabolizers. Table is adapted from Swen J.J., Nijenhuis M., de Boer A., Grandia L. et al. Pharmacogenetics: from bench to byte - an update of guidelines. Clinical pharmacology and therapeutics. 2011;89(5):662–73 (2).

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Table 2. Activity status of *CYP2D6* alleles

Allele type	Alleles
Active	**1, *2, *33, *35*
Decreased activity	****9, *10, *17**, *29, *36, ***41***
Inactive	****3-*8**, *11-*16, *19-*21, *38, *40, *42*

Note: The most clinically significant variants are highlighted in bold.

Drug: Tramadol

Tramadol is an analgesic that is used to treat moderate to moderately severe pain. Tramadol is commonly prescribed for postoperative, cancer, and musculoskeletal pain. In the US, tramadol is classified as a Schedule IV controlled substance (1, 3).

Tramadol is a centrally acting analgesic that is structurally related to codeine and morphine, and belongs to the same drug class of opiate drugs. Tramadol, however, is a synthetic opioid, and it is administered as a racemic mixture of two enantiomers, (+) and (-) tramadol (4).

Although opiates have been used for pain control for several thousands of years, the receptors upon which they act were discovered relatively recently, in the 1960s. The exact mechanism of action of tramadol is not known, but it is thought that both enantiomers contribute to its analgesic effect in different ways. Tramadol has some activity at mu-opioid receptor (less than codeine) and it also inhibits the synaptic reuptake of serotonin and norepinephrine which inhibits pain transmission at the spinal cord (4, 5).

Tramadol is extensively metabolized within the liver and has one main major metabolite, O-desmethyltramadol, known as M1. Both the parent drug and M1 contribute to the analgesic effect, but M1 has a significantly higher affinity for opioid receptors than tramadol (6). The enzyme CYP2D6 catalyzes the production of M1, and other CYP enzymes (CYP2B6 and CYP3A4) catalyze the production of M2, an inactive metabolite (7).

The adverse effects of tramadol therapy are similar to that of other weak opioids. Common side effects include dizziness, nausea, constipation, and headache. But an additional risk of tramadol therapy is the risk of seizures, especially in patients who are already taking antidepressants or other drugs that decrease the seizure threshold. There is also an increased risk of suicide, and therefore tramadol should not be prescribed for patients who are suicidal or prone to addictions—the use of non-narcotic analgesics should be considered instead (1).

Because tramadol has mu-opioid agonist activity, there is a risk of abuse and addiction, even under appropriate medical use. Therefore, as for all patients treated with opioids, there should be careful monitoring of patients taking tramadol. In addition, the longer a patient is on continuous tramadol therapy, the greater the risk of tolerance (the need to increase the dose of drug to maintain a defined level of analgesia in the absence of disease progression). Physical dependence upon tramadol is manifested by withdrawal symptoms after the use of tramadol is stopped abruptly. Symptoms include restlessness, rhinorrhea, lacrimation, and chills (1).

Serotonin syndrome is a potentially life-threatening syndrome that may occur with the use of tramadol, especially if other medications such as antidepressants or other drugs that impair the metabolism of tramadol (CYP2D6 and CYP3A4 inhibitors) are used concurrently. Symptoms include changes in mental status (e.g., agitation, hallucinations, coma), autonomic instability (e.g., tachycardia, labile blood pressure, hyperthermia), neuromuscular aberrations (e.g., hyperreflexia, incoordination) and/or gastrointestinal symptoms (e.g., nausea, vomiting, diarrhea) (1, 8).

Gene: *CYP2D6*

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The *CYP450* genes are very polymorphic and can result in reduced, absent, or increased enzyme activity.

CYP2D6 is responsible for the metabolism of many commonly prescribed drugs, including antidepressants, antipsychotics, analgesics, and beta-blockers. The *CYP2D6* gene is highly polymorphic, with more than 100 star (*) alleles described (9).

*CYP2D6*1* is the wild-type allele and is associated with normal enzyme activity and the "extensive metabolizer" phenotype. The *CYP2D6* alleles **2*, **33*, and **35* are also considered to have near-normal activity.

Other alleles include variants that produce a non-functioning enzyme (e.g., **3*, **4*, **5*, and **6*) (10-13) or an enzyme with reduced activity (e.g., **10*, **17*, and **41*) (2, 14, 15) (see Table 2). There are large inter-ethnic differences in the frequency of these alleles, with **3*, **4*, **5*, **6*, and **41* being more common in Caucasians, **17* more common in Africans, and **10* more common in Asians (16).

Individuals who are intermediate or poor metabolizers carry copies of decreased-functioning and inactive *CYP2D6* alleles (see Table 1 and 2). In these individuals, the metabolic capacity of CYP2D6 is decreased which may result in higher levels of tramadol.

The FDA-approved drug label for tramadol includes a study where concentrations of tramadol were approximately 20% higher in "poor metabolizers" versus "extensive metabolizers", while M1 concentrations were 40% lower. The label also states that other factors, such as the concurrent use of CYP2D6 inhibitors (e.g., fluoxetine and its metabolite norfluoxetine, amitriptyline and quinidine) could also result in increases in tramadol concentrations and decreased concentrations of M1, and that the "full pharmacological impact of these alterations in terms of either efficacy or safety is unknown" (1).

The Dutch Pharmacogenetics Working Group recommendations state that for poor metabolizers, "either select an alternative drug (not oxycodone or codeine) or be alert to the symptoms of insufficient pain relief" (2).

Poor metabolizers are commonly found in European Caucasians (6-10%). The most common allele in this population is the functional *CYP2D6*1* (70%), and the most common nonfunctional alleles include *CYP2D6*4* and **5*, which largely account for the poor metabolizer phenotype in these populations (16). About 2% of African Americans are poor metabolizers, due to a wide ranges of variants that include the nonfunctional **4* and **5* alleles (17-19).

For intermediate metabolizers, the Dutch Pharmacogenetics Working Group recommendations state to be alert to decreased efficacy of tramadol. Consider increasing the dose of tramadol and if the response is still inadequate, either select an alternative drug (not oxycodone or codeine), or be alert to the symptoms of insufficient pain relief (2).

Approximately 30% of Asians and individuals of Asian descent are intermediate metabolizers. In these populations, only half of *CYPD6* alleles are fully functional, with the reduced function **10* variant being very common (~40%, compared to ~2% in Caucasians) (20). As a result, Asians are more likely to be intermediate metabolizers than Caucasians (16).

Individuals who have multiple functional copies of the *CYP2D6* gene are "ultrarapid metabolizers" (UM). Each allele contributes to the metabolism of tramadol. The Dutch Pharmacogenetics Working Group recommendations state that for ultrarapid metabolizers, either reduce the dose of tramadol by 30% and be alert to adverse drug events (e.g., nausea, vomiting, constipation, respiratory depression, confusion, urinary retention), or select an alternative drug (e.g., acetaminophen, NSAID, morphine—not oxycodone or codeine).

The ultrarapid metabolizer phenotype is estimated to be present in up to 28% of North Africans, Ethiopians, and Arabs; up to 10% in Caucasians; 3% in African Americans, and up to 1% in Hispanics, Chinese, and Japanese (16).

Genetic Testing

Genetic testing is available for many of the more common variant *CYP2D6* alleles. Results are typically reported as a diplotype, such as *CYP2D6 *1/*1* (21). A result for copy number, if available, is also important when interpreting *CYP2D6* results.

If the test results include an interpretation of the patient's predicted metabolizer phenotype, this should be confirmed by checking the diplotype and assigning an activity score to each allele (e.g., 0 for nonfunctional, 0.5 for reduced function, and 1 for each copy of a functional allele). The phenotype is defined by the sum of the two scores:

- An extensive (normal) metabolizer phenotype has an activity score of 1 to 2
- An intermediate metabolizer has an activity score of 0.5
- A poor metabolizer has an activity score of 0
- An ultrarapid metabolizer has an activity score greater than 2

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

Statement from the US Food and Drug Administration (FDA): Approximately 7% of the population has reduced activity of the CYP2D6 isoenzyme of cytochrome P- 450. These individuals are "poor metabolizers" of debrisoquine, dextromethorphan, tricyclic antidepressants, among other drugs. Based on a population PK [pharmacokinetic] analysis of Phase I studies in healthy subjects, concentrations of tramadol were approximately 20% higher in "poor metabolizers" versus "extensive metabolizers", while M1 [tramadol metabolite] concentrations were 40% lower. Concomitant therapy with inhibitors of CYP2D6 such as fluoxetine, paroxetine and quinidine could result in significant drug interactions. In vitro drug interaction studies in human liver microsomes indicate that inhibitors of CYP2D6 such as fluoxetine and its metabolite norfluoxetine, amitriptyline and quinidine inhibit the metabolism of tramadol to various degrees, suggesting that concomitant administration of these compounds could result in increases in tramadol concentrations and decreased concentrations of M1. The full pharmacological impact of these alterations in terms of either efficacy or safety is unknown. Concomitant use of SEROTONIN re-uptake INHIBITORS and MAO INHIBITORS may enhance the risk of adverse events, including seizure and serotonin syndrome.

Please review the complete therapeutic recommendations that are located here: (1)

Summary of recommendations from the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP): For ultrarapid metabolizers, either reduce the dose of tramadol by 30% and be alert to adverse drug events (e.g., nausea, vomiting, constipation, respiratory depression, confusion, urinary retention), or select an alternative drug (e.g., acetaminophen, NSAID, morphine—not oxycodone or codeine).

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt and may have inserted text in brackets, to explain some of the terms used. The FDA may not have labeled all formulations containing the generic drug.

For intermediate metabolizers, be alert to decreased efficacy of tramadol. Consider increasing the dose of tramadol and if the response is still inadequate, either select an alternative drug (not oxycodone or codeine), or be alert the symptoms of insufficient pain relief.

For poor metabolizers, either select an alternative drug (not oxycodone or codeine) or be alert to the symptoms of insufficient pain relief.

Please review the complete therapeutic recommendations that are located here: (2)

Nomenclature

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*4*	1846G>A	NM_000106.4:c.506-1G>A	Not applicable—variant occurs in a non-coding region	rs3892097
*CYP2D6*5*	CYP2D6,DEL	NC_000022.10:g.(42534124_42531353)_(42521970_42519196)del	Not applicable—variant results in a whole gene deletion	
*CYP2D6*6*	1707 del T Trp152Gly	NM_000106.4:c.454delT	NP_000097.2:p.Trp152Glyfs	rs5030655
*CYP2D6*10*	100C>T Pro34Ser	NM_000106.4:c.100C>T	NP_000097.2:p.Pro34Ser	rs1065852
*CYP2D6*17*	Includes at least two functional variants*: 1023C>T (Thr107Ile) 2850C>T (Cys296Arg)	NM_000106.4:c.320C>T NM_000106.4:c.886T>C	NP_000097.2:p.Thr107Ile NP_000097.2:p.Cys296Arg	rs28371706 rs16947
*CYP2D6*41*	2988G>A	NM_000106.4:c.985+39G>A	Not applicable—variant occurs in a non-coding region	rs28371725

* In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: http://www.cypalleles.ki.se/

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Trastuzumab (Herceptin) Therapy and *ERBB2 (HER2)* Genotype

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Introduction

Trastuzumab (brand name, Herceptin) is a monoclonal antibody used in the treatment of breast and gastric/gastroesophageal cancer. It targets an epidermal growth factor receptor encoded by the *ERBB2* gene, which is commonly referred to as the *HER2* gene.

The *HER2* gene is overexpressed in 15-20% of breast cancers and is also overexpressed in some cases of gastric cancer. Overall, “HER2 positive” tumors are associated with a faster rate of growth and a poorer prognosis. The use of trastuzumab in treatment regimes improves outcomes, but adverse effects of therapy include cardiac toxicity.

The FDA-approved drug label for trastuzumab states that trastuzumab should only be used to treat patients with tumors that have either HER2 protein overexpression or HER2 gene amplification, as determined by an accurate and validated FDA-approved assay, specific for the type of tumor tested (breast or gastric). This is because these are the only patients studied for whom benefit has been shown (1).

A guideline from ASCO/CAP states that oncologists must request HER2 testing on every primary invasive breast cancer (and on a metastatic site, if stage IV and if specimen available) from a patient with breast cancer to guide decision to pursue HER2-targeted therapy. This should be especially considered for a patient who previously tested HER2 negative in a primary tumor and presents with disease recurrence with clinical behavior suggestive of HER2-positive or triple-negative disease (2).

Drug: Trastuzumab (Herceptin)

Trastuzumab (brand name, Herceptin) is a monoclonal antibody that targets ERBB2 (a tyrosine kinase receptor, also known as HER2 or HER-2/neu). Trastuzumab is only used to treat specific tumors that overexpress ERBB2; these tumors are known as “HER2-positive” tumors.

Trastuzumab is typically used as an adjuvant treatment of early-stage HER2-positive breast cancer. Adjuvant therapies are used after primary treatment (such as surgery) to increase the chance of long-term disease-free survival. An example chemotherapy treatment regime is "AC→TH", which stands for Adriamycin, Cytoxan, then Taxol and Herceptin. Trastuzumab is also used in the treatment of HER2-positive metastatic breast cancer and HER2-positive metastatic gastric cancer (1).

Recently, HER2 targeted therapy has been approved by the FDA for use in the neoadjuvant setting. Neoadjuvant therapy is given before primary therapy, for example, to shrink a tumor to an operable size or to allow for breast-conserving surgery, and to increase the chance of long-term, disease-free survival. In the neoadjuvant setting, pertuzumab, along with trastuzumab and docetaxel (a chemotherapy agent) can be given pre-operatively (3-5).

Before treatment with trastuzumab begins, overexpression of the ERBB2 protein or amplification of the ERBB2 gene must first be determined. The FDA recommends that testing be performed using an FDA-approved test for the specific tumor type (breast or gastric tumor), in a laboratory with demonstrated proficiency with the technology being used. This is because the benefits of trastuzumab have only been proven in patients with tumors that overexpress ERBB2. In addition, although trastuzumab is generally well tolerated, the risks of

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treatment include infusion reactions, pulmonary toxicity, and cardiomyopathy that can result in cardiac failure (1).

Trastuzumab targets the ERBB2 receptor by binding to the juxtamembrane portion of the extracellular domain. This binding limits the receptor's ability to activate its intrinsic tyrosine kinase, which in turn, limits the activation of numerous signaling pathways that can promote the growth of cancerous cells.

A number of proposed mechanisms may underlie the anti-tumor effects of trastuzumab. One such mechanism is that trastuzumab blocks the HER3 receptor from binding to ERBB2. The ERBB2-HER3 dimerized receptor is thought to be highly active, triggering many signaling cascades in the absence of a "true" ligand (6).

Another proposed mechanism is antibody-dependent cellular cytotoxicity (ADCC). Once trastuzumab has bound to a cancer cell, immune cells (typically activated natural killer cells) bind to trastuzumab and initiate lysis of the cancer cell (7). Trastuzumab may also mediate the enhanced internalization and degradation of the ERBB2 receptor, inhibit angiogenesis, and inhibit ERBB2 shedding by preventing the cleavage of ERBB2 and the subsequent release of its extracellular domain (8, 9).

Unfortunately, breast cancer may start to progress again during trastuzumab therapy. Possible mechanisms that may facilitate disease progression during treatment include increased signaling from the HER family of receptors, an upregulation of downstream signaling pathways, and an increased level of insulin growth factor -1 receptor (10, 11).

At the time of writing, four drugs have been approved to target ERBB2 (trastuzumab, lapatinib, pertuzumab, and T-DM1), with more drugs in clinical trials.

Gene: *ERBB2 (HER2)*

The human epidermal growth factor receptor (HER) family consists of four members: the epidermal growth factor receptor (EGFR), HER2, HER3, and HER4 (see Nomenclature). All four members are transmembrane tyrosine kinase receptors, and they regulate a number of important cellular processes, such as cell growth, survival, and differentiation (12).

HER2, along with EGFR, are proto-oncogenes. Proto-oncogenes are a group of genes that, when mutated or expressed at abnormally high levels, can contribute to normal cells becoming cancerous cells. The mutated version of the proto-oncogene is called an oncogene. Proto-oncogenes typically encode proteins that stimulate cell division, inhibit cell differentiation, and halt cell death. All these are important biological processes. However, the increased production of these proteins, caused by oncogenes, can lead to the proliferation of poorly differentiated cancer cells (13).

The official gene symbol for HER2 is ERBB2, which is derived from a viral oncogene with which the receptor shares homology; "v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2." However, clinicians commonly refer to the ERBB2 gene as "HER2" (Human Epidermal growth factor Receptor 2) or "HER2/neu" (neu was the name given to the gene that caused cancer derived from a rodent neuro/glioblastoma). HER2 is an alternate gene symbol for ERBB2 and is more commonly used by the community.

One unique feature of ERBB2 compared to the other receptors in the HER family is the absence of a known ligand. It is therefore thought that this receptor may permanently be in an activated state, or it may become activated during heterodimerization with one of the other members of the HER family (9). And, one unique feature of HER3 is that it has very little enzymatic activity compared to the other tyrosine kinase receptors in the HER family. It is therefore thought that an important role of HER3 is to act as a heterodimerization partner for ERBB2 (14, 15).

When a partner such as HER3 binds to ERBB2, the heterodimer undergoes activation, which stimulates the intrinsic tyrosine kinase activity of the receptor. Autophosphorylation of several key residues of the receptor triggers the downstream activation of many commonly used growth factor signaling pathways, such as the PI3K/AKT/mTOR pathway and the RAS/RAF/MEK/ERK pathway (16, 17). Impaired ERBB2 signaling is associated with the development of neurodegenerative diseases, such as multiple sclerosis and Alzheimer disease, whereas excessive ERBB2 signaling is associated with the development of cancers.

ERBB2 is overexpressed in approximately 15-20% of breast tumors, as a result of amplification of the ERBB2 gene, and tumors with increased ERBB2 usually have a higher growth rate and more aggressive clinical behavior (2, 18-20). Although gene amplification is frequently seen in cancer and other degenerative disorders, the underlying basis for amplification remains largely unknown (21). And in the case of ERBB2, although sequence variants have been identified, it is nearly always the wildtype ERBB2 gene that is overexpressed in tumors (22). In about 1% of breast cancers, activating mutations in ERBB2 can be identified that are likely to drive tumorigenesis, without ERBB2 amplification (23).

Tumor Testing for *ERBB2 (HER2)*

There are two main methods used for HER2 testing: testing for overexpression of the HER2 protein using immunohistochemistry (IHC), or testing for gene amplification using in-situ hybridization (ISH). Each assay type has diagnostic pitfalls that must be avoided, and so the pathologist who reviews the histologic findings should determine the optimal assay (IHC or ISH) for the determination of HER2 status (2, 20).

In an IHC assay, a slice of tumor tissue is stained, along with a control sample that contains high levels of HER2. The tumor sample is then examined by light microscopy to assess the intensity of membrane staining—the amount of staining correlates with the quantity of HER2 protein and is typically graded from 0 to 3+:

- IHC 0 means no visible staining and is an "HER2 negative" result
- IHC 1+ is also an "HER2 negative" result—there is a staining pattern with weak and incomplete staining, or weak and complete staining of very few tumor cells
- IHC 2+ is an "HER2 equivocal result"—there is a staining pattern with moderately intense staining, or intense staining of very few tumor cells
- IHC 3+ is an "HER2 positive result"—there is a staining pattern with intense membrane staining on more than 10% of tumor cells, indicating a higher than normal level of HER2

For an equivacol (IHC 2+) result, either a reflex test must be ordered (same specimen using ISH), or a new test must be ordered (using a new specimen, if available, using IHC or ISH) to confirm the results.

The ISH assay, or FISH assay (fluorescence in situ hybridization), measures HER2 gene amplification by measuring HER2 DNA—the actual number of copies of the HER2 genes are counted. Under the microscope, the genes appear as red signals or dots, in a blue-stained cancer cell nucleus. The result is usually either FISH negative (normal level of HER2 gene) or FISH positive (at least twice as much as normal level of HER2 gene), but in a small number of cases the FISH result will be equivocal due to a low level of HER2 amplification. The use of a control helps distinguish between a negative result and a non-informative result caused by an error. Approximately 25% of patients who have an IHC 2+ result will have a FISH positive result (24).

For the complete algorithms for evaluation of HER2 protein expression using IHC or ISH, please see the American Society of Clinical Oncology (ASCO) / College of American Pathologists (CAP) clinical practice guideline update, located here (2)

Therapeutic Recommendations based on *HER2* Testing

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

Statement from the US Food and Drug Administration (FDA):

Detection of HER2 protein overexpression is necessary for selection of patients appropriate for trastuzumab therapy because these are the only patients studied and for whom benefit has been shown. Due to differences in tumor histopathology, use FDA-approved tests for the specific tumor type (breast or gastric/gastroesophageal adenocarcinoma) to assess HER2 protein overexpression and HER2 gene amplification. Tests should be performed by laboratories with demonstrated proficiency in the specific technology being utilized. Improper assay performance, including use of suboptimally fixed tissue, failure to utilize specified reagents, deviation from specific assay instructions, and failure to include appropriate controls for assay validation, can lead to unreliable results.

Several FDA-approved commercial assays are available to aid in the selection of breast cancer and metastatic gastric cancer patients for trastuzumab therapy. Users should refer to the package inserts of specific assay kits for information on the Intended Use, and the validation and performance of each assay.

Limitations in assay precision make it inadvisable to rely on a single method to rule out potential Herceptin benefit.

Please review the complete therapeutic recommendations that are located here: (1)

FDA-approved medical devices for HER2 are listed here.

Excerpted recommendations from the American Society of Clinical Oncology / College of American Pathologists 2013 clinical practice guideline update:

Key Recommendations for Oncologists

- Must request HER2 testing on every primary invasive breast cancer (and on metastatic site, if stage IV and if specimen available) from a patient with breast cancer to guide decision to pursue HER2-targeted therapy. This should be especially considered for a patient who previously tested HER2 negative in a primary tumor and presents with disease recurrence with clinical behavior suggestive of HER2-positive or triple-negative disease.
- Should recommend HER2-targeted therapy if HER2 test result is positive, if there is no apparent histopathologic discordance with HER2 testing and if clinically appropriate.
- Must delay decision to recommend HER2-targeted therapy if initial HER2 test result is equivocal. Reflex testing should be performed on the same specimen using the alternative test if initial HER2 test result is equivocal or on an alternative specimen.
- Must not recommend HER2-targeted therapy if HER2 test result is negative and if there is no apparent histopathologic discordance with HER2 testing.
- Should delay decision to recommend HER2-targeted therapy if HER2 status cannot be confirmed as positive or negative after separate HER2 tests (HER2 test result or results equivocal). The oncologist should confer with the pathologist regarding the need for additional HER2 testing on the same or another tumor specimen.
- If the HER2 test result is ultimately deemed to be equivocal, even after reflex testing with an alternative assay (i.e., if neither test is unequivocally positive), the oncologist may consider HER2-targeted therapy.

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

The oncologist should also consider the feasibility of testing another tumor specimen to attempt to definitely establish the tumor HER2 status and guide therapeutic decisions. A clinical decision to ultimately consider HER2-targeted therapy in such cases should be individualized on the basis of patient status (comorbidities, prognosis, and so on) and patient preferences after discussing available clinical evidence.

Please review the complete therapeutic recommendations, including Key Recommendations for Pathologists that are located here (2).

Nomenclature

Common gene symbols	Alternative gene symbols
EGFR	*ERBB1* *ERBB* *HER1*
ERBB2	*HER2* *HER-2* *HER-2/neu* *NEU*
ERBB3	*HER3*
ERBB4	*HER4*

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Vemurafenib Therapy and *BRAF* and *NRAS* Genotype

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Introduction

Vemurafenib is a kinase inhibitor used in the treatment of patients with unresectable or metastatic melanoma with the *BRAF* V600E variant.

BRAF is an intracellular kinase in the mitogen-activated protein kinases (MAPK) pathway. BRAF is involved in regulating important cell functions such as cell growth, division, differentiation, and apoptosis. BRAF is also a proto-oncogene—when mutated it has the ability to transform normal cells into cancerous cells.

Variation in the kinase domain of BRAF have been associated with various cancers. The most common *BRAF* variant, V600E, constitutively activates the kinase, and causes cell proliferation in the absence of growth factors that would normally be required. The V600E variant is detected in approximately 50% of melanomas (1, 2).

The FDA-approved drug label for vemurafenib states that the presence of *BRAF* V600E mutation in tumor specimens should be confirmed, using an FDA-approved test, before starting treatment with vemurafenib. The label also states that vemurafenib is not indicated for treatment of patients with wild-type *BRAF* melanoma (3).

Variations in *NRAS*, also an oncogene, are found in up to 30% of all malignancies and in approximately 15-20% of melanomas. *NRAS* variants activate MAPK and have been implicated in in acquired resistance to BRAF inhibitors. Vemurafenib's label warns that one adverse effect associated with therapy may be the progression of pre-existing chronic myelomonocytic leukemia with *NRAS* mutation (3). Other adverse effects include arthralgia, rash, alopecia, photosensitivity reaction, pruritus, and skin papilloma.

Drug: Vemurafenib

Vemurafenib is a BRAF kinase inhibitor indicated for the treatment of patients with unresectable or metastatic melanoma with the *BRAF* V600E variant, as detected by an FDA-approved test. It was one of the first molecularly targeted agents to receive FDA approval for advanced melanoma (3). Off-label uses of vemurafenib include the treatment of other BRAF V600E positive tumors that are not responding to traditional treatments, e.g., refractory hairy cell leukemia (4).

Skin cancer is the most common of all cancers. Although melanoma is the least common type of skin cancer, accounting for approximately 1% of cases, it is responsible for the majority of deaths from skin cancer. In the US, the lifetime risk of melanoma is approximately 2.5% for whites, 0.5% for Hispanics, and 0.1% for blacks (5).

Most cases of malignant melanoma are diagnosed at an early stage, when the tumor is localized and surgical excision can be curative. However, the 5-year survival rate drops from 98% for localized disease, to only 16% for patients with metastatic disease.

For patients with advanced metastatic or unresectable malignant melanoma, treatment options typically include immunotherapy and targeted therapy. Although chemotherapy was once widely used, it does not increase survival and therefore its use is now limited to patients who are not candidates for further treatment with either immunotherapy or targeted therapy, and for whom there is no appropriate clinical trial.

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High-dose interleukin2 (IL2) therapy may be successful in a minority of cases, but can only be used in select patients with good organ function because of the risk of severe toxicity. Immunotherapy drugs include antibodies that target programmed cell death protein 1 (PD1), e.g., nivolumab and pembrolizumab (6); and ipilimumab, a monoclonal antibody that targets cytotoxic T-lymphocyte-associated protein 4 (CTLA4). Oncolytic virus therapy with T-VEC (talimogene laherparepvec) is one of the newer immunotherapy drugs approved for melanoma.

Targeted therapies are designed to inhibit components of the MAPK signaling pathway, primarily when it is constitutively activated in melanomas with the activating BRAF mutation, V600E. Drugs in this category include vemurafenib and dabrafenib, which inhibit BRAF, and trametinib and cobimetinib, which target downstream kinases MEK1 and MEK2, respectively.

Vemurafenib is a potent inhibitor of the kinase domain of the variant *BRAF* V600E. It acts by decreasing signaling through the MAPK pathway, leading to the reduced transcription of genes involved in various cellular responses. Combining vemurafenib with MEK inhibitors may potentiate these effects and has been shown to extend survival (7, 8).

Both targeted therapy with vemurafenib and immunotherapy regimens (e.g., nivolumab plus ipilimumab) have been shown to improve overall survival in patients with metastatic melanoma compared with chemotherapy (9, 10). However, at this time there are no randomized trials that compare targeted therapy with immunotherapy, and there are little data regarding the appropriate combinations and sequencing of these therapies for patients with a *BRAF* V600E variant.

In the BRIM3 trial, vemurafenib improved overall survival (13.6 versus 9.7 months) and progression-free survival (6.9 versus 1.6 months) when compared to cytotoxic chemotherapy (dacarbazine)(11). However, virtually every patient treated with a BRAF inhibitor eventually demonstrated disease progression (12). Most patients developed mechanisms of acquired resistance, which is sometimes associated with NRAS variants, and approximately15% of patients did not achieve tumor regression at all (11, 13-17).

The most common adverse events associated with vemurafenib are skin lesions (benign and malignant), fever, arthralgiab, and fatigue. Skin lesions, such as cutaneous squamous cell carcinoma, tend to occur during the first 8 weeks of treatment. Regular evaluation of the skin is recommended, with excision of suspicious lesions (18). Liver enzymes (transaminases, alkaline phosphatase, and bilirubin) should also be monitored because of the risk of liver injury. Combining BRAF with MEK inhibitors helps reduce the odds of these side effects.

Approximately 50% of cases of metastatic melanoma are found to have the *BRAF* V600E activating variant (1, 2). Because vemurafenib targets the kinase with this variant, patients without *BRAF* variants or with a different type of *BRAF* variant (e.g., V600K) should not be treated with vemurafenib; they will not benefit from vemurafenib therapy and will be needlessly exposed to adverse events. In addition, the FDA drug label warns that BRAF inhibitors have been shown to increase cell proliferation in *BRAF* wild-type cells *in vitro*.

Gene: *BRAF*

RAF is a family of intracellular kinases within the MAPK signaling pathway. The RAF family has three members, ARAF, BRAF, and CRAF (19). RAF, along with RAS (see below), are proto-oncogenes.

Proto-oncogenes are genes that, when mutated or expressed at abnormally high levels, can transform normal cells into cancerous cells. Proto-oncogenes typically encode proteins that stimulate cell division, inhibit cell differentiation, and halt cell death. The increased production of oncogenic proteins can lead to the proliferation of poorly differentiated cancer cells (20).

Germline mutations in BRAF, as well as other components of the MAPK signaling pathway, are associated with birth defects, such as cardiofaciocutaneous syndrome, characterized by heart defects, mental retardation, and a

distinctive facial dysmorphology. Somatic *BRAF* mutations are also associated with several malignancies, including lung adenocarcinoma, mucinous adenoma, ductal carcinoma of the pancreas, colorectal carcinoma, and malignant melanoma.

Variations in *BRAF* are detectable in approximately 50% of malignant melanomas, and drive progression of the disease (1, 2). The *BRAF* variant V600E accounts for approximately 90% of variants. This variant is a substitution of adenine for thymine at position 1799 and results in the substitution of valine for glutamate at codon 600. The variant BRAF protein kinase is constitutively active and a highly potent oncogene, with an increase in kinase activity by as much as 500-fold compared to the wild-type (21). The second most common *BRAF* variant is V600K. Substitutions at other sites are rarer (22, 23).

Several drugs are being developed to target *BRAF* mutations, and so far, two drugs have been FDA- approved: vemurafenib and dabrafenib. Unfortunately, less progress has been made in developing targeted therapies for melanoma with wild-type *BRAF*. There are fewer treatment options available, but these include immunotherapy and MEK inhibitors (6, 24).

Gene: *NRAS*

The RAS family contains three genes, HRAS, NRAS, and KRAS, which are essential components of a number of signaling pathways. They act as signal transducers, coupling cell surface receptors to intracellular signaling pathways.

RAS proteins have intrinsic GTPase activity, they are activated by a guanine nucleotide-exchange factor, and inactivated by a GTPase activating protein. RAS proteins regulate cell signal transduction by acting as a switch; they cycle between "on" (GTP-bound) or "off" (GDP-bound) conformations. In the "on" position, RAS proteins transmit extracellular growth signals to the nucleus, primarily via the MAPK pathway. Cells are subsequently stimulated to grow, divide, mature, and differentiate.

Variations in *RAS* genes lead to RAS proteins that are resistant to GTPase, so that GTP-remains permanently bound and the receptor remains "on" providing a continual growth stimulus to cells. Such activating RAS variants are common, having been detected in colorectal cancer, lung cancer, pancreatic cancer, and melanoma.

Variations in NRAS are detectable in 15–30% of melanomas, clustering at codons 12, 13, and 61 (25, 26). These NRAS variants are the second most common oncogenic “driver” mutation in malignant melanomas, behind alternations in *BRAF* (26).

NRAS variants are associated with more aggressive melanomas, and generally a poorer prognosis (26). Currently, no therapies that specifically target NRAS have been approved. However, in the near future newer targeted therapies will likely provide effective treatment options for *NRAS*-variant melanoma (26, 27). Off-label, MEK inhibitors, especially in combination with other agents, have exhibited some efficacy in NRAS-variant melanoma.

NRAS variants are also associated with a number of other conditions, including Noonan syndrome (type 6), somatic rectal cancer, follicular thyroid cancer, autoimmune lymphoproliferative syndrome, and juvenile myelomonocytic leukemia.

Genetic Testing

The NIH Genetic Testing Registry, GTR, displays genetic tests that are currently available for the genes *BRAF* and *NRAS*.

The FDA-approved label for vemurafenib states that the presence of the *BRAF* V600E mutation should be confirmed in tumor specimens using an FDA-approved test before starting treatment with vemurafenib. The label also states that vemurafenib is not indicated for treatment of patients with wild-type *BRAF* melanoma.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2016 Statement from the US Food and Drug Administration (FDA):

Vemurafenib is indicated for the treatment of patients with unresectable or metastatic melanoma with BRAF V600E mutation as detected by an FDA-approved test.

Limitation of Use: Vemurafenib is not indicated for treatment of patients with wild-type BRAF melanoma.

Patient Selection: Confirm the presence of BRAF V600E mutation in tumor specimens prior to initiation of treatment with Vemurafenib. Information on FDA-approved tests for the detection of BRAF V600 mutations in melanoma is available at http://www.fda.gov/CompanionDiagnostics.

Please review the complete therapeutic recommendations that are located here: (3)

Nomenclature

Selected BRAF variants

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
V600E	p.Val600Glu	NM_004333.4:c.1799T>A	NP_004324.2:p.Val600Glu	rs113488022
V600K	p.Val600Lys	NM_004333.4:c.1798_1799delGTinsAA	NP_004324.2:p.Val600Lys	rs121913227
V600R	p.Val600Arg	NM_004333.4:c.1798_1799delGTinsAG	NP_004324.2:p.Val600Arg	rs121913227
V600D	p.Val600Asp	NM_004333.4:c.1799_1800delTGinsAT	NP_004324.2:p.Val600Asp	rs121913377

Selected NRAS variants

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
NRAS G12V	p.Gly12Val	NM_002524.4:c.35G>T	NP_002515.1:p.Gly12Val	rs121913237
NRAS G13R	p.Gly13Arg	NM_002524.4:c.37G>C	NP_002515.1:p.Gly13Arg	rs121434595
NRAS Q61R	p.Gln61Arg	NM_002524.4:c.182A>G	NP_002515.1:p.Gln61Arg	rs11554290
NRAS Q61K	p.Gln61Lys	NM_002524.4:c.181C>A	NP_002515.1:p.Gln61Lys	rs121913254

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

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Venlafaxine Therapy and *CYP2D6* Genotype

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Introduction

Venlafaxine is an antidepressant used in the treatment of major depressive order, anxiety, and panic disorders. Venlafaxine belongs to the drug class of serotonin and norepinephrine reuptake inhibitors (SNRIs) (1).

The CYP2D6 enzyme metabolizes a quarter of all prescribed drugs, including venlafaxine. This enzyme converts venlafaxine to the active metabolite, O-desmethylvenlafaxine (ODV). Individuals who carry two inactive copies of *CYP2D6* ("poor metabolizers") may have decreased capacity to metabolize venlafaxine, resulting in less active metabolites in their system. In contrast, individuals who carry more than two copies of functional *CYP2D6* alleles ("ultrarapid metabolizers") may have an enhanced capacity to metabolize venlafaxine, resulting in more increased active metabolites in their system.

The FDA states that because the total exposure of venlafaxine and ODV is similar in poor and extensive (normal) metabolizers, there is no need for different venlafaxine dosing regimens for these individuals (1). However, the Dutch Pharmacogenetics Working Group recommends that both poor and intermediate metabolizer genotypes should be treated with an alternative drug, or lower doses of venlafaxine based on clinical response and drug levels. For ultrarapid metabolizer genotypes, they recommend that either the dose of venlafaxine be increased up to 150% of the normal dose, or an alternative drug used (see Table 1 and 2) (2).

Table 1. *CYP2D6* phenotypes and therapeutic recommendations for venlafaxine therapy

Phenotype	Genotype	Recommendations for venlafaxine therapy
Ultrarapid metabolizer	More than two copies of functional alleles	Be alert to decreased venlafaxine and increased O-desmethylvenlafaxine plasma concentration. Titrate dose to a maximum of 150% of the normal dose or select alternative drug (e.g., citalopram, sertraline).
Intermediate metabolizer	One active allele and one inactive allele, or two decreased activity alleles, or one decreased activity allele and one inactive allele	Insufficient data to allow calculation of dose adjustment. Select alternative drug (e.g., citalopram, sertraline) or adjust dose to clinical response and monitor O-desmethylvenlafaxine plasma concentration
Poor metabolizer	Two inactive alleles	Insufficient data to allow calculation of dose adjustment. Select alternative drug (e.g., citalopram, sertraline) or adjust dose to clinical response and monitor O-desmethylvenlafaxine plasma concentration.

Table is adapted from Swen J.J., Nijenhuis M., de Boer A., Grandia L. et al. Pharmacogenetics: from bench to byte - an update of guidelines. Clinical pharmacology and therapeutics. 2011;89(5):662–73 (2).

Table 2. Activity status of *CYP2D6* alleles

Allele type	Alleles
Active	**1, *2, *33, *35*
Decreased activity	**9, *10, *17, *29, *36, *41*
Inactive	**3-*8, *11-*16, *19-*21, *38, *40, *42*

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Drug: Venlafaxine

Venlafaxine is an antidepressant that is used for the treatment of a range of psychiatric disorders that include major depressive disorder, generalized anxiety disorder (GAD), social anxiety disorder, and panic disorder (1).

Venlafaxine is thought to exert its antidepressant effect by blocking the transporter reuptake proteins for key neurotransmitters affecting mood, thereby leaving more active neurotransmitters in the synapse. This is known as the "potentiation of neurotransmission."

Venlafaxine belongs to the drug class of serotonin-norepinephrine reuptake inhibitors (SNRIs). However, because venlafaxine also weakly inhibits dopamine reuptake, it is also referred to as a serotonin-norepinephrine-dopamine reuptake inhibitor (SNDRI).

Venlafaxine is metabolized in the liver to its major active metabolite, O-desmethylvenlafaxine (ODV). Venlafaxine and ODV are both potent inhibitors of neuronal serotonin and norepinephrine reuptake and weak inhibitors of dopamine reuptake. The formation of ODV is catalyzed by the enzyme CYP2D6. A high ratio of venlafaxine to ODV is a marker of low CYP2D6 activity. Other hepatic enzymes (CYP3A4, CYP2C19, and CYP2C9) metabolize venlafaxine and ODV to minor, less active metabolites (1).

As for all antidepressants, the FDA-approved drug label for venlafaxine includes a black box warning about the risk of suicide: "Antidepressants increased the risk compared to placebo of suicidal thinking and behavior (suicidality) in children, adolescents, and young adults in short-term studies of Major Depressive Disorder (MDD) and other psychiatric disorders. Anyone considering the use of venlafaxine or any other antidepressant in a child, adolescent, or young adult must balance this risk with the clinical need." (1)

The toxicity of venlafaxine appears to be higher than for other drugs of the same class. Adverse events include an increase in anxiety, insomnia, and nervousness; the precipitation of mania or hypomania in patients with bipolar disorder; weight loss, reduced appetite, hyponatremia, seizures, cardiac conduction abnormalities, and an increased risk of bleeding events. There is also a risk of discontinuation syndrome, which may occur if therapy is stopped abruptly (a gradual reduction in the dose of venlafaxine is recommended whenever possible) (1, 3).

Gene: *CYP2D6*

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The *CYP450* genes are very polymorphic and can result in reduced, absent, or increased enzyme activity.

CYP2D6 is responsible for the metabolism of many commonly prescribed drugs, including antidepressants, antipsychotics, analgesics, and beta-blockers. The *CYP2D6* gene is highly polymorphic, with more than 100 star (*) alleles described (4).

*CYP2D6*1* is the wild-type allele and is associated with normal enzyme activity and the "extensive metabolizer" phenotype. The *CYP2D6 *2*, **33*, and **35* alleles are also considered to have near-normal activity. Other alleles include variants that produce a non-functioning enzyme (e.g., **3*, **4*, **5*, and **6*) (5-8) or an enzyme with reduced activity (e.g., **10*, **17*, and **41*) (9-11) (see Table 2). There are large inter-ethnic differences in the frequency of these alleles, with **3*, **4*, **5*, **6*, and **41* being more common in Caucasians, **17* more common in Africans, and **10* more common in Asians (12).

Individuals who are intermediate or poor metabolizers carry copies of decreased-function and inactive *CYP2D6* alleles (see Table 1 and 2). In these individuals, the metabolic capacity of CYP2D6 is decreased, which may result in higher levels of venlafaxine and lower levels of ODV.

The FDA-approved drug label for venlafaxine states that although poor metabolizers have increased levels of venlafaxine and decreased levels of ODV compared to individuals with normal CYP2D6 activity, the differences between poor and extensive (normal) metabolizers are not thought to be clinically important because "the sum of venlafaxine and ODV is similar in the two groups and venlafaxine and ODV are pharmacologically approximately equiactive and equipotent." (1) However, the results of some reported studies suggest that side effects are more common in poor metabolizers, and that *CYP2D6* genotyping prior to the initiation of venlafaxine may prevent potential side effects (13, 14). Some of the adverse effects of venlafaxine therapy that have been reported to occur more frequently in poor metabolizers include gastrointestinal side effects, such as vomiting and diarrhea; and cardiovascular side effects, such as hypertension, tachycardia, and prolonged QTc interval (14, 15).

The Dutch Pharmacogenetics Working Group recommendations state that for poor and intermediate metabolizers, there is insufficient data to calculate the dose adjustment for venlafaxine, and an alternative drug should be used (e.g., citalopram, sertraline). Or, the dose of venlafaxine should be adjusted according to the clinical response, and ODV plasma levels should be monitored (2).

Poor metabolizers are commonly found in European Caucasians. The functional *CYP2D6*1* allele is the most common (~70%), and the most common nonfunctional alleles include *CYP2D6*4* and **5*, which largely account for the poor metabolizer phenotype in these populations (16, 17).

In individuals of Asian descent, only about 50% of *CYPD6* alleles are functional, with the reduced function *CYP2D6*10* variant being very common (~40%). As a result, Asians are more likely to be intermediate metabolizers than Caucasians (12). Similarly, in Africans and African Americans, only 50% of *CYPD6* alleles are functional; however, a wider range of variants account for the remaining alleles (18, 19).

Individuals who have multiple functional copies of the *CYP2D6* gene are "ultrarapid metabolizers" (UM). Each allele contributes to the metabolism of venlafaxine to the active metabolite, ODV. Data suggest that the ultrarapid metabolizer phenotype does not have a significant effect on treatment with venlafaxine (efficacy or side effects) but as a precaution, drug levels should be monitored and an increased dose of venlafaxine may be required (13, 14, 20). The Dutch Pharmacogenetics Working Group recommendations state that for ultrarapid metabolizers, there is a need to be alert to decreased venlafaxine and increased ODV concentrations. The dose of venlafaxine should be titrated to a maximum of 150% of the normal dose, or an alternative drug (e.g., citalopram, sertraline) should be considered (2), in patients with normal renal clearance (21).

The ultrarapid metabolizer phenotype is estimated to be present in up to 28% of North Africans, Ethiopians, and Arabs; ~10% in Caucasians; 3% in African Americans, and up to 1% in Hispanics, Chinese, and Japanese (22).

Genetic Testing

Genetic testing is available for many of the more common variant *CYP2D6* alleles. Results are typically reported as a diplotype, such as *CYP2D6 *1/*1* (23). A result for copy number, if available, is also important when interpreting *CYP2D6* results.

If the test results include an interpretation of the patient's predicted metabolizer phenotype, this should be confirmed by checking the diplotype and assigning an activity score to each allele (e.g., 0 for nonfunctional, 0.5 for reduced function, and 1 for each copy of a functional allele). The phenotype is defined by the sum of the two scores:

- An extensive (normal) metabolizer phenotype has an activity score of 1 to 2
- An intermediate metabolizer has an activity score of 0.5
- A poor metabolizer has an activity score of 0
- An ultrarapid metabolizer has an activity score greater than 2

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

Statement from the US Food and Drug Administration (FDA): Plasma concentrations of venlafaxine were higher in CYP2D6 poor metabolizers than extensive metabolizers. Because the total exposure (AUC) of venlafaxine and ODV was similar in poor and extensive metabolizer groups, however, there is no need for different venlafaxine dosing regimens for these two groups.

Please review the complete therapeutic recommendations that are located here: (1).

Summary of recommendations from the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP): For individuals who are poor or intermediate metabolizers, there are insufficient data to calculate a dose adjustment for venlafaxine. Select an alternative drug (e.g., citalopram, sertraline), or adjust the dose of venlafaxine based on the clinical response, and monitor (O-desmethyl)venlafaxine plasma concentration. For individuals who are ultrarapid metabolizers, physicians should be alert to decreased venlafaxine and increased (O-desmethyl)venlafaxine plasma concentration. The dose of venlafaxine should be titrated up to a maximum of 150% of the normal dose or an alternative drug used (e.g., citalopram, sertraline).

Please review the complete therapeutic recommendations that are located here: (2).

Nomenclature

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*4*	1846G>A	NM_000106.4:c.506-1G>A	Not applicable - variant occurs in a non-coding region	rs3892097
*CYP2D6*5*	Not applicable - variant results in a whole gene deletion			
*CYP2D6*6*	1707 del T Trp152Gly	NM_000106.4:c.454delT	NP_000097.2:p.Trp152Glyfs	rs5030655
*CYP2D6*10*	100C>T Pro34Ser	NM_000106.4:c.100C>T	NP_000097.2:p.Pro34Ser	rs1065852
*CYP2D6*17*	Includes at least two functional variants*: 1023C>T (Thr107Ile) 2850C>T (Cys296Arg)	NM_000106.4:c.320C>T NM_000106.4:c.886T>C	NP_000097.2:p.Thr107Ile NP_000097.2:p.Cys296Arg	rs28371706 rs16947
*CYP2D6*41*	2988G>A	NM_000106.4:c.985+39 G>	Not applicable – variant occurs in a non-coding region	rs28371725

* In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

Nomenclature for Cytochrome P450 enzymes is available from the Human Cytochrome P450 (CYP) Allele Nomenclature Database: http://www.cypalleles.ki.se/

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labelled all formulations containing the generic drug.

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Voriconazole Therapy and *CYP2C19* Genotype

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Introduction

Voriconazole (brand name VFend) is a broad-spectrum antifungal agent used to treat invasive fungal infections (IFI). Invasive fungal infections are an important cause of morbidity and mortality in critically ill children and immunocompromised individuals.

Voriconazole is a triazole and is the first line treatment of invasive aspergillosis. It is also licensed to treat candidemia (in individuals who do not have neutropenia), disseminated candidiasis, and esophageal candidiasis. For serious fungal infections caused by Scedosporium and Fusarium species, voriconazole may be used in those who are unable to take, or have not responded to, other therapy (1).

Therapeutic drug monitoring of voriconazole has become the standard of care to ensure efficacy and avoid adverse effects (2, 3). Low serum voriconazole concentrations have been associated with treatment failure, which may have devastating consequences in individuals who are seriously ill with an invasive infection. High serum voriconazole concentrations are associated with adverse effects, such as neurotoxicity.

Interindividual drug serum concentrations vary widely among individuals treated with a dose of voriconazole, which is due in part to genetic variation in the *CYP2C19* gene. Voriconazole is primarily metabolized by the CYP2C19 enzyme, with contributions by CYP2C9 and CYP3A4.

Individuals who lack CYP2C19 activity ("CYP2C19 poor metabolizers") have, on average, 4-fold higher voriconazole exposure than normal metabolizers (Table 1). In contrast, individuals who have increased CYP2C19 activity ("rapid" and "ultrarapid metabolizers") have lower serum concentrations of voriconazole (1, 4). Genetic tests are currently available for the voriconazole response and the *CYP2C19* gene.

The FDA-approved drug label for voriconazole discusses the influence of *CYP2C19* on drug levels but does not provide specific dosing recommendations based on the CYP2C19 metabolizer status (Table 1). The label currently only incorporates the type of infection and the individuals weight into the dosing guidelines (1).

However, dosing recommendations for voriconazole based on CYP2C19 metabolizer type are available from the Dutch Pharmacogenetics Working Group (DPWG, Table 2) and the Clinical Pharmacogenetics Implementation Consortium (CPIC, Table 3) (4, 5).

Table 1. FDA (2019) Drug Label for Voriconazole Therapeutic Recommendations based on *CYP2C19* Genotype

Phenotype	Voriconazole
CYP2C19 poor metabolizers	Studies conducted in Caucasian and Japanese healthy subjects have shown that poor metabolizers have, on average, 4-fold higher voriconazole exposure (AUCτ) than their homozygous normal metabolizer counterparts. Subjects who are heterozygous normal metabolizers have, on average, 2-fold higher voriconazole exposure than their homozygous normal metabolizer counterparts

Please see Therapeutic Recommendations based on Genotype for more information from the FDA. This table is adapted from (1).

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Table 2. DPWG (2019) Recommendations for Voriconazole and *CYP2C19* Genotype

Phenotype	Recommendations
CYP2C19 poor metabolizers	Use 50% of the standard dose and monitor the plasma concentration.
CYP2C19 intermediate metabolizers	Monitor the plasma concentration.
CYP2C19 ultrarapid metabolizers	Use an initial dose that is 1.5x higher and monitor the plasma concentration.

Please see Therapeutic Recommendations based on Genotype for more information from DPWG. This table is adapted from (5).

Table 3. CPIC (2016) Dosing Recommendations for Voriconazole Treatment based on CYP2C19 Phenotype for Adults

CYP2C19 phenotype	Implications for voriconazole pharmacologic measures	Therapeutic recommendations	Classification of recommendations[a]
CYP2C19 ultrarapid metabolizer (*17/*17)	In individuals for whom an ultrarapid metabolizer genotype (*17/*17) is identified, the probability of attainment of therapeutic voriconazole concentrations is small with standard dosing	Choose an alternative agent that is not dependent on CYP2C19 metabolism as primary therapy in lieu of voriconazole. Such agents include isavuconazole, liposomal amphotericin B, and posaconazole.[b]	Moderate[c]
CYP2C19 rapid metabolizer (*1/*17)	In individuals for whom a rapid metabolizer genotype (*1/*17) is identified, the probability of attainment of therapeutic concentrations is modest with standard dosing	Choose an alternative agent that is not dependent on CYP2C19 metabolism as primary therapy in lieu of voriconazole. Such agents include isavuconazole, liposomal amphotericin B, and posaconazole.[b]	Moderate
CYP2C19 normal metabolizer	Normal voriconazole metabolism	Initiate therapy with recommended standard of care dosing.[b]	Strong
CYP2C19 intermediate metabolizer	Higher dose-adjusted trough concentrations of voriconazole compared with normal metabolizers	Initiate therapy with recommended standard of care dosing.[b]	Moderate
CYP2C19 poor metabolizer	Higher dose-adjusted trough concentrations of voriconazole and may increase probability of adverse events	Choose an alternative agent that is not dependent on CYP2C19 metabolism as primary therapy in lieu of voriconazole. Such agents include isavuconazole, liposomal amphotericin B, and posaconazole.[b] In the event that voriconazole is considered to be the most appropriate agent, based on clinical advice, for an individual with poor metabolizer genotype, voriconazole should be administered at a preferably lower than standard dos- age with careful therapeutic drug monitoring.	Moderate

[a] Rating scheme is described in Supplementary Data online (4).
[b] Further dose adjustments or selection of alternative therapy may be necessary due to other clinical factors, such as drug interactions, hepatic function, renal function, species, site of infection, therapeutic drug monitoring, and comorbidities.
[c] Recommendations based upon data extrapolated from individuals with CYP2C19*1/*17 genotype.
Please see Therapeutic Recommendations based on Genotype for more information from CPIC. This table is adapted from (4).

Drug: Voriconazole

Voriconazole is a broad-spectrum antifungal agent that belongs to the drug class of triazole antifungals. There currently are 5 triazole antifungal drugs licensed for use in the United States: fluconazole, isavuconazole,

itraconazole, posaconazole, and voriconazole. These medications vary in how they are administered, the pathogens they target, and their side effects (6).

Compared with other triazole antifungals, voriconazole has enhanced activity against the Aspergillus species, and similar to other triazole antifungals, voriconazole is active against the Candida species. The Infectious Diseases Society of America recommend voriconazole as the first-line therapy for invasive aspergillosis, and as an alternative therapy for candidemia, in individuals who do not have neutropenia (4, 7).

Voriconazole is also used to treat esophageal candidiasis, disseminated candidiasis (in skin, abdomen, kidney, bladder wall, and wounds), and serious infections caused by Scedosporium apiospermum complex and Fusarium species, including *Fusarium solani* in individuals intolerant of, or refractory to, other therapy (1).

A healthy adult has an immune system that can prevent a fungal infection becoming invasive and disseminating. But IFI can be life threatening in adults who have a weakened immune system. Susceptible individuals may be at the extremes of age (very young, or elderly), or be immunocompromised because of a disease or its treatment (e.g., cancer, chemotherapy, immunosuppression following transplant surgery). Genetic conditions may also cause immunodeficiency. For these individuals, early treatment of IFI is associated with increased survival (3, 8, 9).

Triazoles share a similar mechanism of action – they disrupt the synthesis of ergosterol, an important part of the fungal cell membrane. They do this by inhibiting the fungal enzyme that produces ergosterol (lanosterol 14-alpha-demethylase). The damaged fungal cell membrane becomes more permeable, resulting in cell lysis and death.

Triazoles are generally well tolerated but they have a narrow therapeutic index. Gastrointestinal symptoms are most frequently reported, including nausea, abdominal pain, vomiting, and diarrhea. All triazoles have been associated with liver dysfunction and hepatotoxicity. Therefore, careful monitoring of liver enzymes is recommended for everyone receiving triazole therapy (6).

Voriconazole can cause fetal harm and should not be used during pregnancy unless the benefit to the mother outweighs the risk to the fetus. In animal studies, voriconazole was associated with teratogenicity (abnormal development of the embryo), embryo toxicity, and death. If voriconazole is used during pregnancy, or if the individual becomes pregnant while taking voriconazole, they should be informed of the potential hazards to the fetus.

Adverse effects specifically associated with voriconazole therapy include vision changes (e.g., photopsia – flashes of light, and photophobia – increased sensitivity to light), periostitis (inflammation of the periosteum that surrounds bones), and neurological toxicity (e.g., visual hallucinations, encephalopathy, and neuropathy).

Clinically, it is important to distinguish between vision changes, which tend to be minor and reversible, and visual hallucinations, which may be one of the first indications of severe neurotoxicity.

Voriconazole can be administered orally or by IV, and a loading dose is given at the start of therapy. For the treatment of invasive aspergillosis in adults, an IV loading dose of 6 mg/kg every 12 hours for 2 doses is recommended, followed by an IV maintenance dose of 4 mg/kg every 12 hours. Intravenous treatment should be continued for at least 7 days. After the individual has improved clinically, oral voriconazole can be used instead of IV (recommended maintenance dose of 200 mg every 12 hours).

The voriconazole drug label states that dose adjustment may be indicated for cases of non-response (dose increased), for individuals who cannot tolerate the medication, have liver insufficiency, or for adults who weigh less than 40 kg (dose decreased). The dose may also need to be adjusted based on concurrent therapy, as many drugs (particularly those that inhibit or induce CYP3A4, CYP2C9, or CYP2C19) can lead to altered voriconazole levels (1).

The dosing of voriconazole is further complicated by the elimination of the drug being characterized by "non-linear pharmacokinetics". Pharmacokinetics is the study of the movement of drugs in the body, including the processes of absorption, distribution, metabolism, and excretion. The term "linear pharmacokinetics" refers to a graph that shows a straight line when various factors are plotted e.g., the dose of the drug versus the serum concentration of the drug. For voriconazole, the observed "non-linear" pharmacokinetics means that above a certain drug dose, the concentration of the drug in the serum increases disproportionately. This occurs because the enzymes responsible for metabolizing and eliminating voriconazole become saturated (e.g., CYP2C19), (10).

In children, however, voriconazole has been found to show linear pharmacokinetics over a wider range of drug doses. This is thought to be because children have a higher expression of CYP2C19, and therefore an increased capacity to metabolize voriconazole. This means that children will often require higher doses to achieve therapeutic drug concentrations (3, 11).

There is substantial variability in voriconazole serum drug concentrations among individuals receiving standard doses of voriconazole. This is in part due to non-linear kinetics and other factors listed above (liver function, comorbidities, concurrent medications, age of the individual), as well as the presence of inflammation, and interindividual pharmacogenetic variability (12, 13).

Genetic variants in the *CYP2C19* gene play an important role in voriconazole serum concentration variability. Voriconazole is metabolized primarily by CYP2C19, and to a lesser extent by CYP3A4 and CYP2C9. Individuals who lack CYP2C9 activity (up to 20% of individuals of Asian descent and 3-5% in many other populations, Table 4) will have a higher exposure to voriconazole in response to standard doses, and are at a higher risk of adverse effects (3, 4, 9). Genetic variation in the *CYP3A4* gene may also influence voriconazole pharmacokinetics (14-17).

Therapeutic drug monitoring of voriconazole has now become the standard of care in many medical centers to improve treatment efficacy and avoid toxicity. However, if a individuals's *CYP2C19* status is known, sub- and supratherapeutic voriconazole concentrations can potentially be avoided in individuals vulnerable to severe infections. Voriconazole dosing recommendations based on *CYP2C19* genotype and/or phenotype have been published by CPIC and DPWG (see Therapeutic Recommendations based on Genotype). (2, 4, 5, 18-23).

Although the FDA drug label states voriconazole is indicated for individuals aged 12 years and above, voriconazole is used in children with IFI, and the label discusses pediatric use. As such, CPIC have provided therapeutic recommendations for the use of voriconazole based on *CYP2C19* genotype for pediatric individuals (children and adolescents less than 18 years old) (1, 4).

Gene: CYP2C19

The cytochrome P450 (CYP) superfamily is a large and diverse group of hepatic enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP genes are very polymorphic and can result in reduced, absent, or increased drug metabolism.

The CYP2C19 enzyme contributes to the metabolism of a range of clinically important drugs, such as antidepressants, benzodiazepines, antiplatelet agents, some proton pump inhibitors, and antifungal agents such as voriconazole.

The *CYP2C19* gene is highly polymorphic, as there are currently 35 variant star (*) alleles cataloged by the Pharmacogene Variation (PharmVar) Consortium. The *CYP2C19*1* is considered the wild-type allele when no variants are detected and is associated with normal enzyme activity and the "normal metabolizer" phenotype.

The *CYP2C19*17* allele is associated with increased enzyme activity and is found among individuals with 'rapid' (**1/*17*) and 'ultrarapid' (**17/*17*) metabolizer phenotypes. Heterozygous carriers of non-functional alleles (e.g.,

*2 and *3) are classified as 'intermediate metabolizers' (e.g., **1/*2*), and individuals who have 2 non-functional alleles are classified as "poor metabolizers" (e.g., **2/*2*, **2/*3*) (Table 4).

Table 4. CPIC (2016). Assignment of CYP2C19 Phenotype based on Genotype.

Phenotype	Genotype	Examples of diplotypes
CYP2C19 ultrarapid metabolizer (approximately 2–5% of individuals)[a]	An individual with 2 increased function alleles	**17/*17*
CYP2C19 rapid metabolizer (approximately 2–30% of individuals)	An individual with one normal function allele and one increased function allele	**1/*17*
CYP2C19 normal metabolizer (approximately 35–50% of individuals)	An individual with 2 normal function alleles	**1/*1*
CYP2C19 intermediate metabolizer (approximately 18–45% of individuals)	An individual with one normal function allele and one no function allele or one no function allele and one increased function allele	**1/*2* **1/*3* **2/*17*[b]
CYP2C19 poor metabolizer (approximately 2–15% of individuals)	An individual with 2 no function alleles	**2/*2* **2/*3* **3/*3*

[a] CYP2C19 metabolizer status frequencies are based on average multi-ethnic frequencies. See the *CYP2C19* Frequency Tables for population-specific allele and phenotype frequencies (4).
[b] The predicted metabolizer phenotype for the**2/*17* genotype is a provisional classification. The currently available evidence indicates that the *CYP2C19*17* increased function allele is unable to completely compensate for the *CYP2C19*2* no function allele.
This CPIC table is adapted from (4).

Approximately 2% of Caucasians, 4% of African Americans, and 14% of Chinese are *CYP2C19* poor metabolizers, and up to 45% of individuals are CYP2C19 intermediate metabolizers (19).

The most common no function allele is *CYP2C19*2*, which is defined by a c.681G>A variant in exon 5 that creates an aberrant splice site that translates a truncated and non-functioning protein. The *CYP2C19*2* allele frequencies are ~15% in Caucasians and Africans, and ~29–35% in Asians (24).

Another commonly tested no function allele is *CYP2C19*3,* which is defined by a c.636G>A variant in exon 4 that creates a premature stop codon. The CYP2C19*3 allele frequencies are ~2-9% in Asian populations, but rare in other racial groups. Other no function variants occur in less than 1% of the general population, and include CYP2C19*4-*8 (24).

The *CYP2C19*17* allele is an increased function allele characterized by a promoter variant that results in increased gene expression, and *is* commonly tested for with an allele frequency of 4-21%.

Linking Gene Variation with Treatment Response

Although studies have not consistently found an association between the *CYP2C19* genotype and the toxicity or efficacy of voriconazole, *CYP2C19* genotype does contribute to the variation observed in voriconazole pharmacokinetics and potentially, could be used to guide the initial dose selection (25, 26).

The presence of *CYP2C19* variants can lead to increased or decreased voriconazole serum concentrations (27, 28). Low concentrations of voriconazole are associated with treatment failure. High concentrations are not associated with an increase in efficacy but are associated with serious adverse effects such as neurotoxicity (2, 4).

CYP2C19 Poor Metabolizers

Individuals who are CYP2C19 poor metabolizers have increased serum voriconazole concentrations, which are up to 4 times higher than normal CYP2C19 metabolizers. However, this difference is most marked in healthy

volunteers – studies with patients have found conflicting results, most likely due to factors such as drug interactions, other conditions, and organ dysfunction (2, 3).

Several studies have found that increased voriconazole serum concentrations are associated with increased risk of side effects, including hepatotoxicity, visual hallucinations and encephalopathy (4, 18, 29-32). The FDA confirms that CYP2C19 poor metabolizers have higher exposure to voriconazole, but the label does not discuss alternative dosing based on CYP2C19 metabolizer status. However, dosing guidelines based on CYP2C19 genotype have been published by CPIC and DPWG.

Therapeutic recommendations from CPIC for CYP2C19 poor metabolizers include choosing an alternative agent that is not dependent upon CYP2C19 metabolism, or if there is a strong case for using voriconazole, use a lower dose than standard with careful therapeutic drug monitoring. For all genotypes, CPIC recommend bearing in mind that further dose adjustments or selection of alternative therapy may be necessary due to other clinical factors, such as drug interactions, hepatic function, renal function, fungal species, site of infection, therapeutic drug monitoring, and comorbidities (Table 3) (4).

For CYP2C19 poor metabolizers, the DPWG recommend using 50% of the standard dose, again with careful monitoring (see Therapeutic Recommendations based on Genotype) (4, 5).

CYP2C19 Intermediate Metabolizers

Data are lacking for CYP2C19 intermediate metabolizers, therefore CPIC recommend following the standard dosing regimen, with therapeutic drug monitoring. The DPWG also recommends the standard dose with therapeutic drug monitoring (4, 5).

CYP2C19 Rapid and Ultrarapid Metabolizers

Trough concentrations of voriconazole can predict the clinical response, with low levels associated with a lower response rate and treatment failure (18, 30, 31, 33-35). Low levels of voriconazole are found in individuals who are CYPC2C19 rapid (individuals who have one copy of *CYP2C19*17*) or ultrarapid (individuals who have 2 copies of *CYP2C19*17*) metabolizers Several studies have found that the *CYP2C19*17* allele is associated with subtherapeutic voriconazole concentrations (2, 27, 36-38).

For these individuals, attempting to achieve therapeutic drug levels may be unsuccessful, or cause serious delays, allowing the invasive fungal disease to progress (3).

For CYP2C19 rapid and ultrarapid metabolizers, CPIC recommends an alternative antifungal agent that is not dependent on CYP2C19 metabolism, whereas the DPWG recommends using an initial dose of voriconazole that is 1.5 times higher than the standard dose, with therapeutic drug monitoring (Table 3, Therapeutic Recommendations based on Genotype) (4, 5).

Genetic Testing

Clinical genotyping tests are available for several *CYP2C19* alleles. The NIH's Genetic Testing Registry (GTR) provides examples of the genetic tests that are currently available for the voriconazole response and the *CYP2C19* gene. In addition, variant *CYP2C19* alleles to be included in clinical genotyping assays have been recommended by the Association for Molecular Pathology (39).

Usually an individual's result is reported as a diplotype, such as *CYP2C19 *1/*1*, and may also include an interpretation of the predicted metabolizer phenotype (ultrarapid, normal, intermediate, or poor). Table 4 summarizes common CYP2C19 phenotypes.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2019 Statement from the US Food and Drug Administration (FDA)

CYP2C19, significantly involved in the metabolism of voriconazole, exhibits genetic polymorphism. Approximately 15 to 20% of Asian populations may be expected to be poor metabolizers. For Caucasians and Blacks, the prevalence of poor metabolizers is 3 to 5%. Studies conducted in Caucasian and Japanese healthy subjects have shown that poor metabolizers have, on average, 4-fold higher voriconazole exposure (AUCτ) than their homozygous normal metabolizer counterparts. Subjects who are heterozygous normal metabolizers have, on average, 2-fold higher voriconazole exposure than their homozygous normal metabolizer counterparts.

Please review the complete therapeutic recommendations that are located here: (1)

2019 Statement from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

CYP2C19 Poor Metabolizers

The gene variation can reduce the conversion of voriconazole and consequently increase the plasma concentration. This could result in improved efficacy or an increase in the risk of side effects. Initially, the risk of side effects is of particular interest.

Recommendation: Use 50% of the standard dose and monitor the plasma concentration

CYP2C19 Intermediate Metabolizers

The gene variation can reduce the conversion of voriconazole and consequently increase the plasma concentration. This could result in improved efficacy or an increase in the risk of side effects.

Recommendation: Monitor the plasma concentration

CYP2C19 Ultrarapid metabolizers

The gene variation increases the conversion of voriconazole, which increases the risk of ineffectiveness.

Recommendation: Use an initial dose that is 1.5x higher and monitor the plasma concentration

Background information

Mechanism:

Voriconazole is predominantly metabolised by CYP2C19 and otherwise by CYP2C9 and CYP3A4. The most important metabolite, voriconazole-N-oxide, is inactive.

For more information about CYP2C19 phenotypes: see the general background information about CYP2C19 on the KNMP Knowledge Bank or on www.knmp.nl (search for key word “CYP2C19”).

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

Other considerations:

Several studies indicate a higher risk of hepatotoxicity at higher plasma concentrations of voriconazole. However, the relationship between the plasma concentration and the effect or side effects (hepatotoxicity) has not been clearly identified.

The kinetics of voriconazole are non-linear at therapeutic doses.

Please review the complete therapeutic recommendations that are located here: (5).

2016 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC)

Clinical studies have not consistently demonstrated an association between CYP2C19 genotype and adverse reactions. However, as individual patients who are poor metabolizers may have elevated levels leading to toxicity, the use of another antifungal agent is recommended. Under circumstances in which voriconazole is strongly indicated for treatment of an invasive mycosis in a patient with a poor metabolizer phenotype, administration of a lower dosage with meticulous therapeutic drug monitoring may be feasible (Table 3).

Knowledge of CYP2C19 ultrarapid and rapid metabolizer genotypes may prevent subtherapeutic concentrations of voriconazole that may lead to treatment failure. In such cases, an alternative antifungal agent also is recommended, especially as several case reports have documented voriconazole treatment failure in CYP2C19 ultrarapid metabolizers (see Supplementary Table S1 online). Attempting to obtain therapeutic levels in patients with ultrarapid metabolizer genotypes are often unsuccessful. Serious delays in achieving therapeutic concentrations in such patients with active invasive mycoses may result in disease progression.

Several alternative agents may be used instead of voriconazole for treatment of invasive mold infections. These include isavuconazole, lipid formulations of amphotericin B, and posaconazole (Table 3). The antifungal triazole isavuconazole is approved for the primary treatment of invasive aspergillosis and invasive mucormycosis and is available in intravenous and oral dosage forms. As isavuconazole is a substrate of CYP3A4, variant alleles in this gene are unlikely to affect its clearance. Only limited data for isavuconazole are currently available in the pediatric population. Liposomal amphotericin B is an alternative therapy to voriconazole for the primary treatment of invasive aspergillosis. Posaconazole is currently indicated for salvage therapy of invasive aspergillosis. The recently approved posaconazole delayed release and intravenous dosage forms achieve higher concentrations than that of the posaconazole suspension. However, intravenous posaconazole requires administration via a central line due to phlebitis with peripheral administration. Similar to voriconazole, intravenous posaconazole also contains the solubilizer sulfobutylether-beta-cyclodextrin sodium. Posaconazole is cleared largely as unchanged compound with <20% of compound being excreted as a glucuronide conjugate. Uridine 50-diphospho- glucuronosyltransferase glucuronidation of posaconazole is not significantly affected by genetic variation. Administration of posaconazole should still be guided by TDM.

Please review the complete therapeutic recommendations that are located here: (4).

Nomenclature for selected *CYP2C19* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C19*2*	681G>A Pro227Pro	NM_000769.1:c.681G>A	NP_000760.1:p.Pro227=	rs4244285
*CYP2C19*3*	636G>A Trp212Ter	NM_000769.1:c.636G>A	NP_000760.1:p.Trp212Ter	rs4986893

Table continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C19*17*	-806C>T	NM_000769.1:c.-806C>T	Not applicable - variant occurs in a non-coding region	rs12248560

dbSNP: The Single Nucleotide Polymorphism Database
Note: the normal "wild-type" allele is *CYP2C19*1*.
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (40).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for cytochrome P450 enzymes is available from Pharmacogene Variation (PharmVar) Consortium.
Please note that the CYP2C19*2 defining variant (rs4244285) has recently been reported to be in high linkage disequilibrium with an intronic variant implicated in aberrant slicing (rs12769205) (41).

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Warfarin Therapy and *VKORC1* and *CYP* Genotype

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Introduction

Warfarin (brand name Coumadin) is an anticoagulant (blood thinner). Warfarin acts by inhibiting the synthesis of vitamin K-dependent clotting factors and is used in the prevention and treatment of various thrombotic disorders. Warfarin is a drug with narrow therapeutic index; thus, a small change in its plasma levels may result in concentration dependent adverse drug reactions or therapeutic failure. Therefore, the dose of warfarin must be tailored for each patient according to the patient's response, measured as INR (International Normalized Ratio), and the condition being treated.

There is a wide inter-individual variability in the dose of warfarin required to achieve target anticoagulation, and the time it takes to reach target INR. Approximately half of this variability is known to be caused by clinical or lifestyle factors (e.g., a patient's age, weight, BMI, gender, smoking status, existing conditions, and concomitant medications) and by genetic factors (known genetic factors include variants in the *VKORC1*, *CYP2C9*, *CYP4F2* genes, and the rs12777823 variant in the *CYP2C* gene cluster on chromosome 10) (1).

The *VKORC1* and *CYP2C9* genotypes are the most important known genetic determinants of warfarin dosing. Warfarin targets VKORC1, an enzyme involved in vitamin K recycling. A common variant, *VKORC1*, c.-1639G>A, is associated with an increased sensitivity to warfarin and lower dose requirements. The CYP2C9 enzyme metabolizes warfarin and the variants *CYP2C9*2* and **3*, are also associated with lower dose requirements.

The FDA-approved drug label for warfarin states that *CYP2C9* and *VKORC1* genotype information, when available, can assist in the selection of the initial dose of warfarin. The label provides 2 sets of warfarin dosing recommendations, for when the *CYP2C9* and *VKORC1* genotypes are either known (Table 1) or not known (taking into account clinical factors, the initial dose of warfarin is usually 2–5 mg once daily) (1).

In addition, the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP) has published recommendations for the initial standard dose of warfarin. A dose reduction is recommended for individuals who are CYP2C9 poor and intermediate metabolizers (with the exception of intermediate metabolizers with the CYP2C9*1/*2 genotype, no dose change is required), and a dose reduction is recommended for individuals who carry 2 copies of the variant *VKORC1* A allele *(VKORC1,* c.-1639G>A/A*)* (Table 2) (2, 3).

Recently, genetic variation in the *CYP4F2* gene, and a variant near the *CYP2C* gene cluster, rs12777823, have been associated with influencing warfarin therapy. The *CYP4F2*3* variant is associated with a modest increase in warfarin dose requirements in individuals with European or Asian ancestry, while in individuals with African ancestry, the rs12777823 A/G or A/A genotype is associated with decreased warfarin dose requirements.

The 2017 Update of the Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Pharmacogenetics-Guided Warfarin Dosing, provides warfarin dosing recommendations for adults with and without African ancestry, and also for pediatric patients (see Therapeutic Recommendations). CPIC recommends that these dosing guidelines are applied after a warfarin dose has been calculated using a validated pharmacogenetic algorithm, which includes genotype information for *VKORC1,* c.-1639G>A and *CYP2C9*2* and **3* (Figure 1) (4)

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Table 1. The FDA (2017) Drug Label for Warfarin. Three Ranges of Expected Maintenance Warfarin Doses based on CYP2C9 and VKORC1 Genotype.

VKORC1	*CYP2C9*					
	**1/*1*	**1/*2*	**1/*3*	**2/*2*	**2/*3*	**3/*3*
GG	5–7 mg	5–7 mg	3–4 mg	3–4 mg	3–4 mg	0.5–2 mg
AG	5–7mg	3–4 mg	3–4 mg	3–4 mg	0.5–2 mg	0.5–2 mg
AA	3–4 mg	3–4 mg	0.5–2 mg	0.5–2 mg	0.5–2 mg	0.5–2 mg

Ranges are derived from multiple published clinical studies. The *VKORC1, c.–1639G>A* (rs9923231) variant is used in this table. Other co-inherited *VKORC1* variants may also be important determinants of warfarin dose. Patients with *CYP2C9 *1/*3*, **2/*2*, **2/*3*, and **3/*3* may require more prolonged time (>2–4 weeks) to achieve a maximum international normalized ratio (INR) effect for a given dosage regimen than patients without these *CYP* variants.
Please see Therapeutic Recommendations based on Genotype for more information. This table is adapted from the FDA-approved drug label for warfarin (1).

Table 2. The DPWG (2017) Recommendations for Warfarin and *CYP2C9* and *VKORC1* Genotype.

Phenotype/diplotype	Recommendation
CYP2C9 IM	Use 65% of the standard initial dose
CYP2C9 PM	Use 20% of the standard initial dose
CYP2C9*1/*2	No action is required for this gene-drug interaction.
CYP2C9*1/*3	Use 65% of the standard initial dose
CYP2C9*2/*2	Use 65% of the standard initial dose
CYP2C9*2/*3	Use 45% of the standard initial dose
CYP2C9*3/*3	Use 20% of the standard initial dose
VKORC1 C/T	No action is required for this gene-drug interaction
VKORC1 T/T	Use 60% of the standard initial dose

Note: VKORC1 1173C>T is equivalent to c.-1639G>A. Therefore:
"VKORC1 CT" corresponds to VKORC1, c.-1639 G/A
"VKORC1 TT" corresponds to VKORC1, c.-1639 A/A
Please see Therapeutic Recommendations based on Genotype for more information from the Dutch Pharmacogenetics Working Group (DPWG). Table is adapted from (2, 3).

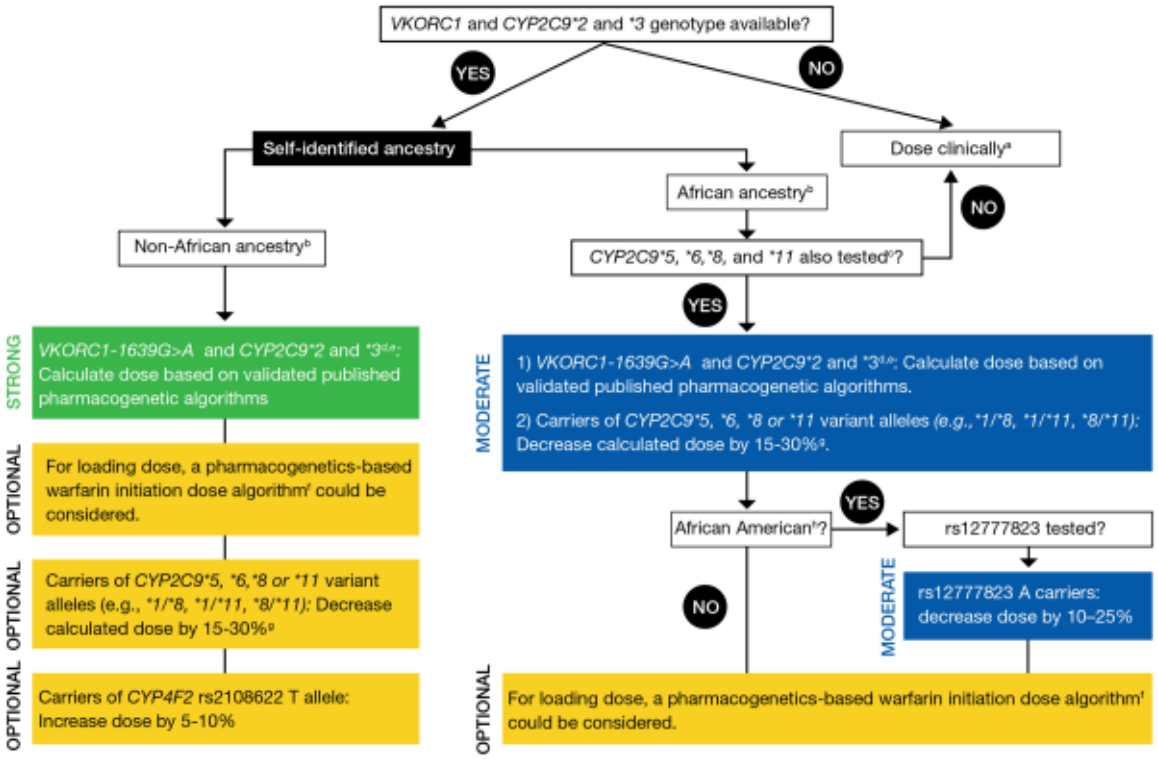

Figure 1. The CPIC (2017) Dosing Recommendations for Warfarin Dosing based on Genotype for Adult Patients. (a) "Dose clinically" means to dose without genetic information, which may include use of a clinical dosing algorithm or standard dose approach. (b) Data strongest for European and East Asian ancestry populations and consistent in other populations. (c) 45–50% of individuals with self-reported African ancestry carry CYP2C9*5, *6, *8, *11, or rs12777823. If CYP2C9*5, *6, *8, and *11 were not tested, dose warfarin clinically. Note: these data derive primarily from African-Americans, who are largely from West Africa. It is unknown if the same associations are present for those from other parts of Africa. (d) Most algorithms are developed for the target INR 2-3. (e) Consider an alternative agent in individuals with genotypes associated with CYP2C9 poor metabolism (e.g., CYP2C9*3/*3, *2/*3, *3/*3) or both increased sensitivity (VKORC1 A/G or A/A) and CYP2C9 poor metabolism. (f) See the EU-PACT trial for pharmacogenetics-based warfarin initiation (loading) dose algorithm with the caveat that the loading dose algorithm has not been specifically tested or validated in populations of African ancestry. (g) Larger dose reduction might be needed in variant homozygotes (i.e., 20–40%). (h) African-American refers to individuals mainly originating from West Africa.
This figure is adapted from (4). Please see Therapeutic Recommendations based on Genotype for more information from CPIC.

Drug: Warfarin

Warfarin is an anticoagulant used in the prevention and treatment of venous thrombosis, pulmonary embolism, and the complications associated with atrial fibrillation and/or cardiac valve replacement. Warfarin is sometimes prescribed to reduce the risk of stroke after a myocardial infarction (MI).

Warfarin has no direct effect on an established thrombus. However, once a thrombus has occurred (e.g., deep venous thrombosis), the goal of warfarin therapy is to prevent further extension of the formed clot and to prevent secondary thromboembolic complications that may be fatal (e.g., pulmonary embolism).

Warfarin is a teratogen – an agent that can cause abnormalities in a developing fetus. Therefore, warfarin use in pregnancy is contraindicated, except in women with mechanical heart valves who have a particularly high risk of thromboembolism. If warfarin is used in pregnancy, or if a patient becomes pregnant while taking warfarin, she should be informed of the potential risks to the fetus (1).

Warfarin exposure in pregnancy can cause fetal death, neonatal death, and warfarin syndrome - a pattern of developmental abnormalities that most commonly affect bone and cartilage, causing nasal hypoplasia, and a

"stippled" appearance to the ends of long bones. The risk of warfarin teratogenicity appears to be greatest between the 6th and 12th week of pregnancy, but toxicity before or after this period is still possible (5, 6).

Warfarin exerts its anticoagulant effect by inhibiting the enzyme encoded by *VKORC1*, which catalyzes the conversion of vitamin K epoxide to the active reduced form of vitamin K, vitamin K hydroquinone. Vitamin K hydroquinone is an essential cofactor in the synthesis of several clotting factors and decreased availability of vitamin K hydroquinone leads to decreased activity of the clotting factors II, VII, IX, and X, and the anticoagulant proteins C and S (7).

Warfarin is administered as a racemic mixture of the *R*- and *S*- stereoisomers. (*S*)-warfarin is 2–5 times more potent than (*R*)-warfarin and is mainly metabolized by CYP2C9. (*R*)-warfarin is mainly metabolized by other cytochrome P450 enzymes (8).

The initial and maintenance doses of warfarin must be tailored to each patient, and monitoring of the international normalized ratio (INR) should be performed in all patients treated with warfarin. The INR is a standardized measurement of prothrombin time, which is the time it takes for blood to clot. In healthy individuals, the INR is approximately one (range: 0.8–1.1). The goal of warfarin therapy is to achieve an INR in a target range for the condition being treated (most commonly 2–3).

The FDA-approved drug label for warfarin carries a boxed warning cautioning of the risk of bleeding, which can be fatal. Bleeding is more likely to occur within the first month, and risk factors include a high intensity of anticoagulation (INR greater than 4), age greater than or equal to 65, and a history of highly variable INRs. Other serious adverse events associated with warfarin therapy include necrosis of the skin and other tissues, particularly when used prematurely to manage thrombosis associated with heparin-induced thrombocytopenia (HIT).

Since warfarin is a drug with a narrow therapeutic index, an optimal starting dose may reduce the time taken to reach a stable INR and reduce the risk of having either a high INR (with a risk of bleeding) or a low INR (with a risk of thrombosis). Known factors that influence an individual's response to the initial dose of warfarin include clinical and lifestyle factors (e.g., age, race, body weight, height, gender, concomitant medications—including those that compete for binding to albumin, comorbidities, diet, nutritional status) and genetic factors (e.g., *CYP2C9* and *VKORC1* genotypes). Therefore, the initial dose should be modified to take into account these and any additional patient-specific factors that may influence warfarin dose requirement.

The FDA-approved drug label for warfarin suggests considering a lower initial and maintenance dose of warfarin for elderly and/or debilitated patients, and in Asian patients. The drug label recommends against the routine use of loading doses because this practice may increase hemorrhagic and other complications and does not offer more rapid protection against clot formation. However, loading doses are used in practice, and are addressed in CPIC recommendations (4).

The drug label also provides a dosing table of expected maintenance daily doses of warfarin based on *CYP2C9* and *VKORC1* genotypes (Table 1). The label states that if the patient's *CYP2C9* and/or *VKORC1* genotypes are known, to consider these doses when selecting the initial dose of warfarin. However, CPIC states that genetics-based algorithms, such as the International Warfarin Pharmacogenetics Consortium (IWPC), predicts warfarin dose better than the table in the drug label (9).

CPIC has provided dosing recommendations that take into account whether the patients *VKORC1* and *CYP2C9*2* and **3* genotype is available, and a patient's self-identified ancestry (African ancestry or non-African ancestry). For patients with African ancestry, the presence of *CYP2C9*5*, **6*, **8*, and **11* alleles, and rs12777823 are also taken into account (4).

Gene: *VKORC1*

Genetic variation in the *VKORC1* gene is the most important *known* genetic factor that influences warfarin dosing. Pharmacogenomic algorithms for warfarin dosing routinely include testing for *VKORC1*.

The *VKORC1* gene encodes the vitamin K epoxide reductase enzyme, which catalyzes the rate-limiting step in vitamin K recycling (converting vitamin K epoxide to vitamin K). This enzyme is also the drug target for warfarin.

A common non-coding variant, *VKORC1*, c.-1639G>A (rs9923231), is associated with an increased sensitivity to warfarin and lower dose requirements (10). The polymorphism occurs in the promoter region of *VKORC1* and is thought to alter a transcription factor binding site, leading to lower protein expression. As a result, patients starting warfarin therapy who are carrying at least one "A "allele at -1639 locus require lower initial and maintenance doses compared with patients carrying a G/G genotype at this locus.

The *VKORC1,* c.–1639G>A allele frequency varies among different ethnic groups. It is the major allele (around 90%) in Asian populations and may be one of the contributing factors for lower warfarin dosing requirements often observed in patients of Asian descent. It is also common in Caucasians (around 40%) and African-Americans (around 14%) (11-13).

Less commonly, missense mutations in *VKORC1* can lead to warfarin resistance and higher dose requirements (14, 15).

The Cytochrome P450 Superfamily

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing or detoxifying lipids, hormones, toxins, and drugs in the liver. The *CYP450* genes are very polymorphic and can result in reduced, absent, or increased enzyme activity.

CYP450 isozymes involved in the metabolism of warfarin include CYP2C9, CYP3A4, and CYP1A2. The more potent warfarin S-enantiomer is metabolized by CYP2C9 while the R-enantiomer is metabolized by CYP1A2 and CYP3A4. The FDA-approved drug label for warfarin states that drugs that inhibit or induce CYP2C9, CYP1A2, and/or CYP3A4 can influence warfarin exposure and increase or decrease the INR.

The influence of genetic variants in *CYP2C9*, *CYP4F2*, and the *CYP2C* gene cluster, is discussed below.

Gene: *CYP2C9*

Genetic variation in the *CYP2C9* gene is a well-known genetic factor that influences warfarin dosing. Pharmacogenomic algorithms for warfarin dosing routinely include testing for *CYP2C9*.

The *CYP2C9* gene is highly polymorphic, with over 60 star (*) alleles described and currently cataloged at the Pharmacogene Variation (PharmVar) Consortium. The *CYP2C9*1* allele is the wild-type allele, and is associated with normal enzyme activity and the normal metabolizer phenotype.

The frequencies of the *CYP2C9* alleles vary between different ethnic groups (16-18). In individuals of European descent, the 2 most common variant alleles associated with reduced enzyme activity are *CYP2C9*2* (c.430C>T; rs1799853) and **3* (c.1075A>C; rs1057910). The **2* allele is more common in Caucasian (10-20%) than African (0-6%) populations (19). The **3* allele is less common (<10% in most populations), but rare in African populations (20).

Compared to normal metabolizers, individuals of European ancestry who carry one or two copies of **2* or **3* are more sensitive to warfarin—they require lower doses and are at a greater risk of bleeding during warfarin initiation (21-25).

In African-Americans, *CYP2C9*5*, **6*, **8*, and **11* variant alleles contribute to the variability in patient response to warfarin (26). These alleles are found more commonly in individuals with African ancestry, and collectively, are more common than the *CYP2C9*2* and **3* alleles.

Gene: *CYP4F2*

The CYP4F2 enzyme is involved in the metabolism of vitamin K in the liver. It is a vitamin K oxidase enzyme and is an important counterpart to VKORC1, a vitamin K reductase enzyme. While VKORC1 catalyzes vitamin K recycling, CYP4F2 limits the excessive accumulation of vitamin K in the liver by catalyzing the production of hydroxylated vitamin K, which is removed from the vitamin K cycle (27).

A genetic variant *CYP4F2*3* (c.1297C>T, rs2108622), has been found to influence warfarin dosing. The frequency of the variant T allele is approximately 30% in Caucasians and Asians, and approximately 7% in African-Americans (28).

The CYP4F2 enzyme with an amino acid change due to missense *3 allele is thought to be less active, leading to a rise in hepatic vitamin K. This leads to a higher dose of warfarin being required to achieve therapeutic anticoagulation (by inhibiting vitamin K-dependent clotting factors) (27).

The first studies of *CYP4F2* and warfarin dosing reported that Caucasian individuals with the variant rs2108622 TT genotype required approximately 1 mg/day more warfarin than individuals with the rs2108622 CC genotype (28). Two more recent meta-analyses concluded that "T carriers" (individuals with CT or TT genotypes) require approximately an 8–11% increase in warfarin dose, compared to CC individuals. However, data did not support *CYP4F2* influencing warfarin requirements in African-Americans (29, 30).

The inclusion of this *CYP4F2* variant in warfarin dosing models moderately improves the accuracy of warfarin dose prediction for individuals of European or Asian ancestry, but not for individuals of African ancestry. Accordingly, CPIC recommends that the dose of warfarin should be increased by 5–10% in non-African-American individuals who carry the *CYP4F2*3* variant (optional recommendation). CPIC makes no recommendation for African-Americans, stating that data do not support an impact of this variant on warfarin dosing in those of African ancestry (moderate recommendation) (4, 29, 30).

Gene: *CYP2C* rs12777823

The genetic variant rs12777823, located in the *CYP2C* gene cluster, is a non-coding variant associated with reduced warfarin dose requirements in African-Americans. The rs12777823 variant was associated with altered warfarin clearance, and individuals with this variant require a lower maintenance dose of warfarin than individuals who do not have this variant (31).

The rs12777823 variant is common in African-Americans (allele frequency 25%) and is also common in other populations; for example, Japanese (32%), and European (15%). However, the association with warfarin dose requirement has only been found for African-Americans: individuals who are heterozygous for the rs12777823 A allele require a dose reduction of warfarin by 7 mg/week, and individuals who are homozygous for the rs12777823 A allele require a dose reduction of warfarin by 9 mg/week (31). Data are lacking for the role of rs12777823 and warfarin response in other populations.

Current pharmacogenomic dosing algorithms do not include rs12777823 status, but analysis has shown that the addition of this variant improves the dosing algorithm published by the IWPC by 21% for African-Americans (31).

CPIC has stated that for African-Americans, a dose reduction of 10–25% in individuals with the rs12777823 A/G or A/A genotype is recommended (moderate recommendation). For non-African-Americans, CPIC recommends that rs12777823 should not be considered, even if the result is available (4).

Genetic Testing

The NIH's Genetic Testing Registry (GTR) provides a list of tests for "warfarin response," and the *VKORC1*, *CYP2C9*, and *CYP4F2* genes.

The *VKORC1* and *CYP2C9* genotypes are important genetic determinants of warfarin dosing. The contribution of *VKORC1* to the variation in dose requirement is larger (approximately 30%) than the contribution of *CYP2C9* (usually less than 10%) (32). The variants that are routinely tested for are *CYP2C9*2*, *CYP2C9*3*, and *VKORC1,* c.–1639G>A. These variants are used in the FDA table to guide therapy, and also in the IWPC algorithm.

Currently, routine lab tests do not test for the presence of rs12777823. Other variants that are not routinely tested for include the *CYP2C9*5, *6, *8* and **11* alleles, the genes *CYP4F2*, *EPHX1*, and *GGCX* (which all have a role in the vitamin K cycle), and the gene *CALU* (a cofactor in the VKOR complex) (26, 33).

In African-Americans, the influence of the CYP2C9**5, *6, *8* and **11* alleles are thought to be as significant as the influence of the *CYP2C9*2* and**3* alleles on warfarin dosing in Caucasians. Requesting testing of these additional *CYP2C9* alleles, and including these genotypes in an expanded dosing algorithm improves warfarin dose prediction in African-Americans, while maintaining high performance in European-Americans (34).

Individuals who are most likely to benefit from genetic testing are those who have yet to start warfarin therapy. However, genotype-guided warfarin dosing is controversial and is generally not carried out preemptively. Some studies have reported that, in general, the current use of genotype-guided dosing algorithms did not improve anticoagulation control in the first few weeks of warfarin therapy (35-41); however, a recent study found genotype-guided warfarin dosing did improve the safety of starting warfarin, compared to clinically guided dosing (42).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2017 Statement from the US Food and Drug Administration (FDA)

Initial and Maintenance Dosing

The appropriate initial dosing of warfarin sodium tablets varies widely for different patients. Not all factors responsible for warfarin dose variability are known, and the initial dose is influenced by:

- Clinical factors including age, race, body weight, sex, concomitant medications, and comorbidities

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

- Genetic factors (*CYP2C9* and *VKORC1* genotypes)

Select the initial dose based on the expected maintenance dose, taking into account the above factors. Modify this dose based on consideration of patient-specific clinical factors. Consider lower initial and maintenance doses for elderly and/or debilitated patients and in Asian patients. Routine use of loading doses is not recommended as this practice may increase hemorrhagic and other complications and does not offer more rapid protection against clot formation.

Individualize the duration of therapy for each patient. In general, anticoagulant therapy should be continued until the danger of thrombosis and embolism has passed.

Dosing Recommendations without Consideration of Genotype

If the patient's *CYP2C9* and *VKORC1* genotypes are not known, the initial dose of warfarin sodium tablets is usually 2 to 5 mg once daily. Determine each patient's dosing needs by close monitoring of the INR response and consideration of the indication being treated. Typical maintenance doses are 2 to 10 mg once daily.

Dosing Recommendations with Consideration of Genotype

Table 1 displays three ranges of expected maintenance warfarin sodium tablets doses observed in subgroups of patients having different combinations of CYP2C9 and VKORC1 gene variants. If the patient's *CYP2C9* and/or *VKORC1* genotype are known, consider these ranges in choosing the initial dose. Patients with *CYP2C9* *1/*3, **2/*2*, **2/*3*, and **3/*3* may require more prolonged time (>2 to 4 weeks) to achieve maximum INR effect for a given dosage regimen than patients without these CYP variants.
Please review the complete therapeutic recommendations that are located here: (1)

2017 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

VKORC1 CT: warfarin

NO action is required for this gene-drug interaction.

The genetic variation results in a reduction in the required dose and an increase in the risk of excessively severe inhibition of blood clotting during the first month of the treatment. However, the effect is small and CT is also the most common genotype, meaning that the standard treatment will primarily be based on patients with this genotype.

VKORC1 TT: warfarin

The genetic variation results in increased sensitivity to warfarin. This results in an increase in the risk of excessively severe inhibition of blood clotting (INR >4) during the first month of the treatment.

Recommendation:

1 use 60% of the standard initial dose

The genotype-specific initial dose and maintenance dose can be calculated using an algorithm, as used in EU-PACT: see https://www.knmp.nl/patientenzorg/medicatiebewaking/farmacogenetica.

From day 6 on the standard algorithm without genotype information can be used to calculate the dose.

CYP2C9 IM: warfarin

This gene variation reduces the conversion of warfarin to inactive metabolites. This can increase the risk of bleeding.

Recommendation:

1 use 65% of the standard initial dose

The genotype-specific initial dose and maintenance dose can be calculated using an algorithm. Algorithms for Caucasian patients usually contain only the *2 and *3 allele. If the activity of the reduced-activity alleles is comparable to the activity of *2 or *3, then the algorithm can be completed as if *1/*2 or *1/*3 is present. See https://www.knmp.nl/patientenzorg/medicatiebewaking/farmacogenetica for Excel files containing calculation modules for oral and equivalent intravenous doses. From day 6 on the standard algorithm without genotype information can be used to calculate the dose.

Modified dose algorithms have been developed for patients of African or (East) Asian heritage.

CYP2C9 PM: warfarin

This gene variation reduces the conversion of warfarin to inactive metabolites. This can increase the risk of bleeding.

Recommendation:

1 use 20% of the standard initial dose

The genotype-specific initial dose and maintenance dose can be calculated using an algorithm. Algorithms for Caucasian patients usually contain only the *2 and *3 allele. If the activity of the reduced-activity alleles is comparable to the activity of *2 or *3, then the algorithm can be completed as if *2 or *3 is present. See https://www.knmp.nl/patientenzorg/medicatiebewaking/farmacogenetica for Excel files containing calculation modules for oral and equivalent intravenous doses. From day 6 on the standard algorithm without genotype information can be used to calculate the dose.

Modified dose algorithms have been developed for patients of African or (East) Asian heritage.

CYP2C9*1/*2: warfarin

NO action is required for this gene-drug interaction.

Genetic variation may lead to a decrease in the required maintenance dose. However, there is insufficient evidence that this causes problems when therapy is initiated as usual.

Please review the complete therapeutic recommendations located here: (2, 3)

2017 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC)

Non-African ancestry recommendation

In patients who self-identify as non-African ancestry, the recommendation is to:

1. Calculate warfarin dosing using a published pharmacogenetic algorithm, including genotype information for VKORC1-1639G>A and CYP2C9*2 and *3. In individuals with genotypes associated with CYP2C9 poor metabolism (e.g., CYP2C9 *2/*3, *3/*3) or both increased sensitivity (VKORC1-1639 A/A) and CYP2C9 poor metabolism, an alternative oral anticoagulant might be considered. The bulk of the literature informing these recommendations is in European and Asian ancestry populations, but consistent data exist for other non-African populations. These recommendations are graded as STRONG.

2. If a loading dose is to be utilized, the EU-PACT loading dose algorithm that incorporates genetic information could be used. This recommendation is OPTIONAL.
3. While *CYP2C9*5*, **6*, **8*, or **11* variant alleles are commonly referred to as African-specific alleles, they can occur among individuals who do not identify as, or know of their, African ancestry. If these variant alleles are detected, decrease calculated dose by 15–30% per variant allele or consider an alternative agent. Larger dose reductions might be needed in patients homozygous for variant alleles (i.e., 20–40%, e.g., CYP2C9*2/*5). This recommendation is graded as OPTIONAL.
4. If the *CYP4F2*3* (i.e., c.1297A, p.433Met) allele is also detected, increase the dose by 5–10%. This recommendation is also considered OPTIONAL.
5. The data do not suggest an association between rs12777823 genotype and warfarin dose in non-African Americans, thus rs12777823 should not be considered in these individuals (even if available).

African ancestry recommendation

In patients of African ancestry, CYP2C9*5, *6, *8, *11 are important for warfarin dosing. If these genotypes are not available, warfarin should be dosed clinically without consideration for genotype. If CYP2C9*5, *6, *8, and *11 are known, then the recommendation is to:

1. Calculate warfarin dose using a validated pharmacogenetic algorithm, including genotype information for VKORC1 c.-1639G>A and CYP2C9*2 and *3;
2. If the individual carries a CYP2C9*5, *6, *8, or *11 variant allele(s), decrease calculated dose by 15–30%. Larger dose reductions might be needed in patients who carry two variant alleles (e.g., CYP2C9*5/*6) (i.e., 20–40% dose reduction).
3. In addition, rs12777823 is associated with warfarin dosing in African Americans (mainly originating from West Africa). Thus, in African Americans a dose reduction of 10–25% in those with rs12777823 A/G or A/A genotype is recommended. These recommendations are considered MODERATE.

In individuals with genotypes that predict CYP2C9 poor metabolism or who have increased warfarin sensitivity (VKORC1 c.-1639 A/A) and CYP2C9 poor metabolism, an alternative oral anticoagulant should be considered (see Supplemental Material for definitions of strength of recommendations). As noted above, for non-African ancestry, if a loading dose is to be used, the EU-PACT algorithm that incorporates genetic information could be used to calculate loading dose. This recommendation is OPTIONAL. The data do not support an impact on clinical phenotype for CYP4F2 on warfarin dosing in those of African ancestry and so no recommendation is made for use of CYP4F2 genotype data in blacks.

Please review the complete therapeutic recommendations, including recommendations for pediatric patients, located here: (4).

Nomenclature

Nomenclature for Selected *CYP2C9* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C9*2*	430C>T Arg144Cys	NM_000771.3:c.430C>T	NP_000762.2:p.Arg144Cys	rs1799853
*CYP2C9*3*	1075A>C Ile359Leu	NM_000771.3:c.1075A>C	NP_000762.2:p.Ile359Leu	rs1057910
*CYP2C9*5*	1080C>G Asp360Glu	NM_000771.3:c.1080C>G	NP_000762.2:p.Asp360Glu	rs28371686

Nomenclature for Selected continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C9*6*	817delA Lys273Argfs	NM_000771.3:c.817delA	NP_000762.2:p.Lys273Argfs	rs9332131
*CYP2C9*8*	449G>A Arg150His	NM_000771.3:c.449G>A	NP_000762.2:p.Arg150His	rs7900194
*CYP2C9*11*	1003C>T Arg335Trp	NM_000771.3:c.1003C>T	NP_000762.2:p.Arg335Trp	rs28371685

HGVS - Human Genome Variation Society, dbSNP - Single Nucleotide Polymorphism Database

Nomenclature for Selected *VKORC1* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
-1639G>A	1173C>T	NM_024006.4:c.-1639G>A	Not applicable - variant occurs in a non-coding region	rs9923231

HGVS - Human Genome Variation Society, dbSNP - Single Nucleotide Polymorphism Database

Nomenclature for Selected *CYP4F2* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP4F2*3*	1297G>A Val433Met	NM_001082.4:c.1297G>A	NP_001073.3:p.Val433Met	rs2108622

HGVS - Human Genome Variation Society, dbSNP - Single Nucleotide Polymorphism Database

Nomenclature for rs12777823

HGVS reference sequence	dbSNP reference identifier for allele location
NC_000010.11:g.94645745G>A (GRCh38) NC_000010.10:g.96405502G>A (GRCh37)	rs12777823

HGVS - Human Genome Variation Society, dbSNP - Single Nucleotide Polymorphism Database

Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (43).

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).

Nomenclature for Cytochrome P450 enzymes is available from Pharmacogene Variation (PharmVar) Consortium.

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First edition (2012):

The Pharmacogenomics Knowledgebase: http://www.pharmgkb.org

The Clinical Pharmacogenetics Implementation Consortium: http://www.pharmgkb.org/page/cpic

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To view the 2016 version of this summary (Created: June 8, 2016) please click here.

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Genetic variants and disease

ABO Blood Group

Laura Dean, MD[1]

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Characteristics

There are four common blood groups in the ABO system: O, A, B, and AB. The blood groups are defined by the presence of specific carbohydrate sugars on the surface of red blood cells, N-acetylgalactosamine for the A antigen, and D-galactose for the B antigen. Both of these sugars are built upon the H antigen—if the H antigen is left unmodified, the resulting blood group is O because neither the A nor the B antigen can attach to the red blood cells.

Individuals will naturally develop antibodies against the ABO antigens they do not have. For example, individuals with blood group A will have anti-B antibodies, and individuals with blood group O will have both anti-A and anti-B. Before a blood transfusion takes place, routine serological testing checks the compatibility of the ABO (and Rh) blood groups. An ABO incompatible blood transfusion can be fatal, due to the highly immunogenic nature of the A and B antigens, and the corresponding strongly hemolytic antibodies (1).

Compared to other blood groups, individuals with blood group O may have a lower risk of pancreatic cancer and thromboembolic disease (2, 3). In addition, in certain African populations, individuals with the blood group O may be protected from life-threatening malaria (4). However, this blood group is not more common in some regions where malaria is endemic. This might be because individuals with blood group O are at higher risk of cholera and severe diarrhea due to *Vibrio cholerae* 01, with individuals with the AB blood group being the most protected (5, 6).

Over 80 *ABO* alleles have been reported. The common alleles include *A1*, *A2*, *B1*, *O1*, *O1v*, and *O2* (7). Whereas the *A* and *B* alleles each encode a specific glycosyl-transferring enzyme, the *O* allele appears to have no function. A single-base deletion in the *O* allele means that individuals with blood group O do not produce either the A or B antigens. Blood type frequencies vary in different racial/ethnic groups. In the US, in Caucasians, the ratio of blood group O, A, B, and AB is 45%, 40%, 11%, and 4% respectively. In Hispanics, the distribution is 57%, 31%, 10%, and 3%; and in Blacks, 50%, 26%, 20%, and 4% (8).

Diagnosis/testing

Serological testing is sufficient to determine an individual's blood type (e.g., blood group A) for the purposes of blood donation and transfusion. Molecular genetic testing can be used to determine an individual's *ABO* genotype (e.g., genotype *AO* or *AA*). This may be useful in the research setting, for example, to investigate the link between ABO blood groups and particular diseases, and also in the forensic setting (9).

Management

Determining an individual's blood group is important prior to blood transfusion and prior to the donation or receiving of a kidney transplant.

Occasionally, a person's blood type may appear to change. For example, the ABO antigens can act as tumor markers. Their presence may be decreased in particular diseases, such as acute myeloid leukemia, AML (10). In contrast, occasionally the B antigen may be acquired in certain infectious diseases. A bacterial infection with

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specific strains of *E. coli* or *Clostridium tertium* can generate a B-like antigen from an individual who has the *A1* allele (11).

Genetic counseling

The ABO blood type is inherited in an autosomal codominant fashion. The *A* and *B* alleles are codominant, and the *O* allele is recessive.

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ACHOO Syndrome

Laura Dean, MD[1]

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Characteristics

Autosomal Dominant Compelling Helioopthalmic Outburst (ACHOO) Syndrome is characterized by uncontrollable sneezing in response to the sudden exposure to bright light, typically intense sunlight (1). This type of sneezing is also known as photic sneezing. About one in four individuals who already have a prickling sensation in their nose will sneeze in response to sunlight, but "pure" photic sneezing is far less common (2).

Sneezing is usually triggered by contact with infectious agents or after inhaling irritants, but the cause of photic sneezing is not fully understood. It may involve an over-excitability of the visual cortex in response to light, leading to a stronger activation of the secondary somatosensory areas (3).

Diagnosis/testing

The diagnosis of ACHOO syndrome is usually made by clinical history. Affected individuals report a "prickling sensation" or sneezing in response to a bright light. This response may be reproduced in the clinical setting by asking the individual to look at a bright light, although findings are unreliable.

The genetic basis of this syndrome is not yet known.

Management

Recommendations for management of ACHOO syndrome include using a hat or sunglasses to shield the eyes from direct sunlight whenever possible. Potential hazards include the possibility of drivers having an accident caused by sneezing brought on by, for example, exiting a road tunnel on a bright day. Similarly, airline pilots may be at risk (4).

Genetic counseling

ACHOO syndrome is inherited in an autosomal dominant manner (1). As such, if one parent is affected, their child has a 50% chance of inheriting the syndrome.

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McCune-Albright Syndrome

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Characteristics

McCune-Albright Syndrome (MAS) is a rare genetic disorder originally characterized as the triad of polyostotic fibrous dysplasia of bone, precocious puberty, and café-au-lait skin pigmentation (1-3). With time other associated endocrinopathies have been recognized, including hyperthyroidism, growth hormone excess, FGF23-mediated phosphate wasting, and hypercortisolism (4, 5).

MAS is caused by an activating mutation in the *GNAS* gene, which encodes the alpha subunit of the stimulatory G protein involved in G-protein signaling (6, 7). A missense mutation, typically Arg201Cys or Arg201His (NM_001077488.3:c.604C>T, rs11554273), impairs the intrinsic GTPase activity of the Gsα protein, resulting in the constitutive activation of the Gsα-cAMP signaling pathway in the cells that contain the mutation.

The mutation arises early in embryogenesis and is distributed in a mosaic pattern. The clinical phenotype is therefore highly variable, depending upon the location and timing of the mutation during embryologic development. Skin manifestations are common and are usually present at or shortly after birth. The café-au-lait spots typically have irregular margins giving them a "coast of Maine" appearance, and usually show an association with the midline of the body.

In MAS, fibrous dysplasia of bone typically occurs at several sites (polyostotic), and commonly presents with fracture, deformity and/or bone pain (8). Radiographs show characteristic expansile lesions with a "ground glass" appearance. Craniofacial fibrous dysplasia can be severe in individuals who have pituitary disorders leading to hypersecretion of growth hormone. Treatment can be challenging and should begin as soon as possible.

In girls, precocious puberty is a common initial manifestation, with recurrent ovarian cysts leading to episodes of vaginal bleeding and breast development. Precocious puberty is less common in boys, presenting with penile enlargement, pubic and axillary hair, acne, body odor, and sexual behavior. However, in both girls and boys, there is a high frequency of gonadal pathology (ovarian abnormalities in girls, and testicular abnormalities in boys) (9).

Diagnosis

The NIH Genetic Testing Registry, GTR, displays genetic tests that are currently available for the *GNAS* gene and the McCune-Albright Syndrome.

Currently, the diagnosis of McCune-Albright syndrome is made clinically in most cases. This is due to the mosaic nature of the disease whereby a negative genetic test result (e.g., in blood) does not exclude the presence of the mutation in other tissues. However, newer techniques such as digital PCR may improve the sensitivity of genetic testing in individuals who have clinical signs of McCune-Albright syndrome (10, 11).

Management

Treatment is individualized based on each patient's clinical presentation. Letrozole (12) and/or tamoxifen (13) may be effective for treatment of precocious puberty in girls. Medications and/or surgery may be used for

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treatment of hyperthyroidism (14, 15), growth hormone excess (16, 17), and hypercortisolism (18). Management of fibrous dysplasia of bone is palliative, with surgery as needed for fracture and deformity (19, 20). Bisphosphonates are effective for treatment of fibrous dysplasia-related pain, but have not been shown to have any long-term effect on the course of the disease (21, 22).

Genetic Counseling

McCune-Albright syndrome is caused by a new (de novo) mutation that occurs after conception, at an early stage of development. Individuals with McCune-Albright syndrome have not been observed to pass the syndrome on to their children.

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Version History

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Methylenetetrahydrofolate Reductase Deficiency

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Characteristics

Methylenetetrahydrofolate Reductase (MTHFR) Deficiency is the most common genetic cause of elevated levels of homocysteine in the plasma (hyperhomocysteinemia).

The MTHFR enzyme plays an important role in processing amino acids, specifically, the conversion of homocysteine to methionine. Genetic variations in the *MTHFR* gene can lead to impaired function or inactivation of this enzyme, which results in mildly elevated levels of homocysteine, especially in individuals who are also deficient in folate (1). In these individuals, a daily supplement of low dose folic acid may reduce and often normalize their homocysteine levels, but this has not been demonstrated to improve health outcomes (2, 3).

A common genetic variant in the *MTHFR* gene is a 677C>T polymorphism (NM_005957.4:c.665C>T, rs1801133). This variant encodes a thermolabile enzyme that is less active at higher temperatures. Individuals who carry two copies of this variant ("TT homozygous") tend to have higher homocysteine levels and lower serum folate levels compared to controls.

More than 25% of Hispanics and around 10-15% of North America Caucasians are estimated to be homozygous for the "thermolabile" variant (TT genotype) (4). The TT genotype is least common in individuals of African descent (6%) (5, 6).

Another common *MTHFR* variant, 1298A>C (NM_005957.4:c.1286A>C, rs1801131), does not cause increased homocysteine levels in heterozygous or homozygous individuals, but combined heterozygosity of 1298A>C and 677C>T results in an outcome similar to TT homozygous individuals (7).

Until recently, it was thought that MTHFR deficiency, by causing elevated homocysteine levels, led to an increased risk of venous thrombosis, coronary heart disease, and recurrent pregnancy loss (8-11). However, more recent analysis has not found an association between elevated homocysteine levels and the risk of venous thrombosis or the risk of coronary heart disease (12).

MTHFR polymorphism genotyping should not be ordered as part of the clinical evaluation for thrombophilia, recurrent pregnancy loss, or for at-risk family members (4).

Rarely, more severe variants in the *MTHFR* gene can be a cause of an autosomal recessive inborn error or metabolism where extremely high levels of homocysteine accumulate in the urine and plasma. This can cause developmental delay, eye disorders, thrombosis, and osteoporosis. But more commonly, homocystinuria is caused by variants in a different gene (cystathionine beta-synthase, *CBS*). To read more about homocystinuria caused by CBS deficiency, please see *GeneReviews*.

Diagnosis

A blood test that measures total homocysteine levels can diagnose hyperhomocysteinemia.

Genetic testing of the *MTHFR* gene may be used to confirm the diagnosis of an inherited hyperhomocysteinemia caused by MTHFR deficiency. However, a 2013 Practice Guideline from the American

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College of Medical Genetics and Genomics (ACMG) states that there is growing evidence that "*MTHFR* polymorphism testing has minimal clinical utility and, therefore should not be ordered as a part of a routine evaluation for thrombophilia" (4).

In an infant or child in whom autosomal recessive severe MTHFR deficiency is suspected, tests for plasma homocysteine and serum amino acids levels would be expected to show a pattern of extremely elevated homocysteine and low methionine. *MTHFR* full gene sequencing (as opposed to targeted polymorphism testing) can confirm the suspected clinical diagnosis.

Management

2013 Statement from the American College of Medical Genetics and Genomics (ACMG) includes the following recommendations:

- *MTHFR* polymorphism genotyping should not be ordered as part of the clinical evaluation for thrombophilia or recurrent pregnancy loss
- *MTHFR* polymorphism genotyping should not be ordered for at-risk family members
- A clinical geneticist who serves as a consultant for a patient in whom an *MTHFR* polymorphism(s) is found should ensure that the patient has received a thorough and appropriate evaluation for his or her symptoms
- If the patient is homozygous for the "thermolabile" variant c.665C→T, the geneticist may order a fasting total plasma homocysteine, if not previously ordered, to provide more accurate counseling
- *MTHFR* status does not change the recommendation that women of childbearing age should take the standard dose of folic acid supplementation to reduce the risk of neural tube defects as per the general population guidelines

For the complete guideline, please see *ACMG Practice Guideline: lack of evidence for MTHFR polymorphism testing*. Genetics in Medicine. 2013;15(4):153-6. (4)

The management of severe autosomal recessive MTHFR deficiency is outside the scope of this review.

Genetic Testing

The NIH Genetic Testing Registry, GTR, displays genetic tests that are currently available for the *MTHFR* gene and for homocysteinemia due to MTHFR deficiency.

Biochemical genetic tests may also be used, which assess the level of activity of the MTHFR enzyme or the level of analyte in the blood. GTR provides a list of biochemical tests that assess the level of homocysteine analytes and the activity of the MTHFR enzyme.

Genetic Counseling

The MTHFR polymorphism has been associated with many different medical complications. Individuals who are "*MTHFR* positive" carry one or two copies of variants in the *MTHFR* gene. However, in general, the following genotypes are unlikely to be of clinical significance:

- 677C>T heterozygote
- c.1286A→C homozygote
- (677C>T);(c.1286A→C) compound heterozygote

Individuals who are TT homozygous with normal homocysteine levels do not have an increased risk of venous thrombosis or recurrent pregnancy loss, according to recent evidence. However, women do have a modestly increased risk of having a child with a neural tube defect and this risk increases if the fetus is also homozygous.

If homocysteine levels are elevated, TT homozygotes may have a mildly increased risk of venous thrombosis or recurrent pregnancy loss, but not other previously associated conditions, such as cardiovascular disease.

Less is known about the c.1286A→C variant, but current evidence suggests that it is milder than the "thermolabile" c.665C→T variant (4).

For all individuals, it is important to determine whether medical disorders have been incorrectly attributed to their positive *MTHFR* status. Referral to a hematologist or maternal–fetal medicine specialist may be needed. And patients should provide their *MTHFR* genotype status to their physician before starting chemotherapy agents that require folate (e.g., methotrexate).

Finally, *MTHFR* positive individuals may decide to take vitamin B and folic acid supplements. Although safe (toxicity is rare), evidence is lacking on whether such supplements reduce the risks associated with hyperhomocysteinemia or *MTHFR* genotype status (4).

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Pitt-Hopkins Syndrome

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Introduction

Pitt-Hopkins syndrome is a rare neurodevelopmental disorder caused by loss of function of one allele of the *TCF4* gene. Most cases result from a *de novo* mutation that leads to a functional loss of one copy of the *TCF4* gene. Other cases result from a deletion of the chromosome region in which the *TCF4* gene is located (18q21.2).

Pitt-Hopkins syndrome is characterized by distinctive facial features (e.g., deep-set eyes, prominent nose, wide mouth with widely spaced teeth), global developmental delay, and moderate-severe intellectual disability. Breathing problems and epilepsy often occur.

Once Pitt-Hopkins syndrome has been suspected clinically, the diagnosis is confirmed by molecular genetic testing of the *TCF4* gene.

Characteristics

Pitt-Hopkins syndrome is rare—approximately 500 cases of Pitt-Hopkins syndrome have been reported worldwide (1). In infancy, low muscle tone can cause feeding problems. In older children, gait looks stiff because of a combination of low muscle tone (hypotonia) and balance problems (ataxia).

Children with Pitt-Hopkins tend to have a happy disposition, with hand flapping and excitability. They may develop abnormal breathing patterns, such as sudden attacks of hyperventilation followed by breath-holding until cyanosis. About half of these children have epilepsy; typically their ECG is normal (2). Subtle changes in the brain may be seen in up to 70% of patients by MRI (e.g., underdeveloped corpus callosum, dilated ventricles) (3).

Most adults with Pitt-Hopkins syndrome have severe cognitive impairment, and although they may vocalize, they are unable to use language. Other issues include gastrointestinal (e.g., constipation), ophthalmic (e.g., strabismus, severe myopia), and behavioral problems (e.g., anxiety, stereotypical movements of the head and hands).

Pitt-Hopkins syndrome may be distinguished clinically from other causes of intellectual disability and developmental delay (e.g., Angelman syndrome, Rett syndrome, Mowat–Wilson syndrome) by: 1) abnormal breathing patterns (onset from 7 months to 7 years); 2) lack of congenital abnormalities; and 3) distinctive facial features (craniofacial dysmorphism). In infants, the first sign of craniofacial dysmorphism may be the prominence of the nose and lower face. As the child grows, they may develop deep-set eyes, a high nasal root with prominent nasal bridge, wide nostrils and down-turned nasal tip; a short philtrum, and a wide mouth with widely spaced teeth.

Genetics

Pitt-Hopkins syndrome is an autosomal dominant disorder caused by haploinsufficiency of the *TCF4* gene. Haploinsufficiency occurs when one copy of the gene has been lost (e.g., by a loss-of-function mutation), and the remaining copy of the gene is not sufficient to prevent the disorder.

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TCF4 has an important role in the development of the nervous system. *TCF4* encodes a transcription factor—a protein that binds to specific DNA sequences and controls the expression of other genes. The TCF4 protein contains a basic helix-loop-helix (bHLH) domain, and is also known as an "E-protein" because it binds to a specific sequence of DNA known as an "E-box".

The TCF4 protein is expressed in the brain, heart, lungs, and muscles. TCF4 is also active during early human development, when it is thought to be involved in a series of developmental processes, including initiating the development of several regions of the nervous system (3).

Mutations in *TCF4* disrupt the ability of the protein to bind to DNA and initiate neuronal differentiation, contributing to the neurological symptoms seen in Pitt-Hopkins syndrome. In addition, other proteins that normally form heterodimers with TCF4 are unable to function normally. One of these proteins, ASCL1, is thought to be involved in development in the brain stem—after defective interaction with TCF4, impaired development of the brain stem may contribute to the breathing problems that characterize Pitt-Hopkins syndrome (3).

A spectrum of mutations can disrupt the *TCF4* gene, which is located on the long arm of chromosome 18 (18q21.2) (4, 5). The gene has 20 exons, of which exons 2 to 19 are coding. Exon 18 is thought to harbor a quarter of disease-causing mutations (6).

Approximately 30% of cases of Pitt-Hopkins syndrome are caused by whole gene deletions of TCF4, and approximately 10% caused by partial gene deletions. Missense mutations are also common, and mainly involve the bHLH domain, whereas nonsense and frameshift mutations are spread throughout the gene. Splice site mutations are less common (approximately 10%), and balanced translocations are a rare cause of Pitt-Hopkins syndrome (3, 7).

Diagnosis

The diagnosis of Pitt-Hopkins syndrome is based on the clinical presentation and confirmed by molecular genetic testing.

Currently, there is not a generally accepted diagnostic criteria, but the hallmarks of the syndrome that support a diagnosis of Pitt-Hopkins syndrome are facial dysmorphism, early onset global developmental delay, moderate to severe intellectual disability, seizures, breathing abnormalities, and a lack of major congenital abnormalities (2, 3, 8).

Management

Infants with Pitt-Hopkins syndrome should receive treatment from a multidisciplinary team specializing in the care of children with cognitive and motor impairment, including physical therapists, occupational therapists, and speech therapists. Medical specialists for pulmonary conditions, epilepsy, gastrointestinal conditions and other medical issues may also be needed.

Genetic Testing

The NIH Genetic Testing Registry, GTR, provides examples of the genetic tests that are currently available for Pitt-Hopkins syndrome and the *TCF4* gene.

Testing options include sequence analysis (to determine the nucleotide sequence of *TCF4*), chromosome microarray analysis (to detect copy number variants by determining the gain or loss of chromosome material), quantitative PCR (to determine the relative amount of DNA or RNA in a sample), and cytogenetic testing/karyotyping (to assess chromosome number and structure).

Sequencing analysis detects approximately 70% of *TCF4* variants, which may include missense, nonsense, and splice site variants, and small intragenic inserts and deletions. Typically, deletions of *TCF4* exons or the whole *TCF4* gene will not be detected by Sanger sequencing.

If a variant is not found by sequencing and a gene deletion is suspected, deletion/duplication analysis should be performed at the exon-level. Methods used include quantitative PCR and chromosome microarray analysis.

If a deletion is not found but Pitt-Hopkins syndrome is still suspected, karyotype analysis may be used to search for balanced translocations disrupting the coding region of *TCF4* (3, 9).

Genetic Counseling

Pitt-Hopkins syndrome is caused by a mutation in the *TCF4* gene, or a deletion of the chromosome region in which *TCF4* is located (18q21.2).

Most cases are caused by a *de novo* mutation (a new mutation, not present in either parent); cases of inheritance from a mosaic parent with a de novo mutation are exceedingly rare (10, 11).

Usually only one member of the family is affected. Parents are typically not affected, and although genetic testing could be offered, it would not be possible to entirely rule out a mutation because of somatic mosaicism (different cell lines may have different variants of *TCF4*).

Prenatal diagnosis and preimplantation genetic diagnosis are possible for pregnancies at increased risk of Pitt-Hopkins syndrome (e.g., if the parents have already had one affected child).

This risk of siblings being affected is low because the mutation is almost always *de novo* and not inherited. However, the risk is higher than that of the general population because of the possibility of mosaicism in parental germline cells (precursor cells of the egg or sperm).

For an individual with Pitt-Hopkins syndrome, the risk of passing on the syndrome to their offspring would be 50%. However, there are no known cases of individuals reproducing (2, 9).

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Version History

To view the 2012 version of the summary, please click here.

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Schizophrenia

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Characteristics

Schizophrenia is a severe neurodevelopmental disorder with a worldwide prevalence of around 0.3-0.7% (1). The etiology of schizophrenia is unknown, but it is thought to result from a combination of complex genetic and environmental factors. This includes physical factors e.g., complications during pregnancy and birth, infection, and autoimmune disease; as well as psychological factors that may trigger psychosis, such as stress and drug abuse (2). Several neurotransmitter systems are thought to be involved in the pathogenesis, including dopamine, glutamate, GABA, and acetylcholine.

Schizophrenia is associated with substantial morbidity and mortality. Antipsychotics are the mainstay of treatment, however, their efficacy is poor for many patients. Antipsychotics are thought to exert their therapeutic effects by the post-synaptic blockade of D2 dopamine receptors in the brain.

The symptoms of schizophrenia fall in to three main categories: positive, negative, and cognitive. Positive symptoms are generally not found in healthy individuals, but may come and go or persist in individuals with schizophrenia. Positive symptoms include reality distortion (e.g., delusions, hallucinations), and thought disorders. These symptoms often respond well to treatment.

Negative symptoms are deficits in normal emotions and behavior, and may be mistaken for depression. Symptoms divide into reduced expression of emotion (e.g., speaking without moving or with a monotonous voice) and avolition (a lack of motivation to start or continue with a task). No treatment has established efficacy for these pathologies.

Cognitive symptoms may also be difficult to recognize. They include poor executive functioning (understanding information and using it to make decisions) and trouble focusing or paying attention. And again, no treatment has established efficacy.

Genetics

Schizophrenia is highly heritable, as shown by family, twin, and adoption studies. For example, for identical twins, if one twin develops schizophrenia, the other twin has about a 50% chance of also developing the disease. The risk of the general population developing the schizophrenia is about 0.3-0.7% worldwide (3).

The search for "schizophrenia genes" has been elusive. Initial linkage studies looked at parts of the genome associated with schizophrenia, and many candidate genes were identified, including *APOE*, *COMT*, *DAO*, *DRD1*, *DRD2*, *DRD4*, *DTNBP1*, *GABRB2*, *GRIN2B*, *HP*, *IL1B*, *MTHFR*, *PLXNA2*, *SLC6A4*, *TP53*, and *TPH1* (4). However, some of these have later been questioned (5).

Microdeletions and microduplications have been found to be three times more common in individuals with schizophrenia, compared to controls. Because these deletions and duplications are in genes that are overexpressed in pathways related to brain development, it is possible that the inheritance of multiple rare variants may contribute to the development of schizophrenia (6).

Several genetic disorders feature schizophrenia as a clinical feature. The 22q11.2 Deletion Syndrome comprises many different syndromes, of which one of the most serious is DiGeorge syndrome. Children born with

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DiGeorge syndrome typically have heart defects, cleft palate, learning difficulties, and immune deficiency. Schizophrenia is a late manifestation, affecting around 30% of individuals (7). Microdeletions and duplications in chromosome 1, 2, 3, 7, 15 and 16 have also been associated with schizophrenia (8).

In 2014, a genome-wide association study looked at the genomes of over 35,000 patients and 110,00 controls. The study identified 108 SNPs that were associated with schizophrenia, 83 of which had not been previously reported. As expected, many of these loci occurred in genes that are expressed in the brain. For example, the SNPs included a gene that encodes the dopamine D2 receptor, *DRD2* (the target of antipsychotic drugs), and many genes involved in glutamine neurotransmitter pathways and synaptic plasticity (e.g., *GRM3, GRIN2A, SRR, GRIA1*). More surprisingly, however, associations were also enriched among genes expressed in tissues with important immune functions (9).

In 2016, a study based on nearly 65,000 people investigated the association between schizophrenia and variation in the Major Histocompatibility Complex (MHC) locus—a region on chromosome 6 that is important for immune function. The study focused on the *C4* gene (complement component 4) that exists as two distinct genes: *C4A* and *C4B*, which encode particularly structurally diverse alleles.

The study found that the alleles which promoted greater expression of *C4A* in the brain were associated with a greater risk of schizophrenia. By using mice models, the study showed that C4 is involved in the elimination of synapses during brain maturation. In humans, "synaptic pruning" is most active during late adolescence, which coincides with the typical onset of symptoms of schizophrenia. It is therefore possible that the inheritance of specific *C4A* alleles could lead to "run away" synaptic pruning, increasing the risk of schizophrenia. Further research may even determine C4 as a potential therapeutic target (10).

Diagnosis

Currently, the diagnosis of schizophrenia is made via a psychiatric assessment using the criteria presented in the American Psychiatric Association Manual of Psychiatric Diseases, which is now in its 5th edition, and is known as DSM-V. To make a diagnosis, specific characteristic symptoms of schizophrenia must be present for at least 6 months, together with a disruption in social or occupational function, in the absence of another diagnosis that could account for the symptoms.

The use of chromosome microarray analysis has been suggested as a diagnostic test for schizophrenia. Microarray analysis can detect copy number variants (CNVs), which are large regions of the genome that have been deleted or duplicated. The prevalence of clinically significant CNVs in schizophrenia is around 5%. For autism and intellectual disability, the prevalence is around 10-20%, and CNV testing with microarray analysis is now a routine first-line diagnostic test for these conditions.

For an individual with schizophrenia, a positive test result for CNV may have implications for medical management, because of the association of CNVs with physical diseases and genetic counseling, and because offspring have a 50% risk of inheriting the CNV (3, 11).

Management

Treatment of manifestations: Antipsychotic medications are the mainstay of treatment and help reduce symptoms and improve behaviors in patients with schizophrenia. The type, dose, and route of administration of antipsychotic medications depends upon the clinical scenario. Adverse effects are common, and may require the dose or type of drug to be altered.

Antipsychotics may be given with counseling and other types of psychosocial interventions. For refractory (treatment-resistant) symptoms, an alternative antipsychotic or an additional antipsychotic may be required.

During pregnancy, antipsychotic drugs should be given only when the benefits derived from treatment exceed the possible risks to mother and fetus. Neonates exposed during the third trimester are at risk for extrapyramidal and/or withdrawal symptoms following delivery. There have been reports of agitation, hypertonia, hypotonia, tremor, somnolence, respiratory distress, and feeding disorder. While in some cases symptoms have been self-limited, in others neonates have required intensive care unit support and prolonged hospitalization.

Surveillance: Routine monitoring for the symptoms and signs of extrapyramidal adverse effects is needed in individuals taking antipsychotics. These adverse effects include akathisia (feeling of restlessness that may be accompanied with motor restlessness), dystonias (involuntary contraction of large muscle groups), and parkinsonian syndrome. Patients should also be monitored for signs of tardive dyskinesia (involuntary facial movements) and drug-specific adverse effects. For clozapine, because of the risk of neutropenia, the patient's white blood cell count and absolute neutrophil count must be regularly monitored. For thioridazine, the risk of prolonged QT interval may lead to Torsades de pointes.

Prevention of secondary complications: Patients should be regularly monitored for weight gain and metabolic problems such as hyperglycemia and hyperlipidemia, which are common side effects of antipsychotic medications.

Genetic Testing

Genetic testing is available for several of the susceptibility loci for schizophrenia, including clinical and research tests registered in the NIH Genetic Testing Registry (GTR). Additional tests may be found in the 'Related section' of the main GTR record for schizophrenia.

GTR also has registered tests for genetic conditions with schizophrenia as a clinical feature.

Genetic Counseling

Genetic counseling is recommended for people who have a family member with schizophrenia. Recurrence risk counseling is based on empiric familial risk for families with individuals with schizophrenia (12).

The lifetime risk of schizophrenia for the general population is estimated to be 0.2 to 0.7% (13).

The recurrence risk of schizophrenia in the siblings of a patient is 10%, and in the children of patients, the risk is approximately 10%. The risk for second-degree relatives is approximately 3-4% (14, 15).

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Version History

To view an earlier version (8 March 2012), please click here.

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Authoring and Peer Review

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Medical Genetics Summaries (MGS) for pharmacogenetics is a freely available collection of articles describing how genetics plays a role in an individual's response to drugs or predisposition to disease. The structured format of each summary makes accessing information such as genetic testing or therapeutic recommendations quick and easy to use. Medical Genetics Summaries use authoritative sources, are guideline-driven and actionable, and are subject to an extensive review process as described below.

Editorial Oversight

The editors of Medical Genetics Summaries advise on subject matter, guide the project through developments in the field, provide final approval prior to the publication of each summary, and assist in recruiting reviewers and in resolution of key issues which may be raised during the review process.

Selection of Topics

The selection of topics for new MGS chapters is guided by two factors. First, the author consults the FDA's "Table of Pharmacogenomic Biomarkers in Drug Labeling" to select new drugs that have not yet been covered in MGS. Second, to prioritize the order of new MGS chapters, the author checks the Genetic Testing Registry (GTR) for drug response records which contain information about genetic testing, but lack summary information about the drug response. After a new MGS chapter is released to the production site, an excerpt from the chapter is displayed in the relevant GTR drug response record. Additional reciprocal links between MGS, GTR, and MedGen are also added.

Structured Format

Each MGS drug response chapter follows a structured format. Each summary has one drug section, but may have one or more gene sections, depending on how many genetic factors have been identified.

1. Introductory paragraphs detail the drug and its uses, how the genetic variants influence an individual's response to the drug, and displays dosing recommendations from the FDA and practice guidelines from authoritative professional societies.
2. The drug section begins with a description of the drug, the drug class, its mechanism of action, the indications for its use, and common side effects. This is followed by a discussion on the factors which influence the drug response.
3. The gene section reviews important facts about the gene — what role it plays in the drug metabolism or action, and the nature of the gene variants and how they impact the drug response. The common or clinically significant variants are then discussed, including their prevalence across different ethnic populations.
4. "Genetic Testing" section is a key part of the summary. Here, the summary clearly describes the genetic testing options that are available, linking to genetic test providers listed in GTR.
5. "Therapeutic Recommendations based on Genotype" excerpts clinically actionable information, e.g. dosing recommendations from the FDA drug label; and therapeutic recommendations from pharmacogenetic societies such as CPIC, CPNDS and DPWG and medical societies, such as ASCO, ACMG, NCCN.
6. Nomenclature table provides information about the different terms used for genetic variants. Terms that are commonly used in the literature and historic terms are linked to the official HGVS terms and rs identifiers when available.
7. Expert reviewers are acknowledged, and information about previous versions of the summary is given.

Writing Process

Each summary is written by our in-house senior medical writer, who is an MD. All phases from authoring to production are tracked in an internal ticket management system. To create the first draft of a summary:

1. The author consults the most recent FDA drug label for the drug. To gain a better understanding for the context of the drug use and impact of genetic factors, the author will use NIH resources and other clinical sites, such as UpToDate.
2. The author then identifies key guidelines and primary papers, using PubMed Clinical Queries, PubMed, CPIC and PharmGKB.
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Each summary undergoes internal review involving one or two NCBI staff members. Once the author has finalized the first draft of a summary, it is submitted for internal review, along with key supporting guidelines (e.g., FDA drug label, key guidelines). The internal reviewers perform the first round of expert review, using track changes to ask questions and make suggestions and corrections. Because this process occurs in a ticket management system, all versions of the document and comments from the author and reviewers are documented.

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Created: June 29, 2017; Updated: September 26, 2019.

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- Celecoxib Therapy and *CYP2C9* Genotype
- Flurbiprofen Therapy and *CYP2C9* Genotype
- Diazepam Therapy and *CYP2C19* Genotype
- Imipramine Therapy and *CYP2D6* and *CYP2C19* Genotype
- Lesinurad Therapy and *CYP2C9* Genotype
- Piroxicam Therapy and *CYP2C9* Genotype
- Warfarin Therapy and *VKORC1* and *CYP* Genotype

van Rhenen, Mandy, MSc; Royal Dutch Pharmacists Association (KNMP), The Hague, Netherlands

- Amitriptyline Therapy and *CYP2D6* and *CYP2C19* Genotype
- Imipramine Therapy and *CYP2D6* and *CYP2C19* Genotype
- Metoprolol Therapy and *CYP2D6* Genotype
- Propafenone Therapy and *CYP2D6* Genotype
- Risperidone Therapy and *CYP2D6* Genotype

Visk, DeeAnn, PhD; San Diego, CA, USA

- Propafenone Therapy and *CYP2D6* Genotype

Wadelius, Mia, PhD; Uppsala University, Uppsala, Sweden

- Allopurinol Therapy and *HLA-B*58:01* Genotype
- Atomoxetine Therapy and *CYP2D6* Genotype
- Esomeprazole Therapy and *CYP2C19* Genotype
- Irinotecan Therapy and *UGT1A1* Genotype
- Omeprazole Therapy and *CYP2C19* Genotype

Wainberg, Mark, PhD, OC, OQ, FRSC; McGill University, Montreal, Quebec, Canada

- Maraviroc Therapy and *CCR5* Genotype

Wikistrand, John, MD, PhD; University of Gothenburg, Gothenbeg, Sweden

- Metoprolol Therapy and *CYP2D6* Genotype

Yin, Ji-Ye; Xiangya Hospital, Central South University, Changsha, Hunan Province, China

- Irinotecan Therapy and *UGT1A1* Genotype

Zujewski, Jo Anne, MD; National Cancer Institute, Bethesda, MD, USA

- Pertuzumab Therapy and *ERBB2* (*HER2*) Genotype