Pharmacogenomics of Cardiovascular Pharmacotherapies

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Learning Objectives

1. Apply Clinical Pharmacogenetics Implementation Consortium (CPIC) guidance in the clinical setting.
2. Associate clinically actionable genetic polymorphisms with response to cardiovascular pharmacotherapies.
3. For a given patient, estimate therapeutic response to antiplatelet therapy using CYP2C19 genotype information.
4. For a given patient, analyze the impact of the SLCO1B1 genotype on the risk of myopathy with statins.
5. For a given patient, estimate the dose of warfarin using VKORC1 and CYP2C9 genotype information.

Abbreviations in This Chapter

CK = Creatine kinase
CPIC = Clinical Pharmacogenetics Implementation Consortium
OAT = Organic anion transporting
PCI = Percutaneous coronary intervention
SLCO1B1 = Solute carrier organic anion transporter family, member 1B1
SNP = Single nucleotide polymorphism
VKOR = Vitamin K epoxide reductase

Self-Assessment Questions

Answers and explanations to these questions can be found at the end of the chapter.

1. A 50-year-old woman had a deep venous thrombosis, and warfarin was prescribed with use of unfractionated heparin before stable INR. Which genetic polymorphism would most likely affect this patient’s warfarin dose requirement?
   A. CYP2C9 polymorphism.
   B. SLCO1B1 polymorphism.
   C. CYP2C19 polymorphism.
   D. No known pharmacogenetic interactions.

2. Which best describes the response to simvastatin in a patient with the *5 variant allele in the SLCO1B1 gene?
   A. Standard effectiveness in LDL lowering and standard risk of myopathy.
   B. Increased effectiveness in LDL lowering and standard risk of myopathy.
   C. Increased effectiveness in LDL lowering and increased risk of myopathy.
   D. Decreased effectiveness in LDL lowering and increased risk of myopathy.

3. Which best represents the maximum recommended daily simvastatin dose in a person whose genotype is *5/*5 for the SLCO1B1 gene?
   A. Contraindicated.
   B. 20 mg.
   C. 40 mg.
   D. 80 mg.

4. A patient with a reported pharmacogenetic test result of CYP2C19 of *2/*3 is receiving clopidogrel. Which best depicts the patient’s risk of an adverse cardiovascular event (e.g., thrombosis) because of treatment failure?
   A. Increased.
   B. Moderate.
   C. Decreased.
   D. No change because of genetic result.

5. Which statement is most accurate about a CYP2C19 *17/*17 genotype and the current CPIC guidelines?
   A. Patients convert clopidogrel to an inactive metabolite to a greater extent than *1/*1; therefore, a decrease in clopidogrel dose is recommended.
   B. Patients convert clopidogrel to an active metabolite to a greater extent than *1/*1, but no change in clopidogrel dose is recommended.
   C. Patients convert clopidogrel to an active metabolite to a lesser extent than *1/*1; therefore, a decrease in clopidogrel dose is recommended.
   D. Patients convert clopidogrel to an active metabolite to a lesser extent than *1/*1; therefore, an alternative antiplatelet drug is recommended.
6. With respect to genetic testing, which is most accurate?

A. CPIC guidelines recommend when a genetic test should be obtained.

B. CPIC guidelines recommend dosing of all drugs that include pharmacogenetic information in the FDA-approved label.

C. CPIC guidelines do not consider whether a commercial genetic test is available for a particular genotype.

D. CPIC guidelines are not designed to provide information on whether a genetic test should be obtained.
I. INTRODUCTION

A. Definitions
   1. Precision medicine
      a. An emerging approach for disease treatment and prevention that considers individual variability in
         genes, environment, and lifestyle for each person
      b. Allows for the prediction of which treatment or prevention strategy for a specific disease might
         work best in a particular individual
      c. In contrast to one-size-fits-all approach
      d. Precision Medicine Initiative
         i. In 2015, President Barack Obama announced the Precision Medicine Initiative.
         ii. Focused in cancer care early on; ultimately has the goal of bringing precision medicine to all
             areas of health and health care on a large scale
         iii. The National Institutes of Health has launched a 1-million-person cohort, recently renamed
             “All of Us,” whereby individuals will provide genetic data, biological samples, and other
             health information, which will then be used by researchers to develop improved diagnosis and
             treatment strategies.
   2. Personalized medicine
      a. The term personalized medicine is sometimes used synonymously with precision medicine to iden-
         tify a form of medicine that uses information about a person’s genes, proteins, and environment to
         prevent, diagnose, and treat disease.
      b. The word “personalized” should not be misinterpreted to imply that treatments and preventions are
         being developed uniquely for an individual.
   3. Pharmacogenomics and pharmacogenetics
      a. Pharmacogenetics studies how variation in one single gene influences response to a single drug.
         Pharmacogenetics has largely been used in relation to the study of inherited genetic differences in
         drug metabolic pathways.
      b. Pharmacogenomics is a broader term that studies how all the genes in the genome can influence
         drug response.
      c. A combination of pharmacology and genomics can be used to develop effective, safe medications
         and dosage regimens that are tailored to variations in an individual’s genetic makeup.
      d. Although often used interchangeably with pharmacogenomics, pharmacogenetics generally
         focuses on a single drug-gene interaction.
   4. Epigenetics and epigenomics
      a. Epigenetics examines the processes regulating the activity (expression) of certain genes, whereas
         epigenomics considers the epigenetic changes across many genes in a cell or entire organism.
      b. Epigenetic changes can help determine whether genes are turned on or off and can influence
         the production of proteins in certain cells, ensuring that only necessary proteins are produced
   5. Single nucleotide polymorphisms (SNPs)
      a. A SNP is a variation in a single nucleotide (adenine [A], thymine [T], cytosine [C], or guanine [G])
         that occurs at a specific position in the genome.
      b. An example of a sequence change is the following: AAGCCTA changes to AAGCTTA.
c. SNPs may fall within coding sequences of a gene.
   i. Synonymous SNPs – Do not affect the protein sequence
   ii. Nonsynonymous SNPs – Do affect the protein sequence
      (a) Missense: A point mutation in which a single nucleotide change results in a codon that
codes for a different amino acid
      (b) Nonsense: A mutation in which a sense codon that corresponds to one of the 20 amino
acids specified by the genetic code is changed to a chain-terminating codon

d. SNPs not in protein-coding regions – Can affect gene splicing, messenger RNA degradation, and
   RNA coding and transcription factor binding

e. SNPs can also occur in intergenic regions, which is the sequence of DNA located between genes.
   Intergenic DNA regions are a subset of noncoding DNA.

6. Candidate gene association study
   a. Focuses on associations between genetic variation within prespecified genes of interest and pheno-
      types or disease states
   b. Candidate gene association studies can include SNPs of interest, or they may also include inser-
      tion-deletion polymorphisms or copy number variation polymorphisms.
   c. Findings should be replicated in several populations for confirmation of effect.

7. Genome-wide association study
   a. A search across the genome for SNPs (genetic variation) that occur more commonly in a population
      with a specific disease or drug response (often called cases) than in people without the disease or
      who have a typical drug response (often called controls)
   b. Allows for investigation of millions of polymorphisms
   c. Findings should be replicated in several populations for confirmation of effect.

8. Allele
   a. An allele is a variant form of a given gene.
   b. Different alleles can result in different phenotypes.
   c. Humans have two sets of chromosomes.
      i. If both alleles at a gene are the same, they are called homozygous with respect to that gene
         location.
      ii. If alleles are different, they are called heterozygous with respect to that gene location.

9. Genotype: The genetic constitution of an organism is called its genotype.

10. Phenotype
    a. The phenotype is a composite of an individual’s observable characteristics or traits.
    b. Results from the expression of an individual’s genetic code, as well as the environment

B. Pharmacogenetic Testing – Can be done preemptively or reactively in response to a drug order
1. Preemptive genotyping testing
   a. Usually accomplished using a multigene, chip-based approach such that several genes and genetic
      polymorphisms are tested at the same time, and the results are then available for whenever they are
      needed in the future
   b. Advantages include reduced cost and availability of results at the time of future drug prescribing.
   c. Disadvantages include lack of reimbursement for preemptive testing and the testing of genes that
      may never be needed to guide therapy; however, there is no additional cost and thus ultimately little
      disadvantage.
   d. Many institutions, primarily in the academic setting, have implemented a preemptive pharmacoge-
      nomics program, including St. Jude, Vanderbilt, the University of Indiana, and the University of
      Florida, for example.
2. Reactive genotyping testing
   a. Usually tests a single gene or a few genes and is ordered at the time of need
   b. Advantages include that the test may be reimbursed by third-party payers, testing only the genotype needed at the time.
   c. Disadvantages include increased cost and turnaround time because results may not be available at the time they are needed to make a treatment or dosing decision.

3. Interpretation of results
   a. Understanding how to interpret the results of genetic tests is critical for successful clinical implementation of pharmacogenetics.
   b. In institutions that have implemented pharmacogenetic testing, where the results are run locally and returned by the electronic health record, clinical decision support is usually built into the electronic health record system, triggered by a specific drug-gene pair; this helps guide clinicians in interpreting the genetic test result and recommends drug/dose according to the results.
   c. When genetic tests are sent to private laboratories for analysis, test results are less predictable regarding how they are provided and usually do not consistently provide clinical decision support to help guide clinicians in prescribing a drug/dose according to the test result.

C. Drug Labeling and Pharmacogenetics
   1. The U.S. Food and Drug Administration (FDA) has placed increased focus on the impact of pharmacogenetics on drug response, adverse effects, and pharmacokinetics over the past decade.
   2. 190 unique FDA-approved drugs currently include pharmacogenomic biomarker information in the drug labeling (https://www.fda.gov/drugs/scienceresearch/researchareas/pharmacogenetics/ucm083378.htm).
      a. Pharmacogenetic biomarkers included in FDA labeling include:
         i. Germline or somatic gene variants (polymorphisms, mutations)
         ii. Functional deficiencies with a genetic etiology
         iii. Gene expression differences
         iv. Chromosomal abnormalities
         v. Selected protein biomarkers that are used to select treatments
      b. Although the FDA-approved label of 190 drugs includes information regarding pharmacogenetic biomarkers, in most of these cases, evidence is sufficient to implement specific pharmacogenetic testing, and guidance is available to make informed decisions about how to interpret the genetic test results for 33 drugs as of June 15, 2017.

D. Clinical Pharmacogenomics Implementation Consortium (CPIC) (https://cpicpgx.org/): CPIC was established to overcome barriers to clinical implementation of pharmacogenetics because of lack of available peer-reviewed, updated, and detailed gene/drug clinical practice guidelines.
   1. CPIC guidelines are centered either on genes or on drug-gene combinations.
   2. Priority for establishing guidelines is based on the availability of commercially available tests offered in Clinical Laboratory Improvement Amendments–approved clinical settings.
   3. CPIC guidelines:
      a. Designed to help clinicians understand how available genetic test results should be used and applied at the individual patient level
      b. Not designed to provide information on whether a genetic test should be ordered or the timing of a specific genetic test
      c. 33 different CPIC guidelines currently exist, each based on an individual drug (https://cpicpgx.org/guidelines/).
   4. Most consider the availability of a CPIC guideline, which is developed on the basis of all of the currently available evidence supporting a particular drug-gene(s) combination, as the threshold for clinical implementation readiness.
II. THREE CPIC GUIDELINES FOR PHARMACOTHERAPIES ARE PRESCRIBED IN CARDIOVASCULAR PATIENTS: CLOPIDOGREL, SIMVASTATIN, AND WARFARIN

A. Clopidogrel and cytochrome P450 2C19 (CYP2C19)
   1. Clopidogrel is a thienopyridine prodrug that requires enzymatic bioactivation, primarily by the hepatic CYP2C19 enzyme, among others (figure 1).

   ![Metabolism of clopidogrel](image)

   **Figure 1.** Metabolism of clopidogrel.


   2. After bioactivation, the active metabolite binds irreversibly to inhibit the P2Y₁₂ adenosine diphosphate receptor on platelets, which prevents aggregation.

   3. Clopidogrel prevents thromboembolism-related cardiovascular events.
      a. In patients with acute coronary syndromes
      b. In patients after stent placement during percutaneous coronary interventions (PCIs)
      c. Wide patient variability in response – Around 25% are nonresponsive, meaning residual platelet aggregation exists post-clopidogrel dosing.
      d. Although clopidogrel is also used in secondary stroke prevention and peripheral artery disease, most pharmacogenetic-related studies involving clopidogrel have been done in the context of patients with acute coronary syndromes treated with PCI.
4. Variability in clopidogrel response is largely because of the highly polymorphic nature of \(CYP2C19\) and is summarized in Table 1.

**Table 1. \(CYP2C19\) Phenotype, Genotype, and Effect on Clopidogrel Response, and CPIC Recommendations for Antiplatelet Therapy**

<table>
<thead>
<tr>
<th>(CYP2C19) Phenotype</th>
<th>Overall Prevalence (approximate)</th>
<th>Genotype</th>
<th>Example</th>
<th>Clinical Effect</th>
<th>CPIC Clopidogrel Dose Recommendation (strength of evidence)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrarapid metabolizer</td>
<td>2%–5%</td>
<td>Two increased-function alleles</td>
<td>*17/*17</td>
<td>Greatest antiplatelet effect, lowest on-treatment platelet reactivity</td>
<td>Label-recommended dosage and administration (strong)</td>
</tr>
<tr>
<td>Rapid metabolizer</td>
<td>2%–30%</td>
<td>One normal-function allele and one increased-function allele</td>
<td>*1/*17</td>
<td>Some increase in antiplatelet effect</td>
<td>Label-recommended dosage and administration (strong)</td>
</tr>
<tr>
<td>Normal metabolizer (wild type)</td>
<td>30%–50%</td>
<td>Two normal-function alleles</td>
<td>*1/*1</td>
<td>Expected antiplatelet effect</td>
<td>Label-recommended dosage and administration (strong)</td>
</tr>
<tr>
<td>Intermediate metabolizer</td>
<td>18%–45%</td>
<td>One normal-function allele and one no-function allele or one no-function allele and one increased-function allele</td>
<td>*1/*2, *1/*3, *2/*17</td>
<td>Some reduction in antiplatelet effect</td>
<td>Alternative antiplatelet therapy if no contraindication (prasugrel or ticagrelor) (moderate)</td>
</tr>
<tr>
<td>Poor metabolizer</td>
<td>1%–15%</td>
<td>Two no-function alleles</td>
<td>*2/*2, *2/*3, *3/*3</td>
<td>Lowest antiplatelet effect (highest on-treatment platelet reactivity), reduced active metabolite</td>
<td>Alternative antiplatelet therapy if no contraindication (prasugrel or ticagrelor) (strong)</td>
</tr>
</tbody>
</table>

*Prevalence varies widely by race. Central, South, and East Asians have the highest prevalence of the *2 allele.

a. FDA-cleared \(CYP2C19\) laboratory tests are currently available.
b. For \(CYP2C19\) genotyping results to be applied clinically in the United States, the test must be performed in laboratories operating under College of American Pathologists/Clinical Laboratory Improvement Amendments regulations.
c. More than 30 genetic variants of \(CYP2C19\) are known, though far fewer have been associated with functional consequences.
d. \(CYP2C19\) nomenclature for clinically relevant polymorphisms:
   i. Normal-function allele: \(CYP2C19*1\), associated with normal metabolism
   ii. No-function alleles: \(CYP2C19*2\) rs4244285 (most common) and \(CYP2C19*3\) rs4986893
   iii. Increased-function allele: \(CYP2C19*17\) rs12248560
   iv. Individuals can carry one or two variant (either loss of function or gain of function) alleles.
   v. The number and type of \(CYP2C19\) mutant alleles in an individual determine the individual’s \(CYP2C19\) metabolizer status (phenotype).
5. The CPIC guideline for clopidogrel ([https://cpicpgx.org/guidelines/guideline-for-clopidogrel-and-cyp2c19/](https://cpicpgx.org/guidelines/guideline-for-clopidogrel-and-cyp2c19/)) includes recommendations for P2Y\(_{12}\) inhibitor therapy according to the \(CYP2C19\) genotype in patients with acute coronary syndromes who have undergone a PCI (Table 1).
a. Label-recommended doses should be effective in patients with ultrarapid, rapid, or normal metabolizer status.
b. Patients with intermediate or poor metabolizer status should be treated with alternative antiplatelet therapy, barring any contraindications (prasugrel or ticagrelor).
c. The FDA-approved clopidogrel label warns of reduced clopidogrel effectiveness in patients with poor metabolizer status and recommends consideration of alternative antiplatelet therapy in these patients; however, it is silent regarding risk and recommendations in patients with intermediate metabolizer status.
d. Some studies suggest that *I7 allele carriers have enhanced platelet inhibition and clopidogrel response, and perhaps an increased risk of bleeding complications; however, results are discordant, and further studies are required to confirm this finding.

6. Adverse cardiovascular outcomes and CYP2C19 genotype-guided clopidogrel dosing
a. Currently, clinical guidelines (American College of Cardiology, American Heart Association) do not include definitive recommendations for genotype-guided clopidogrel use, primarily because of the lack of data from large randomized controlled trials showing improved clinical outcomes with genotype-guided antiplatelet drug selection.
b. Post hoc analyses from randomized clinical trials and registries show a higher risk of adverse cardiovascular events among patients undergoing a PCI treated with clopidogrel who have a CYP2C19 no-function allele than among similarly treated patients without a no-function allele.
c. Outcomes data outside the PCI population are inconsistent.
d. Several emerging studies will soon supply additional information on the association between the CYP2C19 genotype and cardiovascular outcomes.

7. Proton pump inhibitors (PPIs) and clopidogrel
a. PPIs are often prescribed concurrently with clopidogrel because of the increased risk of bleeding.
b. Some PPIs inhibit CYP2C19 activity.
i. Omeprazole and esomeprazole are the strongest inhibitors of CYP2C19.
ii. Pantoprazole is the weakest inhibitor of CYP2C19.
c. Evidence suggests that PPIs interfere with the activation of clopidogrel and diminish its antiplatelet effect.
d. The FDA-approved label recommends avoiding concurrent use of omeprazole and esomeprazole with clopidogrel.

Patient Cases
1. M.J. is a 66-year-old man who presented for a left heart catheterization secondary to unstable angina. He presents after a percutaneous coronary intervention of his left anterior descending artery with placement of a drug-eluting stent in the setting of an ST-segment elevation myocardial infarction (STEMI). Of note, the patient has a documented history of transient ischemic attack. Before admission, the patient’s antiplatelet therapy consisted of aspirin and clopidogrel. The patient has no known drug allergies. His medical history includes a STEMI, hypertension, high cholesterol, and coronary artery disease. His medications include aspirin 81 mg daily, atorvastatin 80 mg daily, clopidogrel 75 mg daily, isosorbide mononitrate 30 mg daily, lisinopril 5 mg daily, metformin 1000 mg twice daily, metoprolol succinate 100 mg daily, and nitroglycerine 0.4 mg sublingually as needed for chest pain. The patient underwent genotyping for CYP2C19, which revealed the *1/*2 genotype. Given this information, which is the best recommendation regarding his antiplatelet therapy?
   A. Continue clopidogrel plus aspirin.
   B. Change to prasugrel plus aspirin.
   C. Change to ticagrelor plus aspirin.
   D. Give aspirin alone.
**Patient Cases (Cont’d)**

2. M.B. is a 57-year-old man who presents to the emergency department with acute chest pain. He was transported to the catheterization laboratory and now presents after percutaneous coronary intervention, during which he received two drug-eluting stents. His medical history includes a history of coronary artery disease and a percutaneous coronary intervention in 2011. His current medications include aspirin 81 mg daily, atorvastatin 80 mg daily, carvedilol 6.25 mg twice daily, clopidogrel 75 mg daily, docusate calcium 4 tablets at bedtime, furosemide 20 g twice daily as needed for swelling/edema, isosorbide mononitrate 30 mg daily, and lisinopril 40 mg daily. The patient has no known drug allergies. The patient underwent genotyping for CYP2C19, which revealed the *1/*17 genotype. Which is the best recommendation for antiplatelet therapy (in addition to aspirin) in this patient?

A. Reduced-dose clopidogrel.
B. Prasugrel.
C. Ticagrelor.
D. Standard-dose clopidogrel.

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**B. Simvastatin myopathy and solute carrier organic anion transporter family, member 1B1 (SLCO1B1)**

1. Statins, including simvastatin, are highly effective for reducing cardiovascular risk in both primary and secondary heart disease.

2. Myopathy is an adverse effect of statins, especially simvastatin.
   a. Defined as muscle pain or weakness with elevated creatine kinase (CK) concentrations. Symptoms can range from mild myalgia with no CK elevation to life-threatening rhabdomyolysis with substantially elevated CK concentrations and muscle injury.
   b. Although statin-induced myopathy is relatively rare in randomized clinical trials (3%-5%), it is more common in clinical practice (10%-15%).
   c. Exact mechanism is unknown, but risk factors include
      i. Higher statin doses
      ii. Use with drugs that increase statin bioavailability. Cyclosporine, for example, is a strong inhibitor of the organic anion transporting (OAT) polypeptide 1B1 transporter and CYP3A4 enzyme and increases the area under the curve for simvastatin acid by 3- to 8-fold.
      iii. Genetic polymorphisms that affect statin pharmacokinetics

3. *SLCO1B1* encodes for the OAT polypeptide 1B1 transporter. Genetic polymorphisms of *SLCO1B1* rs4149056 contained within *SLCO1B1*5, *15, and *17 have been associated with statin myopathies.
   a. No *SLCO1B1* test is currently FDA approved; however, College of American Pathologists/Clinical Laboratory Improvement Amendments–certified laboratories can run the *SLCO1B1* assay and return results.
   b. Relationship between rs4149056 and simvastatin-related muscle toxicity is clearly established.
   c. Relationship between rs4149056 and other statins is less clear.

4. The CPIC guideline regarding simvastatin and *SLCO1B1* is available at https://cpicpgx.org/guidelines/guideline-for-simvastatin-and-slco1b1/ and includes dosing guidelines according to genotype.

5. *SLCO1B1* encodes for OAT polypeptide C and plays a role in statin pharmacokinetics (Figure 2).
Figure 2. Pharmacokinetics of statins.

a. OAT polypeptide 1B1 transports all statins into hepatocytes.
   i. Fluvastatin is more lipophilic and is the statin least affected by the \textit{SLCO1B1} polymorphism.
   ii. Although the \textit{SLCO1B1} polymorphism affects several statins, the strength of evidence is highest for simvastatin; thus, the CPIC guidelines on \textit{SLCO1B1} were written for simvastatin-induced myopathy.

b. \textit{SLCO1B1}*5 and *17 reduce OAT polypeptide 1B1 transport activity and are summarized in Table 2.
   i. Carriers of the \textit{SLCO1B1}*5 allele have increased statin concentrations.
   ii. Increased statin concentrations have been associated with increased risk of statin myopathy.
   iii. Around 16% of whites, 1%–2% of African Americans, and 10%–16% of Asians carry an \textit{SLCO1B1}*5 allele.

c. Relative risk of myopathy with simvastatin 40 mg/day is about 2.5/variant (loss of function) allele. For patients with one or two loss-of-function alleles, the CPIC guideline recommends starting at a lower dose of simvastatin (20 mg/day) or starting an alternative statin (Table 2).
Table 2. *SLCO1B1* Phenotype, Genotype, and CPIC Recommendations for Simvastatin Dosing

<table>
<thead>
<tr>
<th>SLCO1B1 Phenotype</th>
<th>Overall Prevalence (approximate)</th>
<th>Genotype</th>
<th>Example</th>
<th>Clinical Effect</th>
<th>CPIC Statin Dose Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal function (wild type)</td>
<td>55%–88%</td>
<td>Two normal-function alleles</td>
<td>*1a/*1a, *1a/*1b, *1b/*1b</td>
<td>Expected statin effect and normal myopathy risk</td>
<td>Label- and specific disease recommended simvastatin dosage and administration (strong)</td>
</tr>
<tr>
<td>Decreased (intermediate) function</td>
<td>11%–36%</td>
<td>One normal-function allele and one no-function allele</td>
<td>*1a/*5, *1a/*15, *1a/*17, *1b/*5, *1b/*15, *1b/*17</td>
<td>Increased risk of myopathy</td>
<td>Prescribe a lower simvastatin dose (20 mg) or an alternative statin (e.g., pravastatin or rosuvastatin); consider routine CK monitoring (strong)</td>
</tr>
<tr>
<td>Low function</td>
<td>0%–6%</td>
<td>Two no-function alleles</td>
<td>*5/*5, *5/*15, *5/*17, *15/*15, *15/*17, *17/*17</td>
<td>High myopathy risk</td>
<td>Prescribe a lower simvastatin dose (20 mg) or an alternative statin (e.g., pravastatin or rosuvastatin); consider routine CK monitoring (strong)</td>
</tr>
</tbody>
</table>

*Prevalence varies widely by race. Central, South, and East Asians have the highest prevalence of the *2 allele.

Patient Case
3. A 62-year-old white man has a medical history of coronary artery disease, diabetes, peripheral neuropathy, and hyperlipidemia. While presenting to the pharmacy to refill his medications, he states that he has achy muscle pain. Medications include simvastatin 40 mg at bedtime, aspirin 81 mg daily, nortriptyline 50 mg at bedtime, metformin 1000 mg twice daily, and glipizide 10 mg daily. The patient underwent genotyping for *SLCO1B1*, which revealed a *5*/*5 genotype. Which is the best recommendation regarding his lipid therapy?
A. Continue simvastatin 40 mg daily.
B. Decrease to simvastatin 20 mg.
C. Use an alternative statin option.
D. Use a PCSK9 inhibitor instead of a statin.

C. Warfarin and *CYP2C9*, vitamin K epoxide reductase C1 (*VKORC1*), and *CYP4F2*
1. Although newer direct oral anticoagulants are available and effective that usually require less monitoring, because of issues of cost and tolerance and limited FDA-approved indications, warfarin remains a commonly prescribed medication.
2. Warfarin has a narrow therapeutic index with large interpatient variability in the dose required to achieve target anticoagulation.
3. Complications of warfarin therapy are among the most commonly reported adverse events to the FDA and a common cause for emergency department visits.
4. Substantial evidence from candidate gene and genome-wide association studies consistently shows that genetic variability affects warfarin dose response.
5. Although clinical trials evaluating the usefulness of genotype-guided warfarin dosing have resulted in inconsistent evidence, reducing enthusiasm for routinely genotyping individuals undergoing warfarin therapy, the warfarin FDA label includes pharmacogenetic guidance.
a. The European Pharmacogenetics of Anticoagulant Therapy (EU-PACT) study showed that, in a European population, use of a pharmacogenetic dosing algorithm reduced the time to stable warfarin dose, improved the percent time spent in therapeutic range, and decreased the number of episodes in which the international normalized ratio (INR) was greater than 4, compared with standard dosing.

b. The Clarification of Optimal Anticoagulation Through Genetics (COAG) study, which was conducted in an ethnically diverse population, showed that use of a pharmacogenetic dosing algorithm caused no difference in time to stable dose, percent time in therapeutic range, reduction in number of episodes of INR greater than 4 or less than 2, or bleeding risk compared with a clinical dosing algorithm.

c. Several issues may have contributed to the negative findings from COAG and serve as the basis for the current CPIC guidelines, with differing recommendations by ancestry.
   i. A large percentage of patients were African American, yet CYP2C variants (CYP2C9*5, *6, *8, *11 rs12777823) occurring most commonly in African Americans were not genotyped.
   ii. Failure to account for CYP2C variants leads to over-estimation of warfarin dose requirements in African Americans.
   iii. Consistent with this, pharmacogenetic versus clinical dosing in African Americans in the COAG trial led to a significantly increased risk of over-anticoagulation.

d. The Genetics-Informatics Trial (GIFT) was a randomized controlled trial examining the effectiveness and safety of genotype-guided dosing versus clinical algorithm for warfarin dosing in orthopedic patients.
   i. GIFT composite outcome included symptomatic and asymptomatic venous thromboembolism, major hemorrhage, INR of 4 or greater, and death and was the first warfarin pharmacogenetics study powered for clinical outcomes.
   ii. GIFT included genotyping for CYP2C9*2 and *3, CYP4F2*3, and VKORC1-1639 but did not include the African-specific CYP2C9 alleles or rs12777823.
   iii. Results of GIFT, presented in early 2017, showed a 27% reduction in the composite outcome with genotype-guided versus clinical algorithm dosing, documenting the clinical benefits of a genotype-guided approach to warfarin dosing. Press release is available at https://www.sciencedaily.com/releases/2017/03/170320091104.htm.

e. Genes most associated with warfarin response include CYP2C9, VKORC1, CYP4F2, and a SNP in the CYP2C.

f. There are FDA-cleared CYP2C9 and VKORC1 laboratory tests; however, there is no currently FDA-cleared test for CYP4F2 or rs12777823.

g. The CPIC guideline regarding warfarin and genetic variability is available at https://cpicpgx.org/guidelines/guideline-for-warfarin-and-cyp2c9-and-vkorc1/ and includes warfarin dosing guidelines according to genotype.

h. CYP2C9 and warfarin
   i. CYP2C9 is a hepatic drug-metabolizing enzyme and is the enzyme primarily responsible for S-warfarin, which is 3–5 times more potent than R-warfarin (Figure 3).
   ii. CYP2C9*1 is considered a “normal metabolizer” allele.
      (a) Among individuals of European ancestry, CYP2C9*2 (rs1799853) and *3 (rs1057910) are the most common decreased-function alleles and impair warfarin metabolism by 30%–40% and 80%–90%, respectively, resulting in a greater risk of bleeding, lower dose requirement, and longer time to reach stable INR.
      (b) CYP2C9*5, *6, *8, and *11 are also associated with decreased enzyme function and are found with the highest frequency among those of African ancestry.
Figure 3. Warfarin pharmacokinetic and pharmacodynamic pathway diagram.

1. **VKORC1** and warfarin
   - **VKORC1** encodes the VKOR protein, which is the target enzyme of warfarin.
   - Vitamin K epoxide is converted to vitamin K by **VKORC1**.
   - A common **VKORC1** variant, rs9923231 (1639G>A), is associated with a significantly increased response to warfarin (warfarin sensitivity).
   - rs9923231 (1639G>A) frequency varies by race, occurring in about 40% of whites and only 10%–13% of African Americans and Africans.
   - Patients with one A allele (-1639GA) or two A alleles (-1639AA) at rs9923231 require progressively lower warfarin doses than do patients with one normal-function allele (1639GG).

2. **CYP4F2** and warfarin
   - **CYP4F2** catalyzes the metabolism of vitamin K to hydroxyvitamin K\(_1\), thus removing vitamin K from the vitamin K cycle.
   - **CYP4F2** is a counterpart to **VKORC1** by limiting the accumulation of vitamin K.
   - A variant in **CYP4F2**—CYP4F2*3 rs2108622—decreases enzyme activity and is associated with the warfarin dose in individuals of European and Asian ancestry but not African ancestry.
   - Dosing algorithms that include **CYP4F2**, together with clinical factors (age, sex, race, weight, height, smoking status, warfarin indication, target INR, interacting drugs) that affect warfarin response, as well as **CYP2C9** and **VKORC1** improve the accuracy of warfarin dose prediction.

3. **CYP2C** cluster
   - rs12777823 is a SNP in the **CYP2C** cluster that is associated with warfarin dose and reduced warfarin clearance, independent of **CYP2C9**<sup>2</sup> and <sup>3</sup> in African Americans.
   - Although rs12777823 is common in several populations, only those of African ancestry who carry one or two variant alleles at this SNP require a warfarin dose reduction of around 7–9 mg/week.
   - Dosing algorithms that include the **CYP2C** cluster improve the accuracy of warfarin dose prediction in African Americans.

6. Warfarin pharmacogenetic dosing algorithms: The warfarin CPIC guideline recommends that pharmacogenetic warfarin dosing be accomplished through one of the available pharmacogenetic dosing algorithms.
   - The Gage algorithm (primary) and the International Warfarin Pharmacogenetics Consortium algorithm (IWPC) (secondary) are available at www.warfarindosing.org/Source/Home.aspx.
   - Figure 4 includes a summary of the warfarin dosing algorithms in adults, according to patient ancestry and including strength of the evidence for specific gene-based recommendations.
c. Use of warfarin dosing algorithms computes the anticipated stable daily warfarin dose, and the clinician then prescribes a regimen that approximates this anticipated dose. Once additional INRs are obtained, the algorithms can be reapplied for refinement of warfarin daily/weekly dose.

Figure 4. Dosing recommendations for warfarin dosing are based on genotype for adult patients.

**Figure 2 of the 2017 guideline manuscript and reprinted with permission from CPIC, PharmGKB, and Clinical Pharmacology & Therapeutics.**

**Figure 4.** Dosing recommendations for warfarin dosing are based on genotype for adult patients.

- “Dose clinically” means to dose without genetic information, which may include use of a clinical dosing algorithm or a standard dose approach.
- Data are strongest for European and East Asian ancestry populations and are consistent in other populations.
- 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 whether the same associations exist for those from other parts of Africa.
- Most algorithms are developed for the target INR 2–3.
- 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.
- See the EU-PACT trial for a pharmacogenetics-based warfarin initiation (loading) dose algorithm, with the caveat that the loading dose pharmacogenetics algorithm has not been specifically tested or validated in populations of African ancestry.
- Larger dose reductions may be needed in variant homozygotes (i.e., 20%–40%).
- African American refers to individuals mainly originating from West Africa.
Patient Case
4. A 71-year-old white woman (height 68 inches, weight 54.4 kg [120 lb]) was recently given a diagnosis of atrial fibrillation (INR target 2.5), and you are consulted to manage her anticoagulation with warfarin (she cannot afford to take one of the newer oral anticoagulants). She has not yet received any warfarin doses. Her medical history includes diabetes, hyperlipidemia, and hypertension. She smokes 1 pack/day and has a baseline INR of 1.1. Her medications include rosuvastatin, lisinopril, metformin, and carvedilol. As part of a service provided by the anticoagulation clinic, she has undergone CYP2C9 and VKORC1 genotyping, and the results revealed CYP2C9 *2*3 and VKORC1 G>A. Given her pharmacogenomics profile, which would be best regarding her warfarin therapy?

A. Her dose requirement is likely to be 5 mg daily.
B. Her dose requirement is likely to be less than 5 mg daily.
C. Her dose requirement is likely to be greater than 5 mg daily.
D. She should not receive warfarin because she is unlikely to achieve a stable INR.
REFERENCES


ANSWERS AND EXPLANATIONS TO PATIENT CASES

1. **Answer: C**
   This patient needs dual antiplatelet therapy because of his recent drug-eluting stent placement. His genotype makes him an intermediate metabolizer (IM) phenotype because of one loss-of-function allele (*2), which makes him less likely to be able to adequately metabolize clopidogrel and would not have adequate antiplatelet effect. In patients with an IM phenotype, the CPIC clopidogrel-CYP2C19 guideline recommends treatment with an alternative antiplatelet agent, instead of clopidogrel (Answer A is incorrect). With respect to the choice between prasugrel and ticagrelor, because this patient has a history of transient ischemic attack, prasugrel is contraindicated (Answer B is incorrect), and ticagrelor would be best for this patient (Answer C is correct). Aspirin alone in this patient is not the best response because the patient needs dual antiplatelet therapy, given his stent placement, acute coronary syndrome, and STEMI history (Answer D is incorrect).

2. **Answer: D**
   This patient presents after two drug-eluting stents; thus, he needs antiplatelet therapy. His CYP2C19 genotype indicates he has one normal-function allele and one increased-function allele with a rapid metabolizer (RM) phenotype. In patients with an RM phenotype, the CPIC clopidogrel-CYP2C19 guideline recommends treatment with a label-recommended clopidogrel dose (Answer D is correct). The clopidogrel dose should not be reduced because of a single increased-function allele (Answer A is incorrect). In addition, alternative antiplatelet agents need not be used in patients with the RM phenotype (Answers B and C are incorrect). Data regarding increased risk of bleeding in patients with one increased-function allele (or two) are inconclusive; thus, the current evidence does not warrant a decreased clopidogrel dose or an alternative antiplatelet therapy in the setting of increased function allele(s).

3. **Answer: C**
   This patient’s SLCO1B1 genotype indicates he has a low-function phenotype because he has two no-function alleles, and he is at high risk of myopathy, which he is currently experiencing. In patients with a low-function phenotype, the SLCO1B1-simvastatin CPIC guideline recommends either a decreased simvastatin dose (Answer B) or use of an alternative statin (Answer C). In this patient who already has myopathy, and given that all other statins are available in a generic version, it would be best clinically to try an alternative statin to maintain LDL lowering as well as reduce myopathy symptoms (Answer C). Answer A is incorrect because simvastatin 40 mg should not be continued in a patient with a low-function phenotype. Answer D is incorrect because statin use need not be discontinued altogether. Use of a PCSK9 inhibitor in this patient would substantially increase overall medication costs, which is unnecessary.

4. **Answer: B**
   This patient has two CYP2C9 decreased-function alleles, which is associated with impaired warfarin metabolism, resulting in a lower dose requirement and a greater risk of bleeding (Answer B is correct). In addition, her VKORC1 genotype indicates that she will have increased response to warfarin (increased warfarin sensitivity). Answer A is incorrect because 5 mg daily is the usual warfarin dose requirement, and the usual warfarin dose in this patient would likely result in excess bleeding risk. Answer C is also incorrect because this patient is very unlikely to require a warfarin dose greater than 5 mg daily. Answer D is incorrect because it is possible to use tools on the basis of clinical factors and genetic information to estimate a starting warfarin dose and determine dosing adjustments that can result in a stable INR. This patient has already indicated that she cannot afford the more expensive anticoagulant agents, and not being treated with any anticoagulation agent would place this patient at increased of clot formation and stroke.
ANSWERS AND EXPLANATIONS TO SELF-ASSESSMENT QUESTIONS

1. **Answer: A**
   CYP2C9 is primarily responsible for the metabolism of the enantiomer S-warfarin, which is the more potent form of warfarin (Answer A is correct). The *2 and *3 polymorphisms cause decreased warfarin metabolism, resulting in decreased warfarin dose requirements, increased INR levels, and a higher risk of bleeding during warfarin therapy. The SLCO1B1 genotype (Answer B) is associated with simvastatin efficacy and toxicity, and CYP2C19 (Answer C) is associated with clopidogrel metabolism and efficacy. Answer D is incorrect because there are several known pharmacogenetic interactions with warfarin.

2. **Answer: D**
   The SLCO1B1 genotype encodes for a transporter for simvastatin from the gut to the liver. Carriers of the *5 allele have reduced liver uptake of simvastatin from the gut and thus are at a higher risk of developing myopathy because of higher concentrations in the blood (Answer D is correct). This person has a variant and thus is not at standard risk of toxicity (Answers A and B are incorrect). In addition, less drug will get into the liver; thus, the patient might experience reduced, not increased, effectiveness (Answer C is incorrect).

3. **Answer: B**
   The CPIC guideline for simvastatin and SLCO1B1, on the basis of the available data, recommends a maximum daily dose of 20 mg of simvastatin in a person with a *5*5 genotype (Answer B is correct). The current guidelines do not specify that simvastatin would be contraindicated (Answer A). Both 40 mg (Answer C) and 80 mg (Answer D) are too high a dose for individuals with this variant (80 mg is too high for anyone) unless they have already been taking this dose before knowing their genetic information and are tolerating it well; if so, the current dose can be continued.

4. **Answer: A**
   The CYP2C19 *2/*3 genotype is considered a poor metabolizer for clopidogrel; thus, individuals with this genotype will have decreased antiplatelet effect and high platelet activity, making them at an increased risk of an adverse thrombotic event (Answer A is correct). Answers B–D are incorrect for this genotype.

5. **Answer: B**
   The CYP2C19 *17/*17 genotype is considered an ultra-metabolizer status and is associated the greatest antiplatelet effect and the lowest on-treatment platelet reactivity. However, current data support use of the standard clopidogrel dose (75 mg daily) for this genotype (Answer B is correct). Answer A is incorrect because the *17/*17 genotype is not associated with conversion of clopidogrel to an inactive metabolite or with decreased dose. Answer C is incorrect because although the *17/*17 genotype is associated with increased conversion to active metabolite, data do not support that this is associated with an increased bleeding risk; thus, a decreased dose is not recommended. Answer D is incorrect because patients with a *17/*17 genotype do not require treatment with an alternative antiplatelet therapy.

6. **Answer: D**
   The CPIC guidelines are not designed to provide information on whether a genetic test should be ordered or the timing of a specific genetic test (Answer D is correct; Answer A is incorrect). Currently, only 33 of 190 drugs with pharmacogenetic information in the package label have guideline information available (Answer B is incorrect). The CPIC guidelines do give priority to availability of commercially available pharmacogenetic tests offered in Clinical Laboratory Improvement Amendments–approved clinical settings (Answer C is incorrect).