CHAPTER

5 Pharmacogenomics

Jennifer E. Hibma, PharmD,
& Kathleen M. Giacomini, PhD

CASE STUDY

A 35-year-old male with newly diagnosed human immunodeficiency virus (HIV) infection was prescribed an antiretroviral regimen, which included the protease inhibitor atazanavir 300 mg to be taken by mouth once daily, along with ritonavir, a pharmacokinetic enhancer, and two nucleoside analog antiretroviral agents. Liver function and renal function were normal. After 1 year of treatment, the patient experienced visible yellow discoloration of the skin and eyes. Blood samples were drawn, and grade 4 hyperbilirubinemia was documented. When atazanavir was discontinued and the antiretroviral regimen was modified to include lopinavir, the plasma levels of bilirubin returned to the normal range, and skin and eye color were cleared. Could a *UGT1A1*28* polymorphism have led to the adverse effects?

Pharmacogenomics, the study of genetic factors that underlie variation in drug response, is a modern term for **pharmacogenetics.** Pharmacogenomics implies a recognition that more than one genetic variant may contribute to variation in drug response. Historically, the field began with observations of severe adverse drug reactions in certain individuals, who were found to harbor genetic variants in drug-metabolizing enzymes. As a scientific field, pharmacogenomics has advanced rapidly since the sequencing of the human genome. In the last decade, powerful genome-wide association (GWA) studies, in which hundreds of thousands of genetic variants across the genome are tested for association with drug response, led to the discovery of many other important polymorphisms that underlie variation in both therapeutic and adverse drug response. In addition to polymorphisms in genes that encode drug-metabolizing enzymes, it is now known that polymorphisms in genes that encode transporters, human leukocyte antigen (HLA) loci, cytokines, and various other proteins are also predictive of variation in therapeutic and adverse drug responses. In addition to the new discoveries that have been made, the past decade has ushered in **"precision medicine,"** also known as **"stratified** or **personalized medicine,"** in which genetic information is used to guide drug and dosing selection for subgroups of patients or individual patients in medical practice. The Clinical Pharmacogenetics Implementation Consortium (CPIC) published a series of guidelines for using genetic information in selecting medications and in dosing. These highly informative guidelines are being used by practitioners in prescribing drugs to more effectively treat patients. In this chapter, we begin with a case study and then describe genetic variants that are determinants of drug response. Where appropriate, CPIC recommendations are included to provide information on how to use genetic variant data appropriately in therapeutic medicine.

The description in this chapter of DNA sequence variations in germline DNA involves a number of terms that describe the nature of the variations and their locations within the genome. A glossary of commonly used terms is presented in the Glossary Table. Some of the more common and important variations are described in the text that follows.

GLOSSARY

Term	Definition
Allele	One of two or more alternative forms of a gene that arise by mutation and are found at the same genetic locus. Example: *CYP2D6*3* is an important variant allele for a drug-metabolizing enzyme, CYP2D6.
Allele frequency	The fraction or percentage of times a specific allele is observed in proportion to the total of all possible alleles that could occur at a specific location on a chromosome.
Coding single nucleotide polymorphisms (cSNPs)	A single base-pair substitution that occurs in the coding region.
Copy number variations (CNVs)	A segment of DNA in which a variable number of that segment has been found.
Haplotype	A series of alleles found in a linked locus on a chromosome.
Hardy-Weinberg equilibrium	The principle that allele frequencies will remain constant from generation to generation in the absence of evolutionary influences.
Insertions/deletion (indel)	Insertion or deletion of base pairs, which may occur in coding and noncoding regions.
Linkage disequilibrium	The nonrandom association of alleles at two or more loci that descend from a single ancestral chromosome.
Noncoding region polymorphism	Polymorphisms that occur in the 3′ and 5′ untranslated regions, intronic regions, or intergenic regions.
Nonsynonymous SNPs (nsSNPs)	A single base-pair substitution in the coding region that results in an amino acid change.
Polymorphism or variant	Any genetic variation in the DNA sequence; the terms can be used interchangeably.
PM, IM, EM, or UM	Poor, intermediate, extensive, or ultra-rapid metabolizer phenotype.
SNPs	Single nucleotide polymorphisms: base-pair substitutions that occur in the genome.
Synonymous SNPs	Base-pair substitutions in the coding region that do not result in an amino acid change.

GENETIC VARIATIONS IN ENZYMES

PHASE I ENZYMES

As described in Chapter 4, biotransformation reactions mediated by P450 phase I enzymes typically modify functional groups (–OH, –SH, $–NH_2$, $–OCH_3$) of endogenous and xenobiotic compounds, resulting in an alteration of the biological activity of the compound. Phase I enzymes are involved in the biotransformation of over 75% of prescription drugs; therefore, polymorphisms in these enzymes may significantly affect blood levels, which in turn may alter response to many drugs. Polymorphisms in drug-metabolizing enzymes dominated the field of pharmacogenomics for many years, and for some years, metabolic phenotypes such as extensive metabolizer (EM), reflecting an individual's metabolic rate of a particular drug that is a known substrate of a specific enzyme, were used to describe genetic effects on drug metabolism. After genotypic information became available, a new nomenclature was used to characterize an individual's metabolic rate. In particular, diplotypes, consisting of one maternal and one paternal allele, using star (*) allele nomenclature, have been used. Each star (*) allele is defined by specific sequence variation(s) within the gene locus, eg, single nucleotide polymorphisms (SNPs), and may be assigned a functional activity score when the functional characterization is known, eg, 0 for nonfunctional, 0.5 for reduced function, and 1.0 for fully functional. Some genes, such as *CYP2D6*, are subject to whole gene deletions, eg, *CYP2D6*5,* and whole gene duplications or multiplications, eg, **1xN*, **2xN,* where N is the number of copies. If more than one copy of the gene is detected, the activity score is then multiplied by the number of copies observed. Enzyme activity is generally a co-dominant or additive trait. For example, if an individual carries one normal function allele and one nonfunctional allele, he will have an intermediate metabolic activity or be considered an intermediate metabolizer (IM). The sum of allelic activity scores typically ranges between 0 and ≥ 3.0 and is most often used to define phenotypes as follows: 0 = PM (poor metabolizer), 0.5 = IM, 1.0–2.0 = EM, and ≥ 2.0 = UM (ultra-rapid metabolizer).

CYP2D6

As described in Chapter 4, cytochrome P450 2D6 is involved in the metabolism of up to one quarter of all drugs used clinically, including predominantly basic compounds such as β blockers, antidepressants, antipsychotics, and opioid analgesics. Among the CYP enzymes, CYP2D6 is responsible for metabolism of about 20% of clinically used drugs. Similar to other polymorphic enzymes, four clinically defined metabolic phenotypes, ie, PMs, IMs, EMs, and UMs, are used to predict therapeutic and adverse responses following the administration of CYP2D6 substrates.

The gene encoding CYP2D6 is highly polymorphic, with over 100 alleles defined (www.cypalleles.ki.se/cyp2d6.htm); however, greater than 95% of phenotypes can be accounted for with just nine alleles, ie, *CYP2D6* alleles **3*, **4*, **5*, and **6* are nonfunctional; alleles **10*, **17*, and **41* have reduced function; and alleles **1* and **2* are fully functional. As with many polymorphisms, allele frequencies vary across populations (Table 5–1). Some genetic variants are shared among populations at similar allele frequencies, whereas others vary considerably. For example, the most common nonfunctional allele, *CYP2D6*4*, is observed at

TABLE 5–1 Major alleles and frequencies in African, Asian, and European populations.

Gene	Allele(s)	dbSNP[1] Number	Amino Acid	Function	Activity	Fraction in African Populations	Fraction in Asian Populations	Fraction in European Populations
CYP2D6								
	**1*	Reference	—	Normal	1.0	0.39	0.34	0.54
	**1xN*	Gene duplication or multiplication	Increased expression	Increased	1.0 × N	0.015	0.0028	0.0080
	**2*	rs16947, rs1135840	R296C, S486T	Normal	1.0	0.20	0.13	0.27
	**2xN*	Duplication or multiplication	Increased expression	Increased	1.0 × N	0.016	0.0038	0.013
	**3*	rs35742686	Frameshift	None	0.0	0.00030	0.00	0.013
	**4*	rs1065852, rs3892097	P34S, Splicing defect	None	0.0	0.034	0.0042	0.19
	**5*	—	No enzyme	None	0.0	0.061	0.056	0.027
	**6*	rs5030655	Frameshift	None	0.0	0.031	0.0002	0.0095
	**10*	rs1065852, rs1135840	P34S, S486T	Decreased	0.5	0.068	0.42	0.032
	**17*	rs28371706, rs16947, rs1135840	T107I, R296C, S486T	Decreased	0.5	0.20	0.0001	0.0032
	**41*	rs16947, rs1135840, rs28371725	R296C, S486T, Splicing defect	Decreased	0.5	0.11	0.020	0.086
CYP2C19								
	**1*	Reference	—	Normal	—	0.68	0.60	0.63
	**2*	rs4244285	Splicing defect	None	—	0.15	0.29	0.15
	**3*	rs4986893	W212X	None	—	0.0052	0.089	0.0042
	**17*	rs12248560	Increased expression	Increased	—	0.16	0.027	0.21
DPYD								
	**1*	Reference	—	Normal	—			
	**2A*	rs3918290	Splicing defect	None	—	0.00	0.0015	0.0086
	**13*	rs55886062	I560S	None	—	n/a	0.00	0.0010
	—	rs67376798	D949V	None	—	n/a	n/a	0.011
UGT1A1								
	**1*	Reference	TA_6	Normal	—	0.50	0.85	0.68
	**28*	rs8175347	TA_7	Decreased	—	0.39	0.15	0.32
	**36*	rs8175347	TA_5	Increased	—	0.066	0.00	0.00
	**37*	rs8175347	TA_8	Decreased	—	0.036	0.00	0.0010
TPMT								
	**1*	Reference	—	Normal	—	0.94	0.98	0.96
	**2*	rs1800462	A80P	None	—	0.00079	0.00	0.0019
	**3A*	rs1800460, rs1142345	A154T, Y240C	None	—	0.0020	0.00012	0.036
	**3B*	rs1800460	A154T	None	—	0.00	0.00	0.00046
	**3C*	rs1142345	Y240C	None	—	0.050	0.016	0.0042
	**4–*26*	Various	Various	Decreased	—	Various	Various	Various

(*continued*)

TABLE 5–1 Major alleles and frequencies in African, Asian, and European populations. (Continued)

Gene	Allele(s)	dbSNP[1] Number	Amino Acid	Function	Activity	Fraction in African Populations	Fraction in Asian Populations	Fraction in European Populations
G6PD								
	B	Reference	—	Normal	IV	—	—	—
	A	rs1050829	N126D	Normal	III–IV	0.31–0.35	0.00	0.00–0.060
	A- (rs1050829, rs1050828) *A*- (rs1050829, rs137852328) *A*- (rs1050829, rs76723693)		(N126D, V68M) (N126D, R227L) (N126D, L323P)	Decreased (5–10%)	III	0.00–0.30	n/a	n/a
	Mediterranean (rs5030868)		S188P	Decreased (< 1%)	II	0.00–0.052	0.00–0.31	0.00–0.074
	Canton (rs72554665), Kaiping		R459L/R463H	Decreased	II			
	Mahidol		G163S	Decreased (5–32%)	III			
	Chinese-5, Gaohe		L342F H32R	Decreased	III			
SLCO1B1								
	**1a*	Reference	—	Normal	—	0.17	0.27	0.50
	**1b*	rs2306283	N130D	Normal	—	0.78	0.60	0.22
	**5*	rs4149056	V174A	Decreased	—	0.00	0.00	0.01
	**15, *17*	rs4149056, others	V174A others	Decreased	—	0.03	0.13	0.14
HLA-B								
	**57:01*	—	—	positive	—	0.010	0.016	0.068
IFNL3								
	TT/CT	Reference	—	Unfavorable	—	—	—	—
	CC	rs12979860	—	Favorable	—	0.39	0.87	0.63
CYP2C9								
	**1*	Reference	—	Normal	—			
	**2*	rs1799853	R144C	Decreased	—	0.03	0.00	0.13
	**3*	rs1057910	I359L	Decreased	—	0.02	0.04	0.07
VKORC1								
	–1639G	Reference	—	Normal	—			
	–1639A	rs9923231	Reduced expression	Decreased	—	0.11	0.91	0.39

[1]The Single Nucleotide Polymorphism Database (dbSNP) is an online public repository of genomic variation established by the National Center for Biotechnology Information (NCBI), https://www.ncbi.nlm.nih.gov/SNP/.

a frequency of approximately 20% in Europeans and is nearly absent (< 1%) in Asians (Table 5–1). Based on Hardy-Weinberg principles (see Glossary), the percentage of Europeans who are homozygous for the *CYP2D6*4* allele, ie, who carry the **4* allele on both maternal and paternal chromosomes, would be 4%, whereas that of those who are heterozygotes would be 32%. This parallels the lower number of PMs (defined as having two nonfunctional alleles, eg, PMs are homozygous for **3*, **4*, **5*, **6*, or any combination of nonfunctional alleles such as **4*/**5*), observed in Asian populations (~1%) compared with European populations (~5–10%) (Table 5–1). In contrast, the **5* gene deletion is found at similar frequencies (~3–5%) across European, African, and Asian populations, suggesting that this mutation likely took place prior to the separation of the three major races more than 100,000 years ago. Clinically, since some genotyping platforms are specific to a single ethnicity, it is important to ensure alleles applicable to the patient population being treated are tested. Of note, rare or previously undiscovered variants are typically not included in commercial tests, and thus novel or rare polymorphisms, which may exhibit altered function, will be missed.

Example: Codeine is a phenanthrene derivative prodrug opioid analgesic indicated for the management of mild to moderately severe pain (Chapter 31). Codeine, like its active metabolite morphine, binds to μ-opioid receptors in the central nervous

system (CNS). Morphine is 200 times more potent as an agonist than codeine, and conversion of codeine into morphine is essential for codeine's analgesic activity. The enzyme responsible for the *O*-demethylation conversion of codeine into morphine is CYP2D6. Patients with normal CYP2D6 activity (ie, EMs) convert sufficient codeine to morphine (~5–10% of an administered dose) to produce the desired analgesic effect. PMs and IMs are more likely to experience insufficient pain relief, while UMs are at an increased risk for side effects, eg, drowsiness and respiratory depression, due to higher systemic concentrations of morphine. Interestingly, gastrointestinal adverse effects, eg, constipation, are decreased in PMs, whereas the central side effects, eg, sedation and dizziness, do not differ between PMs and EMs. The antitussive properties associated with codeine are not affected by CYP2D6 activity. According to CPIC guidelines, standard starting doses are recommended in EMs and IMs with close monitoring, especially in IMs; and CPIC recommends use of an alternative agent in PMs and UMs (see Table 5–2).

CYP2C19

Cytochrome P450 CYP2C19 is known to preferentially metabolize acidic drugs including proton-pump inhibitors, antidepressants, antiepileptics, and antiplatelet drugs (Chapter 4). Four clinical phenotypes related to CYP2C19 activity (PM, IM, EM, and UM) are closely associated with genetic biomarkers that may assist in guiding individualized therapeutic dosing strategies. The gene that encodes CYP2C19 is highly polymorphic, with over 30 alleles defined (www.cypalleles.ki.se/cyp2c19.htm), yet just four alleles can account for the majority of phenotypic variability, ie, *CYP2C19* allele **2* and **3* are nonfunctional, *CYP2C19* allele **1* is fully functional, and *CYP2C19*17* has increased function. Phenotypes range from PMs who have two deficient alleles, eg, **2/*3*, **2/*2*, or **3/*3*, to UMs who have increased hepatic expression levels of the CYP2C19 protein, due to **1/*17* or **17/*17* alleles (see Table 5–2). Of note, the **17* increased function allele is unable to fully compensate for nonfunctional alleles, and therefore, the presence of a **17* allele in combination with a nonfunctional allele would be considered an IM phenotype (see Table 5–2). The PM phenotype is more common in Asians (~16%) than in Europeans and Africans (~2–5%), which can be expected based on the inheritance patterns of variant alleles across populations, eg, the most common nonfunctional allele, ie, *CYP2C19*2*, is observed approximately twice as frequently in Asians (~30%) compared with Africans and Europeans (~15%), while the apparent gain-of-function **17* allele is observed rarely in Asians (< 3%) but more frequently in Europeans and Africans (16–21%) (see Table 5–1).

Example: Clopidogrel is a thienopyridine antiplatelet prodrug indicated for the prevention of atherothrombotic events. Active metabolites selectively and irreversibly inhibit adenosine diphosphate-induced platelet aggregation (Chapter 34). Clopidogrel is metabolized in the body via one of two main mechanisms; approximately 85% of an administered dose is rapidly hydrolyzed by hepatic esterases to its inactive carboxylic acid derivative, while the remaining ~15% is converted via two sequential CYP-mediated oxidation reactions (predominantly CYP2C19) to the active thiol metabolite responsible for antiplatelet activity.

Genetic polymorphisms in the *CYP2C19* gene that decrease active metabolite formation and consequently reduce the drug's antiplatelet activity are associated with variability in response to clopidogrel. Carriers of the reduced function *CYP2C19 *2* alleles taking clopidogrel are at increased risk for serious adverse cardiovascular events, particularly in acute coronary syndrome managed with percutaneous coronary intervention (PCI); the hazard ratios (HR) are 1.76 for **2/*2* genotype and 1.55 for **2* heterozygotes compared to noncarriers. The risk associated with stent thrombosis is even greater (HR 3.97 for **2/*2* genotype and 2.67 for **2* heterozygotes compared to **1* homozygotes). However, for other indications, eg, atrial fibrillation and stroke, the effects of the *CYP2C19*2* allele are less dramatic. Thus, current clinical recommendations from CPIC are specific for acute coronary syndrome with PCI: Standard starting doses are recommended in EMs and UMs, and CPIC recommends use of an alternative antiplatelet agent, eg, prasugrel or ticagrelor, in PMs and IMs (Table 5–2). The US Food and Drug Administration (FDA)-approved label for clopidogrel recommends alternative antiplatelet drugs for patients who are poor metabolizers of clopidogrel.

Dihydropyrimidine Dehydrogenase (DPD)

Dihydropyrimidine dehydrogenase (DPD, encoded by the *DPYD* gene) is the first and rate-limiting step in pyrimidine catabolism, as well as a major elimination route for fluoropyrimidine chemotherapy agents (Chapter 54). Considerable intergroup and intragroup variation exists in DPD enzyme activity. Many of the alleles identified in the *DPYD* gene either are too rare to sufficiently characterize or have shown conflicting associations with DPD activity. Three nonfunctional alleles have been identified, ie, *DPYD *2A*, **13*, and rs67376798. All three of these variants are rare; however, the **2A* allele is the most commonly observed allele and is often the only variant tested in commercial genotyping platforms (see National Institutes of Health Genetic Testing Registry, http://www.ncbi.nlm.nih.gov/gtr/conditions/C2720286/ or http://www.ncbi.nlm.nih.gov/gtr/conditions/CN077983/). Frequencies of the **2A* allele range from less than 0.005 in most European, African, and Asian populations to 3.5% in a Swedish population (see Table 5–1).

Example: Three fluoropyrimidine drugs are used clinically, namely 5-fluorouracil (5-FU), capecitabine, and tegafur (only approved in Europe). 5-FU is the pharmacologically active compound of each drug, and all are approved to treat solid tumors including colorectal and breast cancer (Chapter 54). 5-FU must be administered intravenously, while both capecitabine and tegafur are oral prodrugs that are rapidly converted to 5-FU in the body. Only 1–3% of an administered dose of the prodrug is converted to the active cytotoxic metabolites, ie, 5-fluorouridine 5′-monophosphate (5-FUMP) and 5-fluoro-2′-deoxyuridine-5′-monophosphate (5-FdUMP), which effectively target rapidly dividing cancer cells and inhibit DNA synthesis. The majority of an administered dose (~80%) is subjected to pyrimidine catabolism via DPD and is excreted in the urine. Complete or

TABLE 5–2 Gene-based dosing recommendations for selected drugs.

Gene	Drug	Diplotype[1]	Likely Phenotype (Activity Score)	Dosing Recommendation	Source of Recommendation
CYP2D6					
	Codeine	**1/*1xN*, **1/*2xN*	UM (> 2.0)	• Alternative analgesic, eg, morphine or nonopioid; increased formation of morphine following codeine administration leads to higher risk of toxicity.	CPIC[2]
		**1/*1*, **1/*2*, **2/*2*, **1/*41*, **2/*5*	EM (1.0–2.0)	• Standard starting dose.	
		**4/*10*, **5/*41*	IM (0.5)	• Standard starting dose; monitor closely for lack of analgesic response due to reduced morphine formation. Consider alternate analgesic, eg, morphine or nonopioid.	
		**3/*4*, **4/*4*, **4/*5*, **5/*5*, **4/*6*	PM (0.0)	• Alternative analgesic, eg, morphine or nonopioid analgesic; greatly reduced morphine formation following codeine administration, leading to insufficient pain relief. Avoid higher doses, as central side effects do not differ in PMs.	
CYP2C19					
	Clopidogrel	**1/*17*, **17/*17* (UM), and **1/*1* (EM)	UM, EM	• Standard dose.	CPIC
		**1/*2*, **1/*3*, **2/*17*	IM	• Alternative antiplatelet agent, eg, prasugrel or ticagrelor.	
		**2/*2*, **2/*3*, **3/*3*	PM	• Alternative antiplatelet agent, eg, prasugrel or ticagrelor.	
DPYD					
	Fluoropy-rimidines	**1/*1*	Normal	• Standard dose.	CPIC
		**1/*2A*, **1/*13*, **1*/rs67376798A	Reduced activity	• Reduce initial dose 50% and titrate based on toxicity or on pharmacokinetic test results (if available).	
		**2A/*2A*, **2A/*13*, **13/*13*, rs67376798A/rs67376798A	Complete deficiency	• Different non-fluoropyrimidine anticancer agent.	
UGT1A1					
	Irinotecan	**1/*1*, **1/*28*	Normal	• Standard starting dose.	
		**28/*28*	Reduced	• Reduce starting dose by at least one dose level. Or,	Drug label
				• Dose > 250 mg/m^2: Reduce starting dose 30% and increase in response to neutrophil count. Dose = 250 mg/m^2: No dose adjustment.	DPWG[3]
	Atazanavir	**1/*1*, **1/*36*, **36/*36*, rs887829 C/C	Normal	No reason to avoid prescribing atazanavir. Inform patient of risks. Based on this genotype, there is a less than 1 in 20 chance of stopping atazanavir for jaundice.	CPIC
		**1/*28*, **1/*37*, **36/*28*, **36/*37*, rs887829 C/T, **1/*6*	Intermediate	No reason to avoid prescribing atazanavir. Inform patient of risks. Based on this genotype, there is a less than 1 in 20 chance of stopping atazanavir for jaundice.	
		28/*28*, **28/*37*, **37/*37*, rs887829 T/T (80/*80*), **6/*6*	Reduced	Consider alternative agent. Based on this genotype, there is a high (20–60%) likelihood of developing jaundice that will result in discontinuation of atazanavir.	
TPMT					
	Thiopurines	**1/*1*	Normal, high activity	• Standard starting dose.	CPIC
		**1/*2*, **1/*3A*, **1/*3B*, **1/*3C*, **1/*4*	Intermediate activity	• Start at 30–70% of target dose and titrate every 2–4 weeks with close clinical monitoring of tolerability, eg, white blood cell counts and liver function tests.	

(*continued*)

TABLE 5–2 Gene-based dosing recommendations for selected drugs. (Continued)

Gene	Drug	Diplotype[1]	Likely Phenotype (Activity Score)	Dosing Recommendation	Source of Recommendation
		*3A/*3A, *2/*3A, *3C/*3A, *3C/*4, *3C/*2, *3A/*4	Low activity	• Malignant disease: Drastic reduction of thiopurine doses, eg, tenfold given thrice weekly instead of daily. • Nonmalignant conditions: Alternative nonthiopurine immunosuppressive agent.	
***G6PDX*-linked trait**		Genotype-to-phenotype predictions limited to males and homozygous females.			
	Rasburicase	*B, A*	Normal	• Standard dose.	Drug label/CPIC
		A-, Mediterranean, Canton	Deficient	• Alternative agent, eg, allopurinol: Rasburicase is contraindicated in patients with G6PD deficiency.	
		Variable	Unknown risk of hemolytic anemia	• Enzyme activity must be measured to determine G6PD status. An alternative is allopurinol.	
SLCO1B1					
	Simvastatin 40 mg	*1a/*1a, *1a/*1b, *1b/*1b	Normal activity	• Standard dose.	CPIC
		*1a/*5, *1a/*15, *1a/*17, *1b/*5, *1b/*15, *1b/*17	Intermediate activity	• Prescribe a lower dose or consider an alternative statin, eg, pravastatin or rosuvastatin; consider routine CK monitoring.	
		*5/*5, *5/*15, *5/*17, *15/*15, *15/*17, *17/*17	Low activity	• Prescribe a lower dose or consider an alternative statin, eg, pravastatin or rosuvastatin; consider routine CK monitoring.	
HLA					
	Abacavir	*Other/*Other	Negative	• Standard dose.	CPIC
		*Other/*57:01, *57:01/*57:01	Positive	• Alternative agent: abacavir is contraindicated in HLA-B*57:01-positive patients.	
IFNL3					
	PEG-IFN-α/RBV	rs12979860/rs12979860	Favorable	• PEG-IFN-α/RBV: Consider cure rates before initiating regimen; ~70% chance for SVR[4] after 48 weeks of therapy. • PEG-IFN-α/RBV + protease inhibitor combinations: Regimen recommended; ~90% chance for SVR after 24–48 weeks of therapy, with 80–90% chance for shortened duration of therapy.	CPIC
		Reference/reference or reference/rs12979860	Unfavorable	• PEG-IFN-α/RBV: Consider cure rates before initiating regimen; ~30% chance for SVR after 48 weeks of therapy. • PEG-IFN-α/RBV + protease inhibitor combinations: Consider cure rates before initiating regimen; ~60% chance for SVR after 24–48 weeks of therapy, with 50% chance for shortened duration of therapy.	
CYP2C9, VKORC1					
	Warfarin	*1/*1, *1/*2, *2/*2, *2/*3, *1/*3, *3/*3, 1639GG, 1639GA, 1639AA	Various	• Apply validated dosing algorithm, eg, www.warfarindosing.org (or IWPC[5]) for international normalized ratio target 2–3) or FDA-approved dosing table per manufacturer's labeling.	CPIC

[1]Diplotypes are shown as the two members of a chromosome pair, eg, *1/*1 indicates both chromosomes contain the *1 allele for that gene, whereas *1/*17 denotes a heterozygote with one *1 allele and one *17 allele.

[2]CPIC: Clinical Pharmacogenetics Implementation Consortium: Full drug-specific recommendations are available online at http://www.pharmgkb.org/page/cpic.

[3]DPWG: Dutch Pharmacogenetics Working Group: Full drug-specific recommendations are available online https://www.pharmgkb.org/page/dpwg.

[4]SVR: sustained viral response.

[5]IWPG: International Warfarin Pharmacogenetics Consortium.

partial deficiency of DPD can lead to dramatically reduced clearances of 5-FU, increased levels of toxic metabolites 5-FUMP and 5-FdUMP, and consequently an increased risk for severe dose-dependent fluoropyrimidine toxicities, eg, myelosuppression, mucositis, neurotoxicity, hand-and-foot syndrome, and diarrhea. In a recent genotype-driven dosing study of over 1600 patients treated with fluoropyrimidine-based chemotherapy, including 18 carriers of *DPYD*2A* who were treated with 50% of the normal dose, the incidence of severe toxicity was significantly reduced from 73% (historical controls) to 28%. CPIC recommendations for therapeutic regimens are shown in Table 5–2.

PHASE II ENZYMES

As described in Chapter 4, phase II enzyme biotransformation reactions typically conjugate endogenous molecules, eg, sulfuric acid, glucuronic acid, and acetic acid, onto a wide variety of substrates in order to enhance their elimination from the body. Consequently, polymorphic phase II enzymes may diminish drug elimination and increase risks for toxicities. In this section, we describe key examples of polymorphic phase II enzymes and the pharmacologic consequence for selected prescription drugs.

Uridine 5′-Diphosphoglucuronosyl Transferase 1 (UGT1A1)

The uridine 5′-diphospho- (UDP) glucuronosyltransferase 1A1 (UGT1A1) enzyme, encoded by the *UGT1A1* gene, conjugates glucuronic acid onto small lipophilic molecules, eg, bilirubin and a wide variety of therapeutic drug substrates so that they may be more readily excreted into bile (Chapter 4). The *UGT1A1* gene locus has over 30 defined alleles, some of which lead to reduced or completely abolished UGT1A1 function. Most reduced function polymorphisms within the *UGT1A1* gene locus are quite rare; however, the **28* allele is common across three major ethnic groups (Table 5–1). Approximately 10% of European populations are homozygous carriers of the **28* allele, ie, *UGT1A1 *28/*28* genotype, and are recognized clinically to have Gilbert's syndrome. The **28* allele is characterized by an extra TA repeated in the proximal promoter region and is associated with reduced expression of the UGT1A1 enzyme. Clinically, Gilbert's syndrome is generally benign; however, affected individuals may have 60–70% increased levels of circulating unconjugated bilirubin due to a ~30% reduction in UGT1A1 activity. Individuals with the *UGT1A1*28/*28* genotype are thus at an increased risk for adverse drug reactions with UGT1A1 drug substrates due to reduced biliary elimination.

Example: Irinotecan is a topoisomerase I inhibitor prodrug and is indicated as first-line chemotherapy in combination with 5-FU and leucovorin for treatment of metastatic carcinoma of the colon or rectum (Chapter 54). Irinotecan is hydrolyzed by hepatic carboxylesterase enzymes to its cytotoxic metabolite, SN-38, which inhibits topoisomerase I and eventually leads to termination of DNA replication and cell death. The active SN-38 metabolite is responsible for the majority of therapeutic action as well as the dose-limiting bone marrow and gastrointestinal toxicities. Inactivation of SN-38 occurs via the polymorphic UGT1A1 enzyme, and carriers of the *UGT1A1*6* and *UGT1A1*28* polymorphisms are consequently at increased risk for severe life-threatening toxicities, eg, neutropenia and diarrhea, due to decreased clearance of the SN-38 metabolite.

Thiopurine *S*-Methyltransferase (TPMT)

Thiopurine *S*-methyltransferase (TPMT) covalently attaches a methyl group onto aromatic and heterocyclic sulfhydryl compounds and is responsible for the pharmacologic deactivation of thiopurine drugs (Chapter 4). Genetic polymorphisms in the gene encoding TPMT may lead to three clinical TPMT activity phenotypes, ie, high, intermediate, and low activity, which are associated with differing rates of inactivation of thiopurine drugs and altered risks for toxicities. While the majority (86–97%) of the population inherits two functional *TPMT* alleles and has high TPMT activity, around 10% of Europeans and Africans inherit only one functional allele and are considered to have intermediate activity. Furthermore, about 0.3% of Europeans inherit two defective alleles and have very low to no TPMT activity (Table 5–1). Over 90% of the phenotypic TPMT variability across populations can be accounted for with just three point mutations that are defined by four non-functional alleles, ie, *TPMT*2*, **3A*, **3B*, and **3C* (Table 5–2). Most commercial genotyping platforms test for these four common genetic biomarkers and are therefore able to identify individuals with reduced TPMT activity.

Example: Three thiopurine drugs are used clinically, ie, azathioprine, 6-mercaptopurine (6-MP), and 6-thioguanine (6-TG). All share similar metabolic pathways and pharmacology. Azathioprine (a prodrug of 6-MP) and 6-MP are used for treating immunologic disorders, while 6-MP and 6-TG are important anticancer agents (Chapter 54). 6-MP and 6-TG may be activated by the salvage pathway enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRTase) to form 6-thioguanine nucleotides (TGNs), which are responsible for the majority of therapeutic efficacy as well as bone marrow toxicity. Alternatively, 6-MP and 6-TG may be inactivated by enzymes such as polymorphic TPMT and xanthine oxidase, leaving less available substrate to be activated by HGPRTase. The *TPMT* gene is a major determinant of thiopurine metabolism and exposure to cytotoxic 6-TGN metabolites and thiopurine-related toxicities. See Table 5–2 for recommended dosing strategies. Recent GWA studies have also implicated variants in the enzyme NUDT15, which catalyzes the hydrolysis of nucleotide diphosphates, as being associated with thiopurine intolerance in children from Japan, Singapore, and Guatemala.

OTHER ENZYMES

G6PD

Glucose 6-phosphate dehydrogenase (G6PD) is the first and rate-limiting step in the pentose phosphate pathway and supplies a significant amount of reduced NADPH in the body. In red blood cells (RBCs), where mitochondria are absent, G6PD is the exclusive source of NADPH and reduced glutathione, which play a

TABLE 5–3 Classification of G6PD deficiency (WHO Working Group, 1989).

World Health Organization Class	Level of Deficiency	Enzyme Activity	Clinical phenotype
I	Severe	<10%	Chronic (non-spherocytic) hemolytic anemia
II	Severe	<10%	Risk of acute hemolytic anemia; intermittent hemolysis
III	Moderate	10–60%	Risk of acute hemolytic anemia; hemolysis with stressors
IV	None	60–150%	Normal
V	None	>150%	Enhanced activity

critical role in the prevention of oxidative damage. Under normal conditions, G6PD in RBCs is able to detoxify unstable oxygen species while working at just 2% of its theoretical capacity. Following exposure to exogenous oxidative stressors, eg, infection, fava beans, and certain therapeutic drugs, G6PD activity in RBCs increases proportionately to meet NADPH demands and ultimately to protect hemoglobin from oxidation. Individuals with G6PD deficiency, defined as less than 60% enzyme activity, according to World Health Organization classification (Table 5–3), are at increased risk for abnormal RBC destruction, ie, hemolysis, due to reduced antioxidant capacity under oxidative pressures.

The gene that encodes the G6PD enzyme is located on the X chromosome and is highly polymorphic, with over 180 genetic variants identified that result in enzyme deficiency. Greater than 90% of variants are single-base substitutions in the coding region that produce amino acid changes, which result in unstable proteins with reduced enzyme activity. As with most X-linked traits, males with one reference X chromosome and females with two reference X chromosomes will have equivalent "normal" G6PD activity. Similarly, hemizygous-deficient males (with a deficient copy of the *G6PD* gene on their single X chromosome) and homozygous-deficient females (with two deficient copies) express reduced activity phenotypes (Table 5–1). However, for heterozygous females (with one deficient allele and one normal allele), genotype-to-phenotype predictions are less reliable due to the X-chromosome mosaicism, ie, where one X chromosome in each female cell is randomly inactivated, leading to G6PD activity that may range from fully functional to severely deficient. G6PD enzyme activity phenotype estimations for heterozygous females therefore may be improved with complementary G6PD activity testing.

G6PD enzyme deficiency affects over 400 million people worldwide, and the World Health Organization has categorized G6PD activity into five classes (Table 5–3). The majority of polymorphic *G6PD*-deficient genotypes are associated with class II for severe deficiency (< 10% enzyme activity) and class III for moderate deficiency (10–60% enzyme activity). Most individuals with reduced function alleles of *G6PD* have ancestries in geographical areas of the world corresponding to areas with high malaria prevalence. Polymorphic alleles gained in frequency over time as they offered some benefit against death from malaria. The estimated frequency of G6PD deficiency is approximately 8% in malaria endemic countries, with the milder *G6PD-A*(–) allele prevalent in Africa, and the more severe *G6PD*-Mediterranean allele widespread across western Asia (Saudi Arabia and Turkey to India). There is a much more heterogeneous distribution of variant alleles in East Asia and Asia Pacific, which complicates G6PD risk predictions; however, the most frequently identified forms in Asia include the more severe class II alleles, eg, Mediterranean, Kaiping, and Canton, as well as some class III alleles, eg, Mahidol, Chinese-5, and Gaohe (Table 5–1).

Example: Rasburicase, a recombinant urate-oxidase enzyme, is indicated for the initial management of high uric acid levels in cancer patients receiving chemotherapy. Rasburicase alleviates the uric acid burden that often accompanies tumor-lysing treatments by converting uric acid into allantoin, a more soluble and easily excreted molecule. During the enzymatic conversion of uric acid to allantoin, hydrogen peroxide, a highly reactive oxidant, is formed. Hydrogen peroxide must be reduced by glutathione to prevent free radical formation and oxidative damage. Individuals with G6PD deficiency receiving rasburicase therapy are at greatly increased risk for severe hemolytic anemia and methemoglobinemia. The manufacturer recommends that patients at high risk (individuals of African or Mediterranean ancestry) be screened prior to the initiation of therapy and that rasburicase not be used in patients with G6PD deficiency (Table 5–2).

GENETIC VARIATIONS IN TRANSPORTERS

Plasma membrane transporters, located on epithelial cells of many tissues, eg, intestinal, renal, and hepatic membranes, mediate selective uptake and efflux of endogenous compounds and xenobiotics including many drug products. Transporters, which often work in concert with drug-metabolizing enzymes, play important roles in determining plasma and tissue concentrations of drugs and their metabolites. Genetic differences in transporter genes can dramatically alter drug disposition and response and thus may increase risk for toxicities. In this section, a key example of a polymorphic uptake transporter and its pharmacologic impact on statin toxicity are described.

ORGANIC ANION TRANSPORTER (OATP1B1)

The OATP1B1 transporter (encoded by the *SLCO1B1* gene) is located on the sinusoidal membrane (facing the blood) of hepatocytes and is responsible for the hepatic uptake of mainly weakly acidic drugs and endogenous compounds, eg, statins, methotrexate, and bilirubin. Over 40 nonsynonymous variants (nsSNPs) have been identified in this transporter, some of which result in decreased transport function. A common reduced function polymorphism, rs4149056, has been shown to reduce transport

of OATP1B1 substrates in vitro as well as to alter pharmacokinetic and clinical outcomes in vivo. The variant results in an amino acid change, Val174Ala, and is associated with reduced membrane expression, likely as a result of impaired trafficking capability. Allele **5* is relatively rare (rs4149056 alone; ~1%), but various other reduced function alleles (**15* and **17*; haplotypes containing rs4149056) are common in most European and Asian populations (between 5% and 15%) (Table 5–1).

Example: HMG-coenzyme A (CoA) reductase inhibitors (statins) are highly effective medications that are widely prescribed to reduce serum lipids for the prevention of cardiovascular events (Chapter 35). Seven statins in use currently are generally safe and well-tolerated, but skeletal muscle toxicity can limit their use. Known risk factors include high statin dose, interacting medications, advanced age, and metabolic comorbidities. Furthermore, the common variant, rs4149056 in *SLCO1B1*, increases systemic exposure of simvastatin (221% increase in plasma area under the curve for patients homozygous for the rs4149056 variant, eg, *SLCO1B1*5/*5*; **5/[*15 or *17]*; or [*15 or *17]/[*15 or *17]) and was identified to have the single strongest association with simvastatin-induced myopathy in a GWA analysis. For individuals receiving simvastatin with reduced OATP1B1 function (at least one nonfunctional allele), CPIC recommends a lower simvastatin dose or an alternative statin (Table 5–2).

BREAST CANCER RESISTANCE PROTEIN (BCRP, *ABCG2*)

BCRP (encoded by the *ABCG2* gene), an efflux transporter in the ATP binding cassette (ABC) superfamily, is located on epithelial cells of the kidney, liver, and intestine as well as on the endothelial cells of the blood-brain barrier. Recent studies have implicated a reduced function variant in *ABCG2*, which encodes an amino acid change from glutamine to lysine at position 141 of the protein (rs2231142), as a determinant of the pharmacokinetics, response, and toxicity of several drugs. The variant has a low frequency in individuals of African ancestry but is found at an allele frequency of about 30% in East Asians including Chinese and Japanese. Notably, the variant has been associated with changes in response to the xanthine oxidase inhibitor, allopurinol, and the statin rosuvastatin. In addition, the variant has been associated with toxicity to various anticancer drugs. Because of its high allele frequency, particularly in Asian populations, and the fact that the transporter is a determinant of the pharmacokinetics of many drugs, it is likely that this variant will become increasingly important in precision medicine.

GENETIC VARIATIONS IN IMMUNE SYSTEM FUNCTION

Genetic predispositions to drug response and toxicities are not limited to genes related to pharmacokinetic processes, eg, drug-metabolizing enzymes and drug transporters. Additional genetic sources of variation may include genes involved in pharmacodynamic processes such as drug receptors and drug targets. For example, a polymorphism in HLA loci is associated with a predisposition to drug toxicity.

DRUG-INDUCED HYPERSENSITIVITY REACTIONS

Hypersensitivity reactions to various drugs can range from mild rashes to severe skin toxicities. The most severe hypersensitivity reactions are liver injury, toxic epidermal necrosis (TEN), and Stevens-Johnson syndrome (SJS), in which drugs or their metabolites form antigens. Drug classes associated with hypersensitivity reactions include sulfonamides, nonsteroidal anti-inflammatory drugs (NSAIDs), antibiotics, steroids, anti-epileptic agents, and methotrexate.

Hypersensitivity reactions have varying prevalence rates in different racial and ethnic populations. For example, carbamazepine-induced skin toxicities have an increased prevalence in East Asian populations. Population-based hypersensitivity reactions have been attributed to genetic polymorphisms in the HLA system, which are part of the major histocompatibility complex (MHC) gene family (see also Chapter 55). Of the several HLA forms, *HLA-B*, *HLA-DQ*, and *HLA-DR* polymorphisms have been associated with many drug-induced hypersensitivity reactions, including reactions to allopurinol, carbamazepine, abacavir, and flucloxacillin (Table 5–4).

Many *HLA-B* polymorphisms have been characterized and have varying allele frequencies depending on the racial and ethnic population. A polymorphism in *HLA-B* may result in altered antigen-binding sites in the HLA molecule, which in turn may recognize different peptides. The selective recognition of particular drug-bound peptides by some *HLA-B* polymorphism products results in population-selective drug hypersensitivity reactions.

Example 1: Abacavir, a nucleoside reverse transcriptase inhibitor used in the treatment of HIV, is associated with hypersensitivity reactions in the skin, particularly SJS, which for

TABLE 5–4 Polymorphisms in HLA genes associated with Stevens-Johnson syndrome, toxic epidermal necrosis, or drug-induced liver injury.

Variant of *HLA* Gene	Drug and Adverse Effect
*HLA-B*57:01*	Abacavir-induced skin toxicity
*HLA-B*58:01*	Allopurinol-induced skin toxicity
*HLA-DRB1 *15:01, DRB5 *01:01, DQB1 *06:02* haplotype	Amoxicillin-clavulanate-induced liver injury
*HLA-B*15:02*	Carbamazepine-induced skin toxicity
*HLA-B *57:01*	Flucloxacillin-induced liver injury
*HLA-DQB1 *06, *02, HLA-DRB1 *15, *07*	Various drugs, subgroup analysis for cholestatic or other types of liver injury
*HLA-DRB1 *07, HLA-DQA1 *02*	Ximelagatran, increased ALT

ALT, alanine transaminase.

many years appeared to be idiosyncratic, ie, of unknown mechanism. Although the drug-bound peptide involved in abacavir hypersensitivity has not been isolated or identified, it appears to interact somewhat specifically with the product of *HLA-B*57:01*, an *HLA-B* polymorphism found more commonly in European populations (Table 5–1). Other *HLA-B* polymorphisms are not associated with abacavir-induced hypersensitivity reactions. However, it is noteworthy that *HLA-B*57:01*, though necessary for SJS or TEN associated with abacavir, is not sufficient. That is, many individuals with the polymorphism do not get the hypersensitivity reaction. This lack of specificity is not understood and clearly warrants further study.

Abacavir hypersensitivity reactions are known to vary in frequency among ethnic groups, consistent with the population frequencies of the *HLA-B*57:01* allele. As a prodrug, abacavir is activated to carbovir triphosphate, a reactive molecule that may be involved in the immunogenicity of abacavir. Abacavir-induced hypersensitivity reactions are probably mediated by the activation of cytotoxic CD8 T cells. In fact, there is an increased abundance of CD8 T cells in the skin of patients with abacavir hypersensitivity reactions. Experiments demonstrating that CD8-positive T cells can be stimulated by lymphoblastoid cell lines expressing *HLA-B*57:01*, but not *HLA-B*57:02* or *HLA-B*58:01*, suggest that the HLA-B*57:01 protein may recognize and bind an abacavir-associated peptide, which is not recognized by the other polymorphisms. Alternatively, the *HLA-B*57:01* gene product complex may present the ligand-bound peptide on the cell surface in a structurally different configuration, which is recognized by cytotoxic T cells.

Because of the importance of abacavir in therapeutics, genetic testing of the *HLA-B*57:01* biomarker associated with abacavir hypersensitivity has been rapidly incorporated into clinical practice, much faster than typical genetic tests (Figure 5–1). CPIC

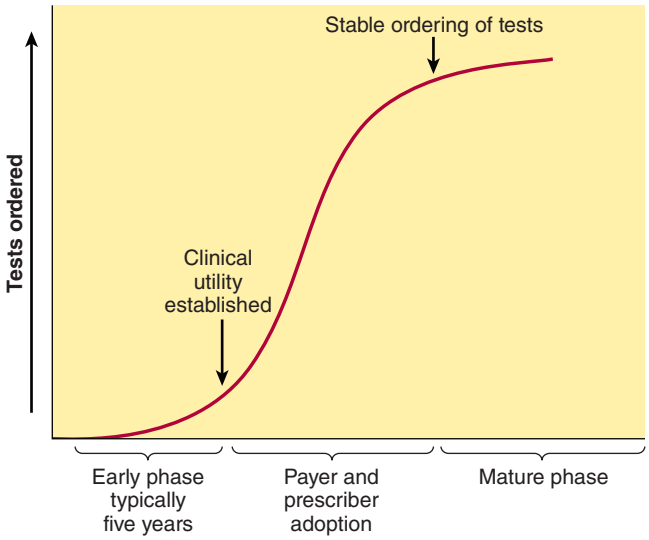

FIGURE 5–1 Increasing use of testing for genetic variants of drug metabolism over time. Adoption of testing in clinical medicine typically undergoes three phases. Testing for *HLA-B*5701* was rapidly adopted. (Adapted, with permission, from Lai-Goldman M, Faruki H: Abacavir hypersensitivity: A model system for pharmacogenetic test adoption. Genet Med 2008;10:874. Copyright 2008 Macmillan Publishers Ltd.)

recommendations based on genotyping results are shown in Table 5–2.

Example 2: Flucloxacillin hypersensitivity reactions may lead to drug-induced liver toxicity. In particular, in 51 cases of flucloxacillin hepatotoxicity, a highly significant association was identified with a polymorphism linked to *HLA-B*57:01* (Figure 5–2). HLA polymorphisms also contribute to liver injury from other drugs (Table 5–4). For example, reaction to the anticoagulant

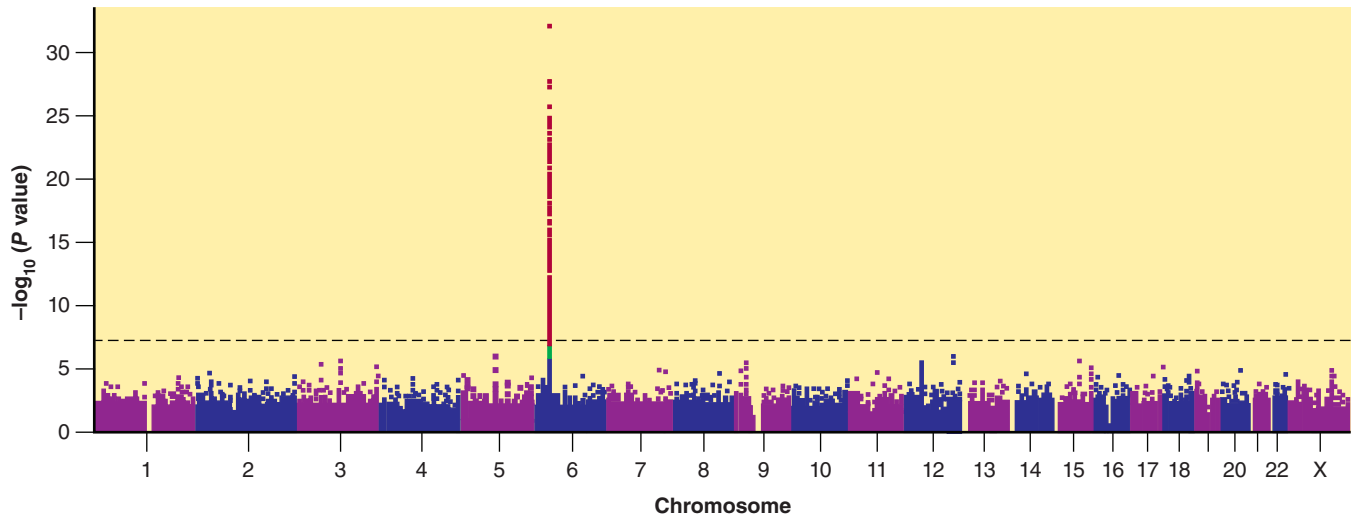

FIGURE 5–2 Results from a flucloxacillin drug-induced liver injury study. Each dot represents an SNP in a genome-wide assay. The *x* axis represents the position of the SNP on chromosomes. The *y* axis represents the magnitude of the association of each SNP with liver damage (Cochran-Armitage trend *P* value) in a case-control study that included 51 liver injury cases and 282 population controls. The high signal peak in chromosome 6 lies in the MHC region and indicates very strong association of injury with that SNP. The horizontal dashed line represents the commonly accepted minimum level for significance in this type of study. (Reproduced, with permission, from Daly AK et al: HLA-B*5701 genotype is a major determinant of drug-induced liver injury due to flucloxacillin. Nat Genet 2009;41:816. Copyright 2009 Macmillan Publishers Ltd.)

ximelagatran is associated with a *HLA-DRB1*07:01* allele. Several drugs used in the treatment of tuberculosis, including isoniazid, rifampin, and ethambutol, also cause liver injury, which appears to be related to HLA polymorphisms.

IFNL3 (IL-28B)

Interferon lambda-3 (IFN-λ3; also known as interleukin-28B), encoded by the *IFNL3* (or *IL28B*) gene, belongs to the family of type III IFN-λ cytokines. Type III IFNs share many therapeutic effects with type I IFNs, eg, IFN-α (Chapter 55), such as being directly induced by viruses and acting through JAK-STAT signal transduction pathways (via distinct heterodimeric receptor signaling complexes) to produce antiviral activity in cells. Type III IFNs play a role in hepatitis C virus (HCV) infection. Genetic variants near the *IFNL3* gene were found to be most significantly associated with HCV treatment response to pegylated-IFN-α (PEG-IFN-α), in combination with ribavirin (RBV). Approximately twofold greater cure rates were observed in patients with a favorable genotype. While the mechanism underlying this association has yet to be fully elucidated, the rs12979860 variant near *IFNL3* is considered the strongest baseline predictor of a cure for patients with HCV-1 receiving PEG-IFN-α/RBV. The favorable allele, the rs12979860 variant, is inherited most frequently in Asians (~90%), and least frequently in Africans (Table 5–1). This frequency distribution is remarkably similar to rates of response to HCV PEG-IFN-α/RBV treatment among the three ethnic groups.

Pegylated interferon with ribavirin: Chronic HCV affects 160 million people worldwide and is a leading cause of cirrhosis of the liver and liver cancer. The goal for HCV antiviral therapy is to resolve the infection, defined clinically as achievement of sustained virologic response (SVR), ie, undetectable HCV RNA measured 6 months after finishing treatment. For patients receiving PEG-IFN-α/RBV regimens, which are associated with many side effects and poor response, clinical decisions of whether to initiate therapy are largely based on likelihood of SVR. Predictors of SVR include viral factors, as well as patient factors. In addition, Europeans homozygous for the favorable genotype (*IFNL3 rs12979860/rs12979860*; SVR: 69%) are more likely to achieve SVR compared with the unfavorable genotype (*IFNL3 reference/reference* or *reference/rs12979860*; SVR: 33% and 27%, respectively), and similar rates are observed in African patients. Guidelines according to CPIC are shown in Table 5–2.

POLYGENIC EFFECTS

In the above examples, variations within single gene loci are described that are significantly associated with altered drug response or toxicity. However, it is expected that polygenic influences, ie, the combinatorial effect of multiple genes on drug response, may more accurately describe individual differences with respect to clinical outcomes. As evidence grows linking newly discovered pharmacogenetic biomarkers with therapeutic response or adverse outcomes, adequately powered clinical studies that consider the impact of newly discovered genes in the context of previously established genetic biomarkers are essential for making strong clinical recommendations. This is best exemplified by warfarin, where the effects of two genes, *CYP2C9* and *VKORC1*, on dose requirement have been clearly defined.

CYP2C9 & VKORC1

CYP2C9 is a phase I drug-metabolizing enzyme that acts primarily on acidic drugs including *S*-warfarin, phenytoin, and NSAIDs (Chapter 4). The gene that encodes CYP2C9 is highly polymorphic, with over 50 alleles defined (www.cypalleles.ki.se/cyp2c9.htm). However, much of the variability in metabolic clearance of CYP2C9 substrates may be accounted for with just two well-studied alleles, *CYP2C9*2* and *3. Allele *CYP2C9*2* encodes an amino acid change (Arg144Cys) located on the outer surface of the CYP2C9 enzyme, which impairs interaction with the microsomal P450 oxidoreductase and leads to reduced metabolism of CYP2C9 substrates, including a 30–40% reduction in *S*-warfarin metabolism. Allele *CYP2C9*3* encodes an amino acid change (Ile-359Leu) on the interior of the enzyme, which results in lowered affinity for many CYP2C9 substrates and a more marked (80–90%) reduction in *S*-warfarin metabolism. Both alleles *2 and *3 are more common in European populations compared with African and Asian populations (7–13% vs < 5%, respectively) and are therefore most useful to explain *CYP2C9* variability in Europeans (Table 5–1). Additional reduced function alleles, eg, *CYP2C9*5*, *6, *8, and *11, occur more frequently in African populations, and as evidence accumulates, their inclusion in genetic tests may improve our ability to explain warfarin variability in Africans.

Vitamin K epoxide reductase complex subunit 1 (VKORC1), encoded by the *VKORC1* gene, is the target of anticoagulant warfarin and a key enzyme in the vitamin K recycling process (Chapter 34, Figure 34–6). Activated vitamin K is an essential cofactor for activation of blood clotting factors II, VII, IX, and X, as well as endogenous anticoagulant proteins C and S. Rare genetic variants in the coding region of *VKORC1* may lead to bleeding disorders, eg, multiple coagulation factor deficiency type 2A, or warfarin resistance. A polymorphism common across all major ethnicities is located in a transcription factor-binding site, *VKORC1*-1639G>A, which results in reduced expression of VKORC1 in the liver. The most important consequences of the *VKORC1* polymorphism are increased sensitivity to warfarin (discussed below). The *VKORC1*-1639G>A polymorphism occurs most frequently in Asian populations (~90%) and least often in Africans (~10%), which explains, in part, the difference in dosing requirements among major ethnic groups (Table 5–1).

Example: Warfarin, a vitamin K antagonist, is the oldest and most widely prescribed oral anticoagulant worldwide. Within a narrow therapeutic range, warfarin is highly effective for the prevention and treatment of thromboembolic disorders (Chapter 34). Nevertheless, interpatient differences in dosing requirements (up to 20-fold) often lead to complications from subtherapeutic anticoagulation and clotting or supratherapeutic anticoagulation and bleeding, which are among the most common causes for emergency room visits in the United States. Understanding the factors that contribute to variability in individual warfarin maintenance doses may improve therapeutic outcomes.

Warfarin dosing algorithms that include clinical and known genetic influences on warfarin dose, ie, polymorphisms in CYP2C9 and VKORC1, clearly outperform empiric-dosing approaches based on population averages, as well as dosing based on clinical factors alone (Table 5–2). The pharmacologic action of warfarin is mediated through inactivation of VKORC1, and since the discovery of the VKORC1 gene in 2004, numerous studies have indicated that individuals with decreased VKORC1 expression, eg, carriers of the -1639G>A polymorphism, are at increased risk for excessive anticoagulation following standard warfarin dosages. Furthermore, warfarin is administered as a racemic mixture of *R*- and *S*-warfarin, and patients with reduced-function CYP2C9 genotypes are at increased risk for bleeding due to decreased metabolic clearance of the more potent *S*-warfarin enantiomer. It is predicted that gene-based dosing may help optimize warfarin therapy management and minimize risks for adverse drug reactions.

EPIGENOMICS

Recently, epigenomics, which is the heritable patterns of gene expression *not* attributable to changes in the primary DNA sequence, has become an active area of research that may provide additional insights into the causes of variability in drug response. Epigenomic mechanisms that can regulate genes involved in pharmacokinetics or drug targets include DNA methylation and histone modifications. Although there is still much to be understood, epigenomics may contribute to our knowledge of diseases as well as our understanding of individual phenotypes such as acquired drug resistance.

FUTURE DIRECTIONS

Discoveries in pharmacogenomics are increasing as new technologies for genotyping are being developed and as access to patient DNA samples along with drug response information has accelerated. Increasingly, pharmacogenomics discoveries will move beyond single SNPs to multiple SNPs that inform both adverse and therapeutic responses. It is hoped that prescriber-friendly predictive models incorporating SNPs and other biomarkers as well as information on demographics, comorbidities, epigenetic signatures, and concomitant medications will be developed to aid in drug and dose selection. CPIC guidelines and Food and Drug Administration-stimulated product label changes will contribute to the accelerated translation of discoveries to clinical practice.

REFERENCES

Altman RB, Whirl-Carrillo M, Klein TE: Challenges in the pharmacogenomic annotation of whole genomes. Clin Pharmacol Ther 2013;94:211.

Bertilsson DL: Geographical/interracial differences in polymorphic drug oxidation. Clin Pharmacokinet 1995;29:192.

Browning LA, Kruse JA: Hemolysis and methemoglobinemia secondary to rasburicase administration. Ann Pharmacother 2005;39:1932.

Camptosar [irinotecan product label]. New York, NY: Pfizer Inc.; 2012.

Cappellini MD, Fiorelli G: Glucose-6-phosphate dehydrogenase deficiency. Lancet 2008;371:64.

Caudle KE et al: Clinical Pharmacogenetics Implementation Consortium guidelines for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing. Clin Pharmacol Ther 2013;94:640.

Chasman DI et al: Genetic determinants of statin-induced low-density lipoprotein cholesterol reduction: The Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) trial. Circ Cardiovasc Genet 2012;5:257.

Crews KR et al: Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for codeine therapy in the context of cytochrome P450 2D6 (CYP2D6) genotype. Clin Pharmacol Ther 2009;91:321.

Daly AK et al: HLA-B*5701 genotype is a major determinant of drug-induced liver injury due to flucloxacillin. Nat Genet 2009;41:816.

Elitek [rasburicase product label]. Bridgewater, NJ: Sanofi U.S. Inc.; 2009.

Gammal RS et al: Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for UGT1A1 and atazanavir prescribing. Clin Pharmacol Ther 2016;99:363.

Giacomini KM et al: International Transporter Consortium commentary on clinically important transporter polymorphisms. Clin Pharmacol Ther 2013;94:23.

Howes RE et al: G6PD deficiency prevalence and estimates of affected populations in malaria endemic countries: A geostatistical model-based map. PLoS Med 2012;9:e1001339.

Howes RE et al: Spatial distribution of G6PD deficiency variants across malaria-endemic regions. Malaria J 2013;12:418.

Johnson JA et al: Clinical Pharmacogenetics Implementation Consortium guidelines for CYP2C9 and VKORC1 genotypes and warfarin dosing. Clin Pharmacol Ther 2009;90:625.

Johnson JA, Klein TE, Relling MV: Clinical implementation of pharmacogenetics: More than one gene at a time. Clin Pharmacol Ther 2013;93:384.

Kim IS et al: ABCG2 Q141K polymorphism is associated with chemotherapy-induced diarrhea in patients with diffuse large B-cell lymphoma who received frontline rituximab plus cyclophosphamide/doxorubicin/vincristine/prednisone chemotherapy. Cancer Sci 2008;99:2496.

Lai-Goldman M, Faruki H: Abacavir hypersensitivity: A model system for pharmacogenetic test adoption. Genet Med 2008;10:874.

Lavanchy D: Evolving epidemiology of hepatitis C virus. Clin Microbiol Infect 2011;17:107.

Matsuura K, Watanabe T, Tanaka Y: Role of IL28B for chronic hepatitis C treatment toward personalized medicine. J Gastroenterol Hepatol 2014;29:241.

McDonagh EM et al: PharmGKB summary: Very important pharmacogene information for G6PD. Pharmacogenet Genomics 2012;22:219.

Minucci A et al: Glucose-6-phosphate dehydrogenase (G6PD) mutations database: Review of the "old" and update of the new mutations. Blood Cell Mol Dis 2012;48:154.

Moriyama T et al: NUDT15 polymorphisms alter thiopurine metabolism and hematopoietic toxicity. Nat Genet 2016;48:367.

Muir AJ et al: Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for IFNL3 (IL28B) genotype and peginterferon alpha based regimens. Clin Pharmacol Ther 2014;95:141.

Relling MV et al: Clinical Pharmacogenetics Implementation Consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing. Clin Pharmacol Ther 2009;89:387.

Russmann S, Jetter A, Kullak-Ublick GA: Pharmacogenetics of drug-induced liver injury. Hepatology 2010;52:748.

Scott SA et al: Clinical Pharmacogenetics Implementation Consortium guidelines for CYP2C19 genotype and clopidogrel therapy: 2013 update. Clin Pharmacol Ther 2013;94:317.

Shin J: Clinical pharmacogenomics of warfarin and clopidogrel. J Pharmacy Pract 2012;25:428.

Swen JJ et al: Pharmacogenetics: From bench to byte—An update of guidelines. Clin Pharmacol Ther 2009;89:662.

Tukey RH, Strassburg CP: Human UDP-glucuronosyltransferases: Metabolism, expression, and disease. Annu Rev Pharmacol Toxicol 2000;40:581.

Tukey RH, Strassburg CP, Mackenzie PI: Pharmacogenomics of human UDP-glucuronosyltransferases and irinotecan toxicity. Mol Pharmacol 2002;62:446.

Wen CC et al: Genome-wide association study identifies ABCG2 (BCRP) as an allopurinol transporter and a determinant of drug response. Clin Pharmacol Ther 2015;97:518.

WHO Working Group: Glucose-6-phosphate dehydrogenase deficiency. Bull World Health Org 1989;67:601.

Wilke RA et al: The Clinical Pharmacogenomics Implementation Consortium: CPIC guideline for SLCO1B1 and simvastatin-induced myopathy. Clin Pharmacol Ther 2009;92:112.

Xu J-M: Severe irinotecan-induced toxicity in a patient with UGT1A1*28 and UGT1A1*6 polymorphisms. World J Gastroenterol 2013;19:3899.

Yang J et al: Influence of CYP2C9 and VKORC1 genotypes on the risk of hemorrhagic complications in warfarin-treated patients: a systematic review and meta-analysis. Int J Cardiol 2013;168:4234.

Reviews

Campbell JM et al: Irinotecan-induced toxicity pharmacogenetics: An umbrella review of systematic reviews and meta-analyses. Pharmacogenomics J 2017;17:21.

Flockhart DA, Huang SM: Clinical pharmacogenetics. In: Atkinson AJ et al (editors): *Principles of Clinical Pharmacology*, 3rd ed. Elsevier, 2012.

Huang SM, Chen L, Giacomini KM: Pharmacogenomic mechanisms of drug toxicity. In: Atkinson AJ et al (editors): *Principles of Clinical Pharmacology*, 3rd ed. Elsevier, 2012.

Meulendijks D et al: Improving safety of fluoropyrimidine chemotherapy by individualizing treatment based on dihydropyrimidine dehydrogenase activity: Ready for clinical practice? Cancer Treat Rev 2016;50:23.

Relling MV, Giacomini KM: Pharmacogenetics. In: Brunton LL, Chabner BA, Knollmann BC (editors): *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 12th ed. McGraw-Hill, 2011.

Relling MV et al: Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for rasburicase therapy in the context of G6PD deficiency genotype. Clin Pharmacol Ther 2014;96:169.

Zanger UM, Schwab M: Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther 2013;138:103.

CASE STUDY ANSWER

Atazanavir inhibits the polymorphic UGT1A1 enzyme, which mediates the conjugation of glucuronic acid with bilirubin. Decreased UGT1A1 activity results in the accumulation of unconjugated (indirect) bilirubin in blood and tissues. When levels are high enough, yellow discoloration of the eyes and skin, ie, jaundice, is the result. The plasma levels of indirect bilirubin concentrations are expected to increase to greater than 2.5 times the upper limit of normal (grade 3 or higher elevations) in approximately 40% of patients taking once-daily atazanavir boosted with ritonavir and at least 5 times the upper limit of normal (grade 4 elevation) in approximately 4.8% of patients. Carriers of the *UGT1A1* decreased function alleles (**28/*28* or **28/*37*) have reduced enzyme activity and have an increased risk of atazanavir discontinuation. Genotyping showed that the patient was homozygous for the *UGT1A1*28* allele polymorphism. This probably led to the high levels of bilirubin and the subsequent discontinuation of atazanavir secondary to the adverse drug reaction of jaundice.