

## Genomic Medicine 2



## Pharmacogenomics

Dan M Roden, Howard L McLeod, Mary V Relling, Marc S Williams, George A Mensah, Josh F Peterson, Sara L Van Driest

Genomic medicine, which uses DNA variation to individualise and improve human health, is the subject of this Series of papers. The idea that genetic variation can be used to individualise drug therapy—the topic addressed here—is often viewed as within reach for genomic medicine. We have reviewed general mechanisms underlying variability in drug action, the role of genetic variation in mediating beneficial and adverse effects through variable drug concentrations (pharmacokinetics) and drug actions (pharmacodynamics), available data from clinical trials, and ongoing efforts to implement pharmacogenetics in clinical practice.

## Introduction

One tenet of clinical medicine is that patients vary in their response to drugs: doses effective in some patients will inevitably be ineffective or cause adverse drug reactions (ADRs) in others. ADRs have been implicated as an important cause of hospital admissions, in one series accounting for 6.5% of all hospitalisations in two large UK hospitals.<sup>1</sup> In the 1990s, a large survey suggested that ADRs occurring in hospitals were the fourth to sixth leading cause of in-hospital mortality in the USA,<sup>2</sup> and a follow-up survey in 2010 showed no improvement.<sup>3</sup> Fewer data are available on the consequences of the lack of efficacy, beyond recognising that only a proportion of a given patient population derives benefit from a given medication. The treatment of common diseases, such as hypertension, arrhythmias, or depression often involves a series of therapeutic trials among different drugs or classes of drugs, and the health-care burden imposed by lack of efficacy during these periods of trial and error can be considerable. For example, ineffective antidepressant therapy has been speculated to increase risk for suicide.<sup>4</sup>

There are many reasons for variability in drug response. The inability of selected drug therapy to target the underlying disease mechanism (which might or might not be known), drug interactions, disease-related changes in drug concentrations or responsiveness, poor compliance, and system errors, such as failure to deliver the correct drug or dose to the patient, are commonly cited. In some instances, therapeutic non-responsiveness and ADRs vary by race or ethnicity and can contribute to disparities in clinical outcomes.<sup>5,6</sup> This Series paper will address how variation in the germline genome affects drug response. Tumour sequencing, identification of driver mutations, and implementation of mutation-specific therapy, which are having a major impact in cancer, have been reviewed in detail elsewhere and will not be addressed further here.<sup>7</sup>

## Mechanisms underlying variable drug responses

Archibald Garrod, who developed the concept of inborn errors of metabolism, speculated a century ago that aberrant metabolism of exogenous substances could account for unusual reactions to food or drugs.<sup>8</sup> During

and after World War 2, the first instances of genetically determined ADRs were described, including haemolytic anaemia in African-American soldiers with G6PD deficiency exposed to antimalarials, malignant hyperthermia during anaesthesia, and prolonged paralysis following treatment with succinylcholine in patients with pseudocholinesterase deficiency. The term pharmacogenetics (panel) was coined by Motulsky<sup>14</sup> at the University of Washington, Seattle, WA, USA and Kalow<sup>15</sup> at the University of Toronto, Toronto, ON, Canada.

One review suggested that common genetic factors contribute to variable serious ADRs in a third of cases.<sup>16</sup> The field of pharmacogenomics aims to define these

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**Department of Medicine**  
(Prof D M Roden MD, J F Peterson MD, S L Van Driest MD), **Department of Pharmacology**  
(Prof D M Roden), **Department of Biomedical Informatics**  
(Prof D M Roden, J F Peterson) and **Department of Pediatrics**  
(S L Van Driest), **Vanderbilt University Medical Center, Nashville, TN, USA; DeBartolo Family Personalized Medicine Institute, Moffitt Cancer Center, Tampa, FL, USA**  
(Prof H L McLeod PharmD); **Pharmaceutical Department,**

## Panel: Comments on nomenclature

The term pharmacogenetics was coined in the 1950s and captures the idea that large effect size DNA variants contribute importantly to variable drug actions in an individual. The term pharmacogenomics is now used by many to describe the idea that multiple variants across the genome that can differ across populations affect drug response. The International Conference on Harmonisation, a worldwide consortium of regulatory agencies, has defined pharmacogenomics as the study of variations of DNA and RNA characteristics as related to drug response, and pharmacogenetics as the study of variations in DNA sequence as related to drug response.<sup>9</sup>

Pharmacogeneticists adopted a star nomenclature (eg, CYP2C19\*2) to describe variants in genes (sometimes termed pharmacogenes) underlying variability in drug response. Some star alleles can include more than one variant (eg, TPMT\*3A designates an allele defined by the presence of two single-nucleotide polymorphisms [SNPs]), and distinguishing this allele from those carrying only one of the SNPs can be challenging.<sup>10</sup> Although the star nomenclature persists, as our understanding of the numbers of variants in important pharmacogenes increases, attempts are being made to reconcile the notation with alternate variant nomenclature, such as the conventional rs designation.<sup>11,12</sup> Most variants studied to date partially or completely inhibit function of the encoded protein. Occasionally, variants increase activity of drug-metabolising enzymes; examples include CYP2C19\*17 and CYP2D6 duplications.

The field is also adopting a standard set of definitions of pharmacogenetic phenotypes; for pharmacokinetic genes these include normal metabolisers, poor metabolisers (carrying two loss-of-function alleles), intermediate metabolisers (carrying one loss-of-function allele), and ultrarapid metabolisers (carrying gain-of-function alleles or gene duplications) and for pharmacodynamic genes these include designations such as positive or negative for high-risk alleles.<sup>13</sup> These are convenient shorthand designations, which often have some overlap in drug response (figure 1A).

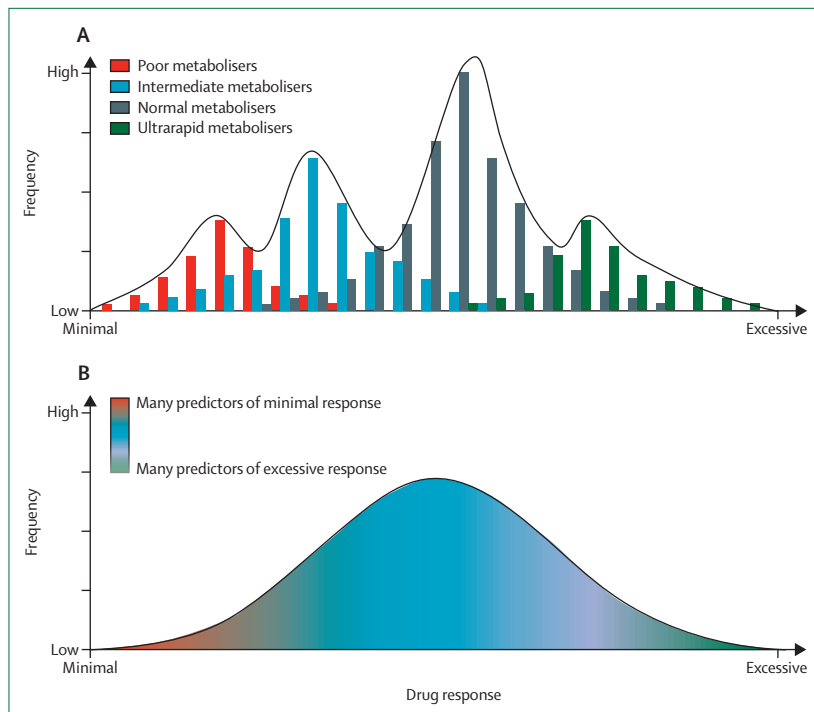


Figure 1: Profile of drug responses as influenced by a single pharmacogene variant (A) or multiple gene variants (B)

St Jude Children's Research Hospital, Memphis, TN, USA (Prof M V Relling PharmD); Genomic Medicine Institute, Geisinger, Danville, PA, USA (Prof M S Williams MD); and Center for Translation Research and Implementation Science, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA (G A Mensah MD)

Correspondence to: Prof Dan M Roden, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN 37232-0575, USA dan.roden@vumc.org

genetic mechanisms, and ultimately to implement genetic testing to improve drug efficacy and reduce toxicity. Furthermore, an understanding of the genetic basis of variable drug response can be used as a tool to expand the use of existing drugs to new indications and to develop new drugs. Well recognised examples of genetically determined variability in drug response often involve single DNA variants common in a population and associated with relatively large effect sizes and clearly definable metaboliser phenotypes (figure 1A). As a result, the implementation of pharmacogenomic information into the clinical flow of medicine has been viewed as within reach. However, several barriers are now identified and need to be overcome to routinely use pharmacogenomic variant data in improving drug prescribing.

Two conceptual pathways describe an organism's overall response to drug exposure. Pharmacokinetics defines variability in the processes (absorption, distribution, metabolism, and elimination) modulating delivery of drug and active metabolites to and removal from their site or sites of action. Pharmacodynamics describes variability in drug action that is not attributable to variable drug concentrations, which can reflect variability in the interaction of active drug with its effector molecules or other mechanisms such as variability in disease mechanisms. The earliest examples of pharmacogenomic variability involved variability in pharmacodynamic processes. With the development of robust methods to measure concentrations of drugs and

their metabolites in plasma and other sites in the 1960s and 1970s came the ability to define patients who are pharmacokinetic outliers in whom unusually high or low plasma concentrations were associated with variable efficacy or ADRs. This in turn led to studies defining variants in key drug metabolising or transport genes as the basis for these responses. More recently, agnostic methods such as the genome-wide association study (GWAS) have validated the role of these candidate genes and have identified new loci associated with variable drug responses.<sup>17</sup> The majority of clinically actionable pharmacogenetic traits described to date have a pharmacokinetic basis (table 1).

## Common genetic variants can produce large drug response effects

### Pharmacokinetic gene variation

Two scenarios illustrate how single gene variants affecting pharmacokinetics can have especially large effects. The first is with administration of a prodrug, a pharmacologically inactive substance that requires bioactivation by drug metabolism to achieve its therapeutic effects (figure 2). Such bioactivation pathways usually involve a single drug-metabolising enzyme and genetic variants that result in loss of function of these enzymes can decrease or block drug action. Examples include codeine bioactivated to its major active metabolite morphine by CYP2D6 and the antiplatelet drug clopidogrel bioactivated by CYP2C19. Although these effects are well established and might contribute to the perception that pharmacogenomic variants are within reach for implementation, it is important to recognise that there is a spectrum of even these large pharmacogenomic effects. Thus, in the case of clopidogrel, increasing the dose resulted in an antiplatelet effect in heterozygotes for *CYP2C19\*2* (the terminology for variants is further explained in the panel), encoding a+ common loss-of-function variant, because they still have demonstrable CYP2C19 activity. By contrast, a dose increase did not generate an antiplatelet effect in individuals homozygous for the variant because they completely lack CYP2C19 activity.<sup>18</sup> A GWAS of clopidogrel inhibition in 429 patients with ADP-related platelet activation resulted in very strong signals ( $p < 10^{-13}$ ) at the *CYP2C19* locus.<sup>19</sup> Although the pharmacological effect of *CYP2C19\*2* is large, the total variability in clopidogrel antiplatelet effect attributable to this variant was only 12%.<sup>19</sup> This effect is large for a single genetic variant; however, the finding also emphasises that other genetic and environmental factors have a role in observed variability in clopidogrel drug action.

Most variants studied to date confer partial or complete loss of function. However, gain-of-function variants in bioactivation pathways have been described and can be associated with excess drug response. Examples include *CYP2C19\*17*, which has been associated with bleeding during clopidogrel therapy,<sup>20</sup> and *CYP2D6* duplications,

which have been associated with an excess narcotic effect, including respiratory arrest, due to rapid and increased accumulation of morphine during codeine therapy (figure 2).<sup>21</sup>

The second situation in which single pharmacokinetic variants can exert very large effects is during administration of an active drug with a narrow therapeutic range (ie, a small margin between therapeutic and toxic doses), which undergoes elimination by a single drug metabolising system (figure 2). The antileukaemic drug 6-mercaptopurine is bioinactivated by TPMT and xanthine oxidase. Loss-of-function *TPMT* variants result in decreased inactivation, higher parent drug concentrations, and increased generation of cytotoxic thioguanine nucleotide metabolites; these nucleotides are incorporated into DNA and associate with drug effect. Individuals homozygous for loss-of-function variants in *TPMT* will exhibit life-threatening bone marrow toxicity with usual drug doses due to cytotoxic thioguanine nucleotide accumulation.<sup>22</sup> These nucleotides are themselves metabolised by *NUDT15*, and *NUDT15* loss-of-function variants have also been associated with toxicity.<sup>22,23</sup> The thiopurine immunosuppressant drug azathioprine is metabolised to 6-mercaptopurine and variants in *TPMT* and *NUDT15* are similarly associated with risk of haematological toxicity.<sup>22</sup>

Similarly, variants in *DPYD* increase plasma concentrations, and toxicity risk, of 5-fluorouracil and other fluoropyrimidines such as capecitabine.<sup>24</sup>

Notably, loss-of-function variants can be mimicked by interactions with drugs that inhibit the same drug metabolism pathways, described as a phenocopy. Examples of phenocopies include *CYP2D6* inhibition by some selective serotonin-reuptake inhibitors, *CYP2C19* inhibition by many proton-pump inhibitors, and xanthine oxidase inhibition by allopurinol, which by inhibiting an alternate pathway for azathioprine and 6-mercaptopurine metabolism, can increase generation of cytotoxic thioguanine nucleotides and thereby increase toxicity.

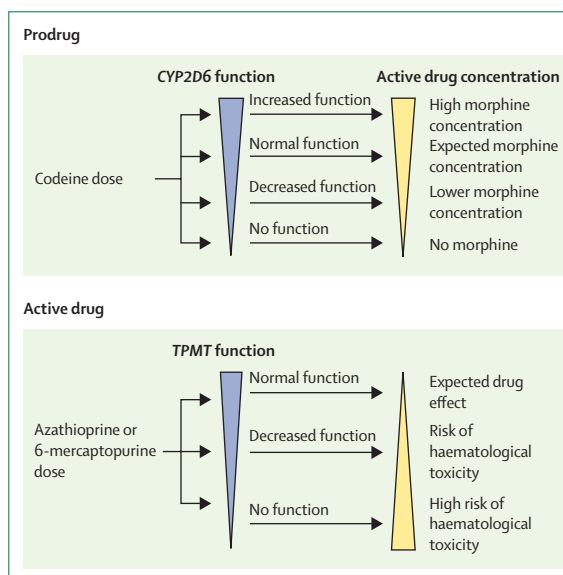
Drugs metabolised predominantly by a single enzyme but with wide therapeutic margins can have substantial variability in pharmacokinetics because of pharmacogenomic variants. However, because of the wide therapeutic margin, these pharmacokinetic differences might not drive clinically relevant variability in drug efficacy or toxicity. Similarly, drugs with narrow therapeutic margins that are inactivated by multiple enzymatic pathways are also less susceptible to unusual responses caused by pharmacogenomic variants, unless a combination of genetic variants or interacting drugs affects multiple pathways. For example, drug interactions or a disease inhibiting one metabolic pathway combined with genetic variation inhibiting a second pathway can account for unusual drug responses.<sup>25</sup>

Drug transport into and out of cells by specific drug transport molecules is another important potential

Drug	
<b>Pharmacokinetic mechanisms</b>	
<i>CYP2B6</i>	Efavirenz
<i>CYP2C19</i>	Clopidogrel, SSRIs, TCAs, voriconazole, proton pump inhibitors*
<i>CYP2C9</i>	Celecoxib <sup>†</sup> , phenytoin, warfarin
<i>CYP2D6</i>	Codeine, oxycodone, tramadol, SSRIs, TCAs, ondansetron, tamoxifen, atomoxetine
<i>CYP3A5</i>	Tacrolimus
<i>DPYD</i>	5-fluorouracil, capecitabine, tegafur
<i>TPMT</i> and <i>NUDT15</i>	Azathioprine, mercaptopurine, thioguanine
<i>SLCO1B1</i>	Simvastatin
<i>UGT1A1</i>	Atazanavir
<b>Pharmacodynamic mechanisms</b>	
<i>CFTR</i>	Ivacaftor
<i>CYP4F2</i>	Warfarin
<i>G6PD</i>	Rasburicase
<i>HLA-B</i>	Abacavir, allopurinol, carbamazepine, phenytoin
<i>IFNL3 (IL28B)</i>	Interferon
<i>RYR1</i> and <i>CACNA1S</i>	Inhaled anesthetics, succinylcholine
<i>VKORC1</i>	Warfarin
SSRI=selective serotonin reuptake inhibitor. TCA=tricyclic antidepressant.	
*Guidelines in progress.	

**Table 1: Drugs and genes with guidelines from the Clinical Pharmacogenetics Implementation Consortium for use in clinical practice**

For the Clinical Pharmacogenetics Implementation Consortium see <https://cpicpgx.org>



**Figure 2: The impact of variable pharmacokinetic gene function on the effect of bioactivation of prodrug versus inactivation of an active drug**

mediator of variable drug concentrations at effector sites and thus drug action. The drug efflux transporter *OATP1B1* encoded by *SLCO1B1* is responsible for removal of simvastatin from the systemic circulation. The common *SLCO1B1*\*5 loss-of-function variant has been associated with elevated simvastatin plasma concentrations and an increased risk for simvastatin

myopathy,<sup>26,27</sup> and contributes to variability in methotrexate clearance in children treated for acute leukaemia.<sup>28</sup>

Warfarin is a well studied example of a drug in which variable actions are determined by both pharmacokinetic and pharmacodynamic gene variants, and in which variant frequency is highly dependent on ancestry. Warfarin is administered as a racemate, and bioinactivation of the more active S-enantiomer is accomplished by CYP2C9. Gene variants that decrease CYP2C9 activity are therefore associated with an increase in S-warfarin plasma concentration and a resultant intensified pharmacological effect, manifest as an increase in the international normalised ratio (INR) or bleeding risk. The *CYP2C9\*2* and *CYP2C9\*3* variants are most common in European ancestry populations; *CYP2C9\*3* reduces CYP2C9 activity to a greater extent than does the *CYP2C9\*2* variant. Thus, patients heterozygous for *CYP2C9\*2* might exhibit only a small pharmacogenomic effect, whereas patients homozygous for *CYP2C9\*3* might exhibit drastic decreases in warfarin dose requirement, and can be difficult to anticoagulate because of day-to-day variability in INR.<sup>29,30</sup> In populations of African ancestry, these variants are rarer and other variants have been reported.<sup>31,32</sup>

Pharmacodynamic variation also influences warfarin effect. Traditional genetic linkage methods identified loss-of-function variants in *VKORC1* as the cause of the rare syndrome of familial warfarin resistance, an absence of a rise in INR even with exposure to very large doses of warfarin;<sup>33</sup> subsequent studies showed that *VKORC1* encodes the warfarin target. A common promoter polymorphism in *VKORC1* is associated with variability in hepatic mRNA concentrations and in warfarin dose requirement.<sup>34</sup> Moreover, rarer reduction-of-function coding region variants in *VKORC1*, associated with increased warfarin dose requirements, have been described and vary by ancestry; for example, a variant encoding D36Y is common (minor allele frequency of 5%) in Ashkenazi populations.<sup>35</sup>

Multiple GWAS of variability in warfarin steady state dose requirements have yielded very strong signals at *CYP2C9*, *VKORC1*, and at *CYP4F2* (a gene responsible for bioinactivation of vitamin K).<sup>36–39</sup> In African-American patients, a GWAS identified a separate signal (whose specific function remains to be defined) near *CYP2C8–CYP2C9*.<sup>32</sup> An estimated 50% of the variability in warfarin dose requirement has been attributed to common genetic variation identified in these studies.

#### Other pharmacodynamic gene variants

As mentioned above, some of the earliest well defined pharmacogenetic syndromes involve pharmacodynamic mechanisms. The risk of malignant hyperthermia on exposure to inhaled anaesthetics or succinylcholine is mediated by variants in *RYR1* or *CACNA1S*.<sup>40</sup> Variants reducing G6PD function caused a high incidence of haemolytic anaemia in African-American soldiers exposed

to antimalarials during World War 2 and increase the risk for haemolytic anaemia and methaemoglobinaemia with rasburicase, a recombinant urate oxidase used to treat hyperuricemia.<sup>41</sup> Variants in *IFNL3* (also known as *IL28B*) predict response to pegylated interferon alpha and ribavirin in hepatitis C although the introduction of newer therapeutics has reduced the impetus for genotyping.<sup>42</sup>

ADRs described to this point are related to exaggerated drug effect, sometimes due to high plasma concentrations, such as bleeding with anticoagulants or hypotension with antihypertensives, and these have been termed type A ADRs. Type B ADRs are those unrelated to the drug's known and intended pharmacological effects and are often considered non-dose-dependent. Type B reactions include serious immunologically mediated ADRs such as the Stevens-Johnson syndrome and toxic epidermal necrolysis (SJS/TEN). Candidate gene and GWAS approaches that use very small case numbers, often less than 100, and large numbers of drug-exposed controls, have implicated specific *HLA* variants in SJS/TEN. These studies also highlight the importance of ancestry in drug response. For example, *HLA-B\*15:02* confers risk of carbamazepine-related SJS/TEN in southeast Asia where the allele is relatively common.<sup>43</sup> In European ancestry populations, however, this allele is rare, and a different *HLA* risk allele (*HLA-A\*31:01*) has been implicated.<sup>44</sup> In these cases, the *HLA* variant is judged necessary, but not sufficient to induce the immunological response.<sup>45</sup> In fact, a very strong association exists between flucloxacillin-related hepatotoxicity and *HLA-B\*57:01*,<sup>46</sup> but it has been estimated that only one case will develop for every 13 000 patients with the *HLA-B\*57:01*-positive genotype who have been exposed to the drug.<sup>45</sup> For other drugs, the number needed to test is smaller (eg, in the case of abacavir,<sup>47</sup> the number needed to test in patients with *HLA-B\*57:01* is 13). Variable susceptibility to type B reactions also depends on plasma drug concentration. For example, *HLA* variants associate with ADRs caused by the antiseizure medication phenytoin, a CYP2C9 substrate, and several studies have reported that risk of ADRs is increased in patients who also carry CYP2C9 loss-of-function alleles.<sup>48,49</sup>

## Implementing pharmacogenomics

### Clinical trial data

Because preclinical and clinical mechanistic studies support the role of genetic variation as a contributor to variable drug responses, retrospective analyses and prospective trials have been mounted to test the hypothesis that pharmacogenomically guided therapy will improve clinical drug outcomes.

After candidate gene studies identified *HLA-B\*57:01* as a strong risk factor for abacavir-related SJS/TEN,<sup>50</sup> a randomised controlled trial (RCT) was done in 1956 patients to compare conventional antiretroviral regimens, including abacavir, to a pharmacogenomically guided

strategy in which abacavir was dropped from treatment if the *HLA-B* risk allele was present.<sup>47</sup> A rash, thought to be related to abacavir, developed in 7.8% of controls and 3.4% of patients in the pharmacogenomically guided group. However, subsequent protocol-mandated skin testing confirmed that the rash was related to abacavir in 2.7% of controls and in none of the patients in the pharmacogenomically guided group. This unambiguous outcome resulted in the US Food and Drug Administration (FDA) label requiring preprescription testing for *HLA-B\*57:01* in all individuals starting abacavir treatment and not using the drug in genotype-positive patients.

An RCT compared standard therapy to pharmacogenomically guided dosing in 783 patients starting treatment with azathioprine or 6-mercaptopurine for inflammatory bowel disease.<sup>51</sup> *TPMT* intermediate metabolisers (defined in the panel) received 50% of the standard dose while poor metabolisers received 0–10% of the standard dose. Overall, serious ADRs or disease progression did not differ in the genotype-guided versus standard therapy groups. However, among the 78 patients with *TPMT* loss-of-function variants (77 intermediate metabolisers and one poor metaboliser), a benefit of pharmacogenomically guided therapy was clear: the incidence of serious haematological ADRs was 22.9% in the control group versus 2.6% in the pharmacogenomically guided group (relative risk 0.11, 95% CI 0.01–0.85). These results highlight the fact that any benefit of pharmacogenomic testing will be confined to the subset in whom the target genetic variants are present, and that the apparent benefits will be diluted if testing is evaluated in the entire population comprising mostly low-risk patients. As discussed further in this Series paper, most patients harbour one or more functionally important variants in key pharmacogenes, suggesting that pre-emptive testing of a panel of multiple pharmacogenes should be a strategy to be considered for pharmacogenetic implementation.

Retrospective analyses of the effect of common genetic variants on outcomes after clopidogrel was initiated for acute coronary syndrome have shown a consistent effect of loss of function genotypes.<sup>52,53</sup> Investigators in the Implementing Genomics in Practice (IGNITE) network summarised outcomes of genotyping to direct the choice of antiplatelet therapies between clopidogrel and alternate therapies in patients with *CYP2C19* loss-of-function alleles. Among 1815 patients at seven institutions, those with loss-of-function alleles (31.5%) had more cardiovascular events if treated with clopidogrel compared with treatment with alternate drugs (23.4/100 patient-years vs 8.7/100 patient-years, hazard ratio 2.26, 95% CI 1.18 to 4.32;  $p=0.013$ ).<sup>54</sup> One small prospective RCT reported a large decrease in late coronary events with a pharmacogenomically driven strategy for clopidogrel.<sup>55</sup> Nevertheless, to date, cardiovascular professional societies have not recommended genetic testing to guide clopidogrel therapy, despite the fact that some have

argued the evidence is stronger than for other recommended tests.<sup>56</sup>

Multiple large RCTs have evaluated the effect of a pharmacogenomically driven strategy including intensive INR monitoring versus a conventional clinical approach for warfarin. The first three large trials<sup>57–59</sup> used a primary endpoint of time in therapeutic INR range or time required to achieve stable anticoagulation. Two studies used a clinical algorithm as the control,<sup>57,58</sup> and one used a clinically conventional fixed-dose regimen.<sup>59</sup> The fixed-dose study showed a significant improvement in the primary outcome, whereas no difference in outcome was reported in the other two studies. The largest of these trials, the US-based Clarification of Optimal Anticoagulation Through Genetics (COAG), included 27% African-American patients and integrated *CYP2C9* variants that are much more common in European ancestry individuals, while other *CYP2C9* variants that have a role in patients of African origin were not assayed.<sup>60</sup> As a result, the null result in COAG has been speculated to reflect, in part, a lack of considering ancestry-specific genetics.<sup>61</sup>

Several other RCTs have reported that pharmacogenomically guided warfarin therapy improves outcome. The Genetic Informatics Trial (known as GIFT)<sup>62</sup> randomly assigned 1650 patients following hip or knee replacement to a warfarin dose strategy guided clinically or by genotype and focused on the primary outcome of warfarin-related ADRs (major bleeding, INR >4, venous thromboembolism, and death) rather than time in therapeutic range. The primary endpoint occurred in 10.8% of patients in the genotype-guided group versus 14.7% in the clinically guided group ( $p=0.02$ ). An RCT in southeast Asia showed that a pharmacogenomically guided strategy resulted in fewer dose titrations in the first 2 weeks of therapy (the primary endpoint for the trial).<sup>63</sup>

In all these warfarin trials, the frequency of serious bleeding was low, and none of the trials were powered to detect an effect of genotype on bleeding itself. Retrospective analyses of large numbers of patients presenting with warfarin-related bleeding, ascertained through administrative databases or electronic health records (EHRs), have reported a significant effect of *CYP4F2 V433M* (odds ratio [OR] 0.62, 95% CI 0.43–0.91)<sup>64</sup> and of *CYP2C9\*3* (adjusted OR 2.05, 95% CI 1.04–4.04).<sup>65</sup> A smaller study of African-Americans with bleeding attributed to warfarin at INR values of less than 4 identified variants thought to regulate expression of *EPHA7*, a gene expressed in the vascular endothelium.<sup>66</sup>

The feasibility of a pharmacogenetically driven strategy with dose adjustment based on four *DPYD* variants was evaluated in 1103 patients receiving fluoropyrimidines. There were 85 variant carriers, and although they had a higher incidence of serious toxicity compared with non-carriers, the rates were lower than those seen in historical controls.<sup>24</sup>

These trials have identified many major issues (table 2). A genetic testing strategy for an individual drug can only show benefit in patients with the variant genotype. In the case of drug metabolising enzymes and drug transporters, the pharmacogenomic effect size is much larger in homozygotes than in heterozygotes. Although trials can be mounted with surrogate endpoints, such as time in therapeutic range, acceptance by the clinical practice community, and thus the payer community, is more likely to occur if data are available on a hard outcome such as death. However, the study of these clinical endpoints might require very large studies even if only high-risk populations are included. These issues

contribute to slow uptake of genetic testing for warfarin and clopidogrel, as does increasing availability of alternate therapies, which appear to be at least as effective without known major pharmacogenomic issues identified to date. By contrast, uptake is more likely when alternate drugs are not available or when ADRs are serious and clearly related to genetic variants, particularly if a regulatory agency or professional society recommends testing, as in the case of abacavir.

#### Current status

Experiments that implement pharmacogenomics have used a point-of-care strategy or pre-emptive strategy. The

	Example	Perceived obstacle	Potential solutions
<b>Pharmacogenes</b>			
Majority of individuals in most populations are wild type	Less than 1% of individuals are TPMT poor metabolisers <sup>67</sup>	Very large numbers needed to test for successful prospective trials and for clinical benefit	Prespecify plan to analyse subset with variant; and conduct trials across multiple drugs and genes, which inform panel-based testing
Rare variants with uncertain effect	46 of 64 haplotypes for <i>CYP2C9</i> have unknown function <sup>68</sup>	Insufficient data to ascertain phenotype with absolute certainty	Assay only variants with known function; include uncertainty on clinical reports; and functional studies
Spectrum of effects due to variants within one gene	Distinct variants in <i>CYP2C19</i> confer complete loss of function, partial loss of function, or gain of function	Need to express genetic effect as quasi-continuous trait	Use activity scores to annotate variant effect
Complexity of gene assays	Different assay technologies required for <i>CYP2C19</i> , <i>CYP2D6</i> , and HLA	Lack of comprehensive local infrastructure for multiple laboratory developed tests	Development of off-the-shelf assays for pharmacogenes; and reliance on send-out laboratories for some or all pharmacogenomic testing
<b>Drug effects</b>			
Hard endpoints are rare	No deaths recorded in the 1650 patients randomly assigned to treatment with warfarin in the GIFT trial <sup>62</sup>	Robust methods to prove impact of genotype-guided therapy on hard endpoints not well developed	Use surrogate, but clinically relevant, endpoints such as major bleeding, length of hospitalisation, symptom control, or health-care cost; and do large retrospective analyses of hard endpoints using EHR-linked biobank data
Efficacy endpoints poorly defined outside of clinical trials	Serial assessment of depression symptoms inconsistently documented in EHR data	Cannot do retrospective analyses on efficacy	Prospective data collection with oversampling of participants with pharmacogenetic variants
<b>Health-care institutions and local health information technology</b>			
Results for each gene require interpretation to discrete clinical guidance	Clinical decision support for warfarin provides dosing calculation, not genetic test results	Lack of technological infrastructure for interpretation from gene test results to functional effect to dosing guidance	Widespread sharing of technical solutions and clinical decision support across institutions
Functional predictions and clinical guidance evolve with new evidence	New evidence for the role of <i>NUDT15</i> variants in thiopurine toxicity <sup>23</sup>	Need to continually assess evidence, which is consistently expanding to include more drugs and more genes	Continued support for development of guidelines to guide appropriate testing
Provider resistance to receiving or using pharmacogenomic information	No agreement among health-care providers about who should take responsibility for results <sup>69</sup>	Limited ordering of pharmacogenomic testing or lack of use of pharmacogenomic guidance	Identification and recruitment of clinical champions for specific drug-gene interactions; increased provider education; and interruptive prescriber alerts making the pharmacogenomic-informed choices the default
Evolving EHR systems	EHR system changes or upgrades might cause loss of reporting or decision support functionality	Large ongoing costs of system maintenance	Commitment from EHR vendors for continual support of pharmacogenomic implementation; and computable guidelines for pharmacogenomics
<b>Health-care systems</b>			
Patient movement across EHR systems	A patient's pharmacogenomic results do not follow them when they receive care in another system	Loss of potential benefit of test or potential for repeat testing	Provision of pharmacogenetic results to patients; and portability of results for transfer to other EHR systems
Diversity of pharmacogenomic assays	Depending on <i>TPMT</i> genotype interpretation, a patient might be labelled as poor or intermediate metaboliser	Lack of consistency of results across CLIA-approved tests	Standardisation of minimal test requirements; and standardisation of interpretation of variant effects
Reimbursement challenges	Pharmacogenomic testing is variably reimbursed across clinical scenarios, states, genes-drugs, and payers	Pharmacogenomic testing is not cost-effective	Increase data available on cost benefit and improve and standardise analyses to promote reimbursement; and develop comprehensive cost-effectiveness model as opposed to models for individual drug-gene pairs

TPMT=thiopurine s-methyltransferase. GIFT=Genetic Informatics Trial. EHR=electronic health records. CLIA=Clinical Laboratory Improvement Amendments.

**Table 2: Issues, obstacles, and potential solutions in pharmacogenomic implementation**

point-of-care strategy uses genetic testing, generally with very rapid turnaround times, for a small number of individual variants when a target drug such as clopidogrel is prescribed.<sup>54</sup> Conversely, the pre-emptive strategy generates variant data for multiple pharmacogenes ideally before prescription of any target drug.<sup>70,71</sup> Variant data are then embedded in EHRs and coupled to clinical decision support, which delivers advice when a target drug is prescribed in a patient with variant genetics. Implementing such a pre-emptive strategy requires well curated data relating individual genetic variants (and their combinations as haplotypes or diplotypes), designation of predicted metaboliser phenotype status (eg, normal metabolisers, poor metabolisers; panel), and advice on alternate therapeutic strategies in patients with genetic variants. Thus, a barrier to early adoption was the need for extensive curation of the pharmacogenomic evidence, expert design of the pharmacogenomic test, curation of predicted consequences of the genetic variants, clinical expertise regarding drug prescribing and alternatives, and technical expertise to support laboratory testing, reporting, and decision support. Many of these needs are now being met by evidence curation by PharmGKB and by the development of guidelines in the USA and in Europe by the Clinical Pharmacogenetics Implementation Consortium<sup>72</sup> and the Dutch Working Group<sup>73</sup> on pharmacogenetics. These largely independent efforts have generated similar guidelines across multiple drugs.<sup>74</sup>

Efforts to implement pharmacogenomics have also been supported by economic analyses for many of the common pharmacogenomic scenarios, such as *CYP2C19*-tailored selection of antiplatelet agents following percutaneous coronary intervention<sup>75</sup> or selection of abacavir for HIV therapy.<sup>76</sup> Although most analyses find testing to be cost-effective when genetic test costs were minimised, they have not always led to changes in guideline recommendations or reimbursement policies.<sup>77</sup> Indeed, lack of evidence for cost-effectiveness, and thus lack of reimbursement, has been identified as a major barrier for implementation of pharmacogenetic testing: one systematic review of cost-effectiveness studies in pharmacogenomics made the comment that “these issues imply that cost-effectiveness analyses on their own cannot answer the question of whether or not a certain strategy should be used and funded, but should be considered in conjunction with other factors such as the available resources, the number of patients who benefit from the intervention and other ethical considerations”.<sup>77</sup>

Regulatory responses to pharmacogenomic variant data are evolving. Although the US FDA includes pharmacogenomic information in over 100 drug labels,<sup>78</sup> it has also included black-box warnings against the use of certain drugs or doses even when ADR risk is thought to be genetically mediated. Thus, for example, the label limits simvastatin doses to no more than 40 mg per day because higher doses increase the risk of myopathy, although this risk is nearly completely confined to patients with an

*SLCO1B1* risk variant.<sup>77</sup> Similarly, codeine can produce respiratory depression particularly post-tonsillectomy and in young patients. The label now recommends against the use of the drug in this setting<sup>21</sup> although the risk seems confined to those with the ultrarapid metaboliser phenotype.<sup>79</sup> This labelling might result in prescription of more potent opioids with attendant risks of other adverse effects.<sup>80</sup>

Although *HLA-B\*15:02*, associated with carbamazepine SJS/TEN, is especially prevalent in southeast Asia, there is controversy about whether compulsory testing is cost-effective.<sup>81,82</sup> In Hong Kong, implementation of a testing programme resulted in a decrease in the prescription of carbamazepine (and a decrease in related SJS/TEN), but an increase in the prescription of other antiseizure medications and no overall change in SJS/TEN.<sup>83</sup> These data emphasise a need for implementation programmes to include an educational component.

Thus, issues such as return on investment for adopter health-care systems and reimbursement across payers remain unsettled. In oncology, adoption has been faster perhaps in part because tumour genetic testing allows definition of subsets of patients in whom therapy will not be effective, thus placing a limit on widespread use of expensive therapies. By contrast, pharmacogenomic variants identifying patients at risk for ADRs during treatment with the older, cheaper drugs like warfarin or clopidogrel identifies individuals who will benefit from a more expensive drug. The fragmented nature of health-care reimbursement in the USA represents a further barrier in that pharmacogenomic test results generated at one site might not be available if the patient moves to a different provider in another health-care or EHR system.

Several reports have shown that when pharmacogenomic testing across multiple drug–gene pairs is done, most individuals have variants that would be important if they were prescribed specific target drugs.<sup>68,84,85</sup> These data add to the appeal of the pre-emptive pharmacogenomic strategy. Identifying patients in whom the strategy is likely to be effective (ie, those in whom target drugs are likely to be prescribed over the next several years) is one challenge.<sup>86</sup> Another is practitioner reluctance to switch prescriptions in the face of pharmacogenomic variant data. Reasons for such reluctance include individual preference, late delivery of genotype data, lack of familiarity with pharmacogenomic information, and expense or risk of alternate therapies.<sup>69</sup>

Engineering the EHR to accommodate pharmacogenomic data and to deliver clinical decision support is another challenge. Addressing this challenge includes developing and implementing robust methods for translating raw genetic data into predicted drug responses (eg, by assignment of predicted pharmacogenetic phenotypes from variants in pharmacogenes). Although single gene-based systems can accomplish this task using human interpretation or non-machine-

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readable (often in pdf format) reports, multiplexed programmes increasingly rely on automated omic ancillary systems<sup>87</sup> to integrate genomic data into EHR-based clinical workflows. A survey of ten health-care systems that adopted pharmacogenomic clinical decision support identified non-specific barriers, such as staffing and coordination across multiple teams, rather than pharmacogenomic-specific ones.<sup>88</sup> Maintenance and updating of variant translations and clinical decision support recommendations is another EHR challenge shared with any use of genetic information in clinical care.

### Role of genomics in the drug development process

Only a very small number of drug candidates entering clinical trials ultimately achieve regulatory approval. Available evidence strongly supports the idea that drugs with targets validated by human genetic studies have a much higher likelihood of successful marketing than drugs lacking such evidence.<sup>89,90</sup> Thus, accumulating this evidence is becoming an increasingly important part of the drug development process. Approaches that are being explored include not only GWAS but also EHR-based phenome scanning (ie, examination of the association between specific variants in candidate drug target genes and phenotypes across the EHR).<sup>91,92</sup>

The identification of rare sequence variants that appear to associate with important human phenotypes has also provided the basis for new drug development. Perhaps the most notable example is *PCSK9*, in which gain-of-function variants were initially associated with striking elevation in LDL cholesterol and familial hypercholesterolaemia.<sup>93</sup> Subsequently, the Dallas Heart Study<sup>94</sup> showed that rare truncation (ie, loss-of-function) variants, occurring largely in African-Americans, were associated with striking decreases in LDL cholesterol and in the Atherosclerosis Risk in Communities cohort a striking decrease in lifetime risk of coronary artery disease. These data propelled to the market the development of PCSK9 inhibitors for treatment of elevated LDL cholesterol. Notably, the indications extend beyond familial hypercholesterolaemia itself, and although the drugs are indicated across ancestries, the original discovery was enabled by studying an African-American cohort. Other drug targets implicated or validated by identifying rare sequence variants associated with unusual phenotypes include *APOC3* for hypertriglyceridaemia,<sup>95</sup> *NPC1L1* (encoding the ezetimibe target) for cholesterol transport,<sup>96</sup> *SLC30A8* for prevention of obesity-related diabetes,<sup>97</sup> *ANGPTL4* for or hyperlipidaemia,<sup>98,99</sup> and *HSD17B13* for reduced risk of chronic liver injury.<sup>100</sup>

Another area in which human genetics is playing a major role in the development of new drugs is in the development of new therapies for rare mendelian diseases. In cystic fibrosis, one relatively minor mechanism for dysfunction of the CFTR protein is

altered conductance of channels that traffic normally to the cell surface. Ivacaftor, a conductance defect corrector, has been associated with improvement in functional status,<sup>101</sup> and is now marketed for patients who carry specific germline variants that have been tested in clinical trials or show ivacaftor-mediated improvement in function in vitro. The most common functional defect in cystic fibrosis is failure of channels to traffic to the cell surface, and lumacaftor has been developed and marketed (with ivacaftor) for this indication.<sup>102</sup> A preliminary study suggests lumacaftor can also correct mistrafficking of cardiac potassium channels in one form of the long QT syndrome suggesting this drug or others correcting mistrafficking of cell proteins can have more widespread applicability.<sup>103</sup>

### Conclusions and future directions

The field of pharmacogenomics has been focused on a few common gene variants with large effect sizes. However, the fundamental impact of pharmacogenomic variants varies from heterozygotes with reduction-of-function alleles to homozygotes for complete loss-of-function alleles in genes crucial for the disposition of individual drugs. This spectrum of effects has complicated the design and conduct of large clinical trials that often focus on individual drugs.

Genome science is providing new tools for understanding variability in drug response. One obvious area is the increasing use of exome or genome sequencing with the attendant recognition of very large numbers of rare missense variants in all genes. It is likely that some variant drug responses must reflect the effect of such rare variants, alone or in combination, but most have not yet been characterised. Pharmacogenomics has focused on a few candidate genes, generally derived from a clear understanding of the mechanisms of underlying variability in drug action, notably in pharmacokinetics, pharmacodynamics, and immunopharmacogenomics. The extent to which an understanding of variability in drug action will be improved by moving beyond a candidate gene approach to considerations of the contribution of variants in multiple genes (figure 1B) remains to be determined. One interesting example is the use of genetic risk scores, derived from multiple genetic variants that individually contribute a small amount to a variable phenotype but might confer larger effect sizes when present in combination. A GWAS identified no individual large effect size variants for drug-induced QT prolongation and associated polymorphic ventricular arrhythmias,<sup>104</sup> but a subsequent analysis with a genetic risk score, derived from 61 individual variants identified in a GWAS of the QT interval itself, readily separated cases from controls.<sup>105</sup> Similarly, a genetic risk score derived from baseline neuropsychiatric traits predicted response to antidepressant therapies.<sup>106</sup> A set of 13 variants increased the area under the receiver



operating curve from 0.64 to 0.81 in a clinical trial studying drug response in patients with advanced breast cancer.<sup>107</sup> The extent to which these multigene markers can identify the genetic architecture of disease and its response to drugs remains an interesting but as yet largely unexplored area in the field of drug response and toxicity. It might also be useful to intensively study individuals with clear outlier responses to drug exposure (eg, to measure plasma drug and metabolite concentrations or to search for rare as-yet-uncharacterised variants in key pharmacogenes).

Several trials are ongoing that might further inform the field. TAILOR-PCI (NCT01742117) and POPular Genetics (NCT01761786) are comparing the effect of a pharmacogenomically informed strategy to conventional strategies in the use of clopidogrel and other antiplatelet therapies. These trials aim to enrol 5270 and 2700 patients respectively and should report the findings by mid-2020. The CETP inhibitor dalcetrapib was tested in 15 871 patients and did not show any difference in a primary cardiovascular endpoint.<sup>108</sup> However, a subsequent analysis of 5749 patients who provided DNA samples identified variants in *ADCY9* as markers of a potentially beneficial response to drug therapy,<sup>109</sup> and in vitro and animal studies have supported a role for *ADCY9* in this drug's action.<sup>110</sup> A large trial, dal-GenE (NCT02525939), is underway to screen approximately 35 000 patients to identify around 6000 with the predicted response allele, and to then randomly assign these patients to dalcetrapib or placebo. The study cohort has been accrued and is currently in follow-up.

The PREemptive Pharmacogenomic Testing for Preventing Adverse Drug Reactions (PREPARE) study of the EU's Ubiquitous Pharmacogenomics Study group is evaluating a pre-emptive pharmacogenomic testing strategy in 12 genes to reduce the incidence of ADRs related to 43 target drugs.<sup>111</sup> PREPARE, which uses a crossover design, is being done at seven sites across Europe, and is randomly assigning patients to a pharmacogenomically guided strategy, with dose adjustments, compared with a conventional dosing strategy. The study was powered to detect a 30% decrease in clinically relevant ADRs, from 4% to 2.8%, and is scheduled to report in 2020. IGNITE is currently planning an evaluation of panel-based testing for management of depression, chronic pain, and acute postoperative pain.

Large personalised medicine programmes that include extensive genotyping or sequencing are being put in place across the globe. Some focus on single diseases, some are more broad-based, but do not include a return of results capability, and others plan whole genome sequencing with return of results to participants and health-care providers. The whole genome programmes include Genome England, aiming to sequence up to 5 000 000 whole genomes, and the US All of Us Program, recruiting 1 000 000 participants.

Variability in response, notably in ADR risk, is a near-inevitable feature of contemporary drug therapy and

includes a prominent genetic component. Defining that genetic component and understanding how best to apply that knowledge in a clinical context are ongoing challenges to pharmacogenomic science. The advent of inexpensive genotyping and sequencing and the development of increasingly sophisticated EHR systems holds the promise of implementing pharmacogenomic variant information that will become a routine part of the practice of genomic medicine.

#### Contributors

All authors contributed to the organisation and scope of the manuscript, editing of the initial draft, and revision. DMR generated the initial draft and reviewed the manuscript.

#### Declaration of interests

HLM is a member of the board of directors of Cancer Genetics Inc and a scientific advisor to Pharmazam. JFP is a consultant for Color Genomics. SLVD has received a speaking honorarium from Merck. All other authors declare no competing interests.

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