

# UCSF

## UC San Francisco Previously Published Works

### Title

Genomic architecture of pharmacological efficacy and adverse events

### Permalink

<https://escholarship.org/uc/item/50m5m315>

### Journal

Pharmacogenomics, 15(16)

### ISSN

1462-2416

### Authors

Chhibber, Aparna  
Kroetz, Deanna L  
Tantisira, Kelan G  
[et al.](#)

### Publication Date

2014-12-01

### DOI

10.2217/pgs.14.144

Peer reviewed



## Genomic architecture of pharmacological efficacy and adverse events

The pharmacokinetic and pharmacodynamic disciplines address pharmacological traits, including efficacy and adverse events. Pharmacogenomics studies have identified pervasive genetic effects on treatment outcomes, resulting in the development of genetic biomarkers for optimization of drug therapy. Pharmacogenomics-based tests are already being applied in clinical decision making. However, despite substantial progress in identifying the genetic etiology of pharmacological response, current biomarker panels still largely rely on single gene tests with a large portion of the genetic effects remaining to be discovered. Future research must account for the combined effects of multiple genetic variants, incorporate pathway-based approaches, explore gene–gene interactions and nonprotein coding functional genetic variants, extend studies across ancestral populations, and prioritize laboratory characterization of molecular mechanisms. Because genetic factors can play a key role in drug response, accurate biomarker tests capturing the main genetic factors determining treatment outcomes have substantial potential for improving individual clinical care.

**Keywords:** genetic architecture • genomics • heritability • linear mixed modeling • pharmacogenomics • polygenic architecture • polygenic modeling

The NIH has envisioned translation of the detailed information collected about the human genome into improvements in human health and well being [1]. Pharmacogenomics (PGx), the study of how genetic architecture influences pharmacological traits and outcomes, provides immediate applications for directing clinical decision making. Pharmacogenomic information can aid choices about selecting pharmacological treatments, optimal time courses and drug dosage on the basis of a patients' genetic architecture.

Interpatient variability manifests itself in different ways for pharmacological traits, described quantitatively by pharmacokinetics (PK) and pharmacodynamics (PD). Pharmacological traits include efficacy, adverse events and the balance between efficacy and toxicity, defining the therapeutic 'window.' Further definitions of these terms are presented in **Box 1**. By connecting genetic

variation to measurable interpatient variability, a course of action can be defined on an individual basis.

Investigating the genetic architecture of PGx traits can be pursued in multiple ways, and each of the approaches taken has advantages and limitations, discussed further in this review. PGx studies typically reveal SNP biomarkers that link genetic variation to treatment outcomes. Elucidating the underlying molecular genetic mechanism underlying the association between genetic variants and pharmacological traits is a major goal in the PGx field. In contrast, genome-wide association studies (GWAS) of complex diseases have yielded numerous variants with significant associations, but for a vast majority of these results, the causative variants and mechanisms remain unknown [6]. In addition, pharmacogenomic variants tend to exert stronger effects on drug response phenotypes than those discovered for complex disor-

Aparna Chhibber<sup>1</sup>, Deanna L Kroetz<sup>1</sup>, Kelan G Tantisiria<sup>2</sup>, Michael McGeachie<sup>2</sup>, Cheng Cheng<sup>3</sup>, Robert Plenge<sup>4</sup>, Eli Stahl<sup>5</sup>, Wolfgang Sadec<sup>6</sup>, Marylyn D Ritchie<sup>\*7</sup> & Sarah A Pendergrass<sup>7</sup>

<sup>1</sup>Department of Bioengineering & Therapeutic Sciences, Department of Pharmaceutical Chemistry, University of California San Francisco, San Francisco, CA, USA

<sup>2</sup>Department of Medicine, Brigham & Women's Hospital, Harvard Medical School, Cambridge, MA, USA

<sup>3</sup>Department of Biostatistics, St Jude Children's Research Hospital, Memphis, TN, USA

<sup>4</sup>Division of Rheumatology, Immunology & Allergy, Division of Genetics, Brigham & Women's Hospital, Harvard Medical School, Cambridge, MA, USA

<sup>5</sup>Department of Genetics & Genomic Sciences, Mount Sinai Hospital, New York, NY, USA

<sup>6</sup>Center for Pharmacogenomics, College of Medicine, The Ohio State University, Columbus, OH, USA

<sup>7</sup>Department of Biochemistry & Molecular Biology, Center for Systems Genomics, Eberly College of Science, The Huck Institutes of the Life Sciences, The Pennsylvania State University, University Park, PA 16801, USA

\*Author for correspondence:

Tel.: +1 814 863 5107

Fax: +1 814 863 6699

[marylyn.ritchie@psu.edu](mailto:marylyn.ritchie@psu.edu)

**Box 1. Definitions**

- Pharmacological trait: Measurable variation in response to pharmacological treatments.
- Pharmacokinetics (PK): The degree or rate of absorption, metabolism, distribution and elimination of a drug within a living system.
- Pharmacodynamics (PD): The relationship between drug concentration and effect on a living system, or the microorganisms affected within a living system by a drug.
- ADME: Absorption, distribution, metabolism and elimination.
- Efficacy: The quality of the effect of a pharmacological treatment on a living system in relation to the quantity of the drug.
- Adverse event: A detrimental response to a drug within a living system.
- Idiosyncratic adverse event: An unexpected response to a drug within a living system.
- Therapeutic window: The range of drug concentrations efficacious with minimal toxicity.
- Efficacy/toxicity balance: Drug levels have an impact on drug efficacy, but also toxicity.
- Pharmacogenomics (PGx): Study of the relationship between genetic variation and drug response, including but not limited to pharmacological traits, PK, PD, efficacy, toxicity and/or adverse events.
- NIH Pharmacogenomics Research Network (PGRN): A collaboration between an ever growing number of study sites, all investigating the pharmacogenomics of a variety of traits [2,3].
- The Pharmacogenomics Knowledgebase (PharmGKB): A database of comprehensively collected information about the relationships between genes and PT, PK, PD, efficacy, adverse events and/or disease [4,5].
- Polygenic genetic architecture: The contribution of multiple common SNPs to phenotypic variance in aggregate.
- Polygenic modeling: Method that develops an additive polygenic risk score based on SNPs that pass a p-value threshold in a discovery set of samples, tested in an independent set of samples.
- Mixed Linear Modeling (MLM): Estimation of an additive genetic variance under a mixed linear model with a random effect representing the polygenic component of underlying trait variation.

ders – perhaps because a relatively limited number of genes influence PK and PD traits. Moreover, drugs are targeted to specific pathways thought to be involved in complex disease phenotypes, thereby narrowing the number of candidate genes, with each displaying a proportionally larger effect size. We assume here that complex disorders such as cardiovascular diseases and cancer represent a collection of disease subtypes each with similar symptoms – where drug therapy would typically target a specific subtype.

A series of limitations and challenges confront the field of PGx. One current limitation is the wide use of single common-variant/outcome trait association testing. Alternative modeling methodologies and strategies that incorporate multiple genetic markers, as well as the inclusion of lower-frequency variants, may prove effective for enhancing PGx trait prediction. A challenge for PGx studies includes the difficulty of estimating the heritability of PGx traits, since it is untenable to administer medications to unaffected individuals. Attaining adequate statistical power presents a challenge when faced with the frequently small sample size in PGx studies, especially for adverse drug reaction studies. Effective biomarker identification has also remained a challenge, with potential biomarkers that have not shown clinical efficacy [7,8], and important work yet to be done understanding the biology underlying effective biomarkers [8]. In addition, the PGx field needs to broaden investigations of PGx traits

across samples of diverse ancestry/race/ethnicity, to derive relevant actionable information for clinicians. Finally, ‘risk’ is difficult to define for PGx traits, as metrics for drug efficacy can be difficult to ascertain when compared with the probability (rate) of risk of a common complex disease.

Herein, we describe the current understanding of the genetic architecture of PGx traits. We discuss in detail some of the aforementioned challenges and limitations while also pointing out opportunities and future directions for the field of PGx. These include new methods development such as polygenic modeling, pathway analyses, and systems biology approaches for the development of further robust biomarkers, all with the goal of discovering the etiology underlying interindividual variations in drug response, and designing robust biomarker panels predictive of treatment outcomes.

**What have we learned?****Heritability of PGx traits: challenges & successes**

The rationale for PGx is the underlying assumption that genetic variation plays a substantial role in pharmacological outcome. The heritability of a PGx trait should be measurable if variant transmission from parent to offspring is the basis of the genetic architecture influencing PGx traits. While determining the heritability provides the rationale for a PGx study, estimating the heritability of PGx traits is nontrivial.

Defining the heritability of PGx traits encounters hurdles distinct from those found in the analysis of complex disorders. By definition, any drug response trait represents a gene–environment interaction, where the drug is only one component of multiple environmental exposures, and multiple genes may contribute to the PGx response. Moreover, each drug, even closely related ones such as the statins, has different degrees of heritability and must be studied individually. Also, drug effects are highly dependent on the dosage, and hence, genetic factors differ with drug dose, as shown for the impact of *SLCO1B1* variants on simvastatin's muscle toxicity, only detectable when high doses are needed to control cholesterol levels [9]. Lastly, drug therapy commonly involves multiple drugs. As a result, predictive biomarker tests of the future will have to evolve to consider complex gene–gene–environment interactions.

Few drugs can be used in a familial setting to monitor the variability of drug response as most family members will not have a condition warranting treatment. Drug treatment of a group of individuals without a need for treatment is limited for safety and ethical reasons. In addition, sample size is often low for PGx studies, especially when investigating adverse events. Access to large numbers of related individuals taking a specific drug is limited to communities that have a common need for a particular drug, such as lipid lowering treatments [10]. This is distinct from estimating heritability for complex diseases or outcomes where twin and family studies are facilitated, even when trait complexity can present challenges for heritability estimation.

A few examples of familial studies of drug response clearly indicate the heritability of some PGx traits, underscoring considerable influence of genetic factors; these are presented in Table 1. For example, dicumarol was monitored in the plasma for a group of identical and fraternal twins [11], revealing little difference in the variability of dicumarol half-life in plasma response between identical twins, minor differences between fraternal twins and wide differences between nonrelated subjects demonstrating a significant heritable component. Investigating heritability of PGx traits across communities of related individuals where a specific drug is commonly administered is another way to determine heritability of certain PGx traits. For instance, a study of the heritability of platelet response, measured by *ex vivo* platelet aggregometry, involved the administration of clopidogrel to 429 Amish persons and revealed the platelet response to be highly heritable [10].

To circumvent limitations of familial studies for PGx traits, estimation of heritability in an *ex vivo* manner has been successful. This approach has been applied for

measuring drug cytotoxicity within familial-derived lymphoblastoid cell lines (LCL) [13]. Further work with the LCL approach has led to a detailed understanding of effective LCL study design, enabling identification of loci related to variability of PGx traits which in turn guide studies in humans and model organisms. For example, heritability of chemotherapeutic cisplatin-induced cytotoxicity has been estimated at approximately 57% through the LCL approach, with evidence for multiple causative variants [14]. Table 2 presents a series of heritability results from cell line experiments. Detailed work has characterized factors that confound interpretation of these experiments, such as the portion of the genetic variation of drug-induced cytotoxicity accounted for by heritability of variation in cellular growth rate [13]. In addition, cellular assays are amenable to high-throughput testing of multiple drugs. For example, one study investigated the cytotoxic effect of 29 chemotherapeutic agents on 125 LCL from 14 extended families, and found a range of heritabilities from <15% (gemcitabine) to >60% (epirubicin) [15].

Furthermore, HapMap cell lines from multiple ancestries can be used in these cellular assay based studies to represent multiple ancestries, allowing for characterization of the relationship between PGx traits across ancestry groups. For example, an exploratory analysis used HapMap cells to investigate genetic variants and their functional consequences for the enzyme deoxycytidine kinase (DCK) in two ancestries, European and African (Yoruba) [18]. DCK is a rate-limiting enzyme in the activation of nucleoside analogs. Cytarabine (ara-C), a chemotherapeutic agent commonly used in acute myeloid leukemia, is one such nucleoside analog. DCK activity was lower for subjects heterozygous for coding changes compared with homozygous subjects, and DCK activity in general was higher in the African cell lines when compared with the European cell lines.

Another approach available for determining the genetic component influencing PGx traits involves Repeated Drug Administration (RDA). In this method, a drug is administered multiple times to unrelated individuals, and the variability in the PGx trait of interest between and within individuals is compared [19]. RDA information can be used to calculate the Relative Genetic Component (rGC), an estimate on a scale of 0 to 1 of the genetic component of a PK or PD parameter. This measurement has also been referred to as 'intra-class correlation' or ICC [20]. The rGC measurement is calculated through the following formula: (variability between individuals - variability within individuals)/variability between individuals. The measurement can be interpreted as a rough estimate of heritability, where a trait with high rGC will likely have high heritability. The rGC measurement can

Table 1. Heritability of pharmacogenomics traits.

Study type	Trait	Drug	Heritability measure	Heritability estimate	SE	Ref.
Pedigree	Response (platelet aggregation)	Clopidogrel	$h^2$	0.73	±0.12	[10]
Twins	Half-life	Dicumarol	$h^2$	0.97	NA	[11]
Twins	Half-life	Antipyrine	$h^2$	0.98	NA	[11]
Pedigree	Platelet response measured by phenotypes indirectly related to inhibition of COX-1	Aspirin (acetylsalicylic acid)	$h^2$	0.266–0.762	SE	[12]
	PRP aggregation 2 µg/ml collagen			0.451	±0.080	[12]
	PRP lag time 2 µg/ml collagen			0.309	±0.095	[12]
	Whole blood aggregation 1 µg/ml collagen			0.365	±0.073	[12]
	PRP aggregation 10 µmol/l ADP			0.475	±0.083	[12]
	Whole blood aggregation 10 µmol/l ADP			0.434	±0.070	[12]
	PRP aggregation 10 µmol/l epinephrine			0.535	±0.077	[12]
	β-thromboglobulin release			0.65	±0.086	[12]

NA: Not applicable; PRP: Platelet rich plasma; SE: Standard error.

also be calculated from monozygotic twin pairs, when dizygotic twins are not available [19]. For example, the genetic component of variation in renal clearance of amoxicillin, ampicillin, metformin, terodiline, digoxin and iohexol, was investigated using this approach [21]. Results from these rGC based studies are summarized in Table 3. Limitations of this approach include the high variability of PGx traits over limited time periods even in the absence of genetic factors, potentially leading to large error estimates.

### The PGx landscape: drug efficacy & adverse events

Among the many PGx success stories is the use of genetic information to facilitate prescription of optimal warfarin dosage levels to prevent cardioembolic stroke, myocardial infarction and venous thrombosis as well as prevent adverse events. Warfarin is widely prescribed after placing arterial stents or after myocardial infarction. However, warfarin causes serious side effects including hemorrhage, especially during drug initiation when patients are titrated to the optimal dosage level [33,34]. Variants in *CYP2C9* influence the PK [35] and *VKORC1* variants influence the PD of warfarin [36–39]. It is noteworthy that the two-gene biomarker test for warfarin dosing still represents an exception; most other genetic biomarker PGx tests only include one gene, and the identification of multigene robust biomarkers remains an important area for expansion within PGx research. An algorithm for estimating individualized warfarin dosages was defined using

clinical and PGx data [40]. As a result, the FDA updated the label for warfarin, detailing the use of pharmacogenetic testing for clinical decision making [33]. Recent studies have evaluated genotypic biomarkers for warfarin dosing with different conclusions; one study indicated genotype-guided dosing of warfarin was ineffective when compared with dosing without genotypic information [41]. A separate study indicated genotype-guided dosing was associated with a patients being within the therapeutic range for a greater period of time when compared with the standard initiation of warfarin [42].

In another example, clopidogrel is prescribed to prevent atherothrombotic events after myocardial infarction but exhibits notable variability in successfully preventing further cardiovascular events. This has at least in part been attributed to genetically determined variation in the drug metabolizing enzyme *CYP2C19*, largely responsible for converting clopidogrel to its active metabolite. The most common loss-of-function allele is *CYP2C19\*2* (rs4244285), associated with increased risk of cardiovascular events [43]. Indeed the *CYP2C19\*2* variant is considered a major determinant of prognosis for patients <45 years of age on clopidogrel treatment after myocardial infarction [44].

While the list of PGx traits continues to grow, translating the complex and sometimes conflicting research results from PGx to clinical action requires accessible information that is updated as new findings come to light. FDA labels are already being modified in response to emerging PGx findings, listed here [45]. However,

FDA label changes are only one way to provide information to clinicians for implementing PGx results in treatment decisions. The Clinical Pharmacogenetics Implementation Consortium (CPIC) [46], the Royal Dutch Association for the Advancement of Pharmacy (DPWG) [47] and other professional medical societies, have also been publishing pharmacogenetic dosing guidelines for an increasing number of drugs [48].

CPIC in particular has carefully reviewed the criteria for translation of PGx traits, and as a result, has developed a framework for identifying key evidence justifying clinical implementation. Published CPIC guidelines target specific gene/drug pairs (Table 4), reviewing the existing research for each gene/drug pair [49]. In addition, CPIC provides a standardized web-interface of gene/drug pair summary information, including outcome phenotype based on genotype, dosing recommendations and allele frequency differences and impact of specific variants across distinct ancestry. CPIC continues to review ongoing research on gene/drug pairs to determine whether the existing information needs updating or new gene–drug pairs can be recommended for clinical use.

The effect size of genetic variants affecting PGx traits tends to exceed that of SNPs derived from GWAS of complex human disorders. In Figure 1, we illustrate this trend by comparing the odds ratios for efficacy and toxicity related PGx results with those from the NHGRI GWAS catalog, where the phenotypic outcome covers a range of non-PGx complex traits [65]. Figure 1 displays larger relative strength of effect size for PGx traits compared with the range of effect sizes observed with complex-trait GWAS. This observation is consistent with our expectation that PGx variants affect targeted subsets of genes and pathways; however, ascertainment bias cannot be excluded resulting from the different methods used for discovery of the genetic variants.

### Functional role of genomic architecture in PGx traits

The PGx field faces challenges in understanding the mechanistic role of genomic variation in PGx traits. Much of the focus of interpretation of the relationship between genetic variability and outcome for both complex disease and PGx has been centered on protein-coding regions. In PGx studies there has been a particular focus on candidate gene approaches targeting Absorption, Distribution, Metabolism and Excretion (ADME) genes in addition to GWAS. This makes particular sense for PGx traits, as genetic variation can have an impact on the protein structure of drug-metabolizing enzymes resulting in changes in enzymatic activity. Furthermore, there are known

**Table 2. Cell-line based estimates of heritability for drug cytotoxicity.**

Drug	h <sup>2</sup> heritability estimate	Ref.
5-fluorouracil	0.26–0.65 (dose dependent)	[16]
Docetaxel	0.21–0.70 (dose dependent)	[16]
Cisplatin	0.47	[14]
Daunorubicin	0.18–0.63 (dose dependent)	[17]
5-Fluorouracil	29.2	[15]
Arsenic trioxide	24.4	[15]
Azacitidine	20.7	[15]
Bleomycin	17.3	[15]
Busulfan	14.2	[15]
Carboplatin	43.2	[15]
Cladribine	27.3	[15]
Cytarabine	41.7	[15]
Daunorubicin	37.1	[15]
Docetaxel	30.1	[15]
Doxorubicin	35.3	[15]
Epirubicin	59.5	[15]
Etoposide	41.3	[15]
Floxuridine	27	[15]
Fludarabine	13.5	[15]
Gemcitabine	8.1	[15]
Hydroxyurea	43.2	[15]
Idarubicin	45.8	[15]
Mitomycin	26.7	[15]
Mitoxantrone	46.5	[15]
Oxaliplatin	50	[15]
Paclitaxel	45.9	[15]
Rapamycin	15.1	[15]
Temozolomide	63.5	[15]
Teniposide	36.4	[15]
Topotecan	46.1	[15]
Vinblastine	31.2	[15]
Vincristine	23.1	[15]
Vinorelbine	34.1	[15]

important PK pathways where the impact of genetic variation has been demonstrated on a protein-coding modification level. One example is CYP2D6, an enzyme involved in the metabolism of up to 25% of clinical drugs, where nonsynonymous variants can result in enzymatic changes and subsequent changes in catalytic activity [66]. Many of these very important pharmacogenes (VIP) are summarized in the Pharmacogenomics Knowledge Base (PharmGKB)

Table 3. Repeated drug administration and resultant relative genetic component measurements.

Study type	Trait	Drug	Relative genetic component estimate	95% CI	Ref.
RDA	Renal clearance	Metformin	0.94	NA	[22]
RDA	Renal clearance	Amoxicillin	0.91	NA	[23]
RDA	Renal clearance	Ampicillin	0.64	NA	[23]
RDA	Renal clearance	Terodiline	0.37	NA	[24]
RDA	Renal clearance	Iohexol	0.2	NA	[25]
RDA	Renal clearance	Digoxin	0.12	NA	[26]
RDA	Metabolism by CYP1A2	Caffeine	0.69	NA	[19,27]
RDA	Metabolism by NAT2	Caffeine	0.95	NA	[19,27]
RDA	Metabolism by xanthine oxidase	Caffeine	0.24	NA	[19,27]
RDA	Metabolism by UGT2B7	Oxazepam	0.98	NA	[19,27]
RDA	Metabolism by ADH	Ethanol	0.57	NA	[19,27]
RDA	Metabolism by CYP2D6	Dextromethorphan	0.97	NA	[19,27]
RDA	Metabolism by CYP3A4 (adriamycinol-AUC[0-Inf] to adriamycin-AUC[0-Inf])	Adriamycin	0.55	0.00–0.86	[28]
RDA	Metabolism by CYP3A4 (terminal elimination half-life (unbound drug))	Cyclosporine	0.83	0.12–0.97	[28]
RDA	Metabolism by CYP3A4 (erythromycin N-demethylation rate)	Erythromycin	0.89	0.65–0.98	[28]
RDA	Metabolism by CYP3A4 (serum AUC[0–24])	Ethinylestradiol	0.79	0.48–0.94	[28]
RDA	Metabolism by CYP3A4 (serum AUC[0–24])	Ethinylestradiol	0.94	0.83–0.98	[28]
RDA	Metabolism by CYP3A4 (serum AUC[0–24])	Ethinylestradiol	0.86	0.48–0.96	[28]
RDA	Metabolism by CYP3A4 (ethylmorphine N-demethylation metabolic ratios)	Ethylmorphine	0.98	0.87–1.00	[28]
RDA	Metabolism by CYP3A4 (plasma clearance)	Midazolam	0.96	0.92–0.98	[28]
RDA	Metabolism by CYP3A4 (plasma AUC[0–24])	Nifedipine	0.82	0.55–0.94	[28]
RDA	Metabolism by CYP3A4 (plasma AUC[0-Inf])	Nifedipine	0.98	0.95–0.99	[28]
RDA	Metabolism by CYP3A4 (plasma AUC[0–24])	Nitrendipine	0.66	0.00–0.92	[28]
RDA	Pharmacokinetics of nevirapine (plasma AUC[0–6])	Nevirapine	European Americans: 0.904 African-Americans: 0.902	European Americans: 0.64–0.97 African-Americans: 0.42–0.98	[29]
Twins	AUC[0–12]	d0-digoxin	0.89	NA	[30]

AUC: Area under the curve; NA: Not applicable; RDA: Repeated drug administration.

**Table 3. Repeated drug administration and resultant relative genetic component measurements (cont.).**

Study type	Trait	Drug	Relative genetic component estimate	95% CI	Ref.
Twins	AUC[0–12]	d3-digoxin	0.79	NA	[30]
Twins	Oral clearance	Digoxin (oral)	0.36	NA	[31]
Twins	Renal clearance	Digoxin (oral)	0.19	NA	[31]
Twins	Renal clearance	Metformin	0.95	NA	[32]

AUC: Area under the curve; NA: Not applicable; RDA: Repeated drug administration.

[67]. However, GWAS and now full-genome sequencing have identified many biomarkers that do not cause functional protein-coding modification, or are located in genes whose role in drug disposition, response or toxicity was previously not well characterized. For example, a distal enhancer variant >100 kb downstream of the coding region of *CYP2D6* strongly increases gene expression in the liver, accounting for cases of ultra rapid metabolism [68]. Likewise, in a genome wide association study of flucloxacillin-induced liver injury, a novel association between *ST6GAL1*, an enzyme with a possible role in B-cell immune response, and the drug induced liver toxicity was identified [69]. In some cases, there are genetic variants that account for substantial outcome variability, and are already used as a clinical biomarker, but we have a more limited understanding of the impact of that genetic factor, such as the functional mechanisms of HLA variants and drug response variability [70,71].

Resolving the mechanistic etiology of the impact of genetic variation on PGx traits is a critical focus for future PGx studies. Exploration of the function of nonprotein coding genetic regions will be essential, including regulatory regions and noncoding RNA [72]. Regulatory variants may account for a large portion of genetic variability, and should be incorporated into analyses as knowledge of the functional impact of genetic variation on genetic enhancers, promoters and gene expression is accrued and shared through projects such as ENCODE and related databases [73–76].

### Beyond PGx GWAS: polygenic analyses

New methodologies will continue to drive advances in the field of PGx. The majority of PGx results have arisen from investigation of the association between single, common, genetic variants and pharmacological outcome. As found with GWAS for common complex traits [6], this approach may have varied or limited success in future studies, as the genetic architecture of any trait can be complex. On a biological level, a variety of potential genetic mechanisms influencing PGx traits fail to be captured when investigating only the relationship between single, common, genetic-variants and out-

comes. Thus, we need to diversify the methodologies being used to better define polygenic traits.

One alternate approach considers polygenic genetic architecture, or the contribution of multiple common SNPs to phenotypic variance in aggregate. Two methods have already been used for a variety of complex outcomes for non-PGx traits: mixed linear modeling (MLM) and polygenic modeling. Both methods test a polygenic model for the relationship between multiple SNPs and outcome, as illustrated in Figure 2. MLM estimates the additive genetic variance under a mixed linear model with a random effect representing the polygenic component of trait variation. The software tool GCTA (Genome-wide Complex Trait Analysis) has been developed for use of MLM in estimation of the proportion of phenotypic variance accounted for by genome-wide association genotypic data [77]. The MLM/GCTA approach has been used successfully for identifying the collective contribution of GWAS-polymorphisms to traits including height [78], Crohn's disease, bipolar disorder and Type 1 diabetes [79] and other complex outcomes [80,81].

Polygenic modeling develops an additive polygenic risk score for a given trait based on a group of SNPs filtered by a GWAS-based p-value threshold in a discovery sample set. The polygenic risk score is then tested in an independent set of samples. This approach has been successfully used to detect the contribution of multiple variants with small effects to the heritability of diseases/traits/outcomes such as schizophrenia [82], multiple sclerosis [83], height [84], body mass index [85] and rheumatoid arthritis [86]. Polygenic modeling analyses for complex traits yield results consistent with simulated genetic models in which hundreds of associated loci harbor common causal variants and a smaller number of loci harbor multiple rare causal variants [86]. The heritability estimates derived from these polygenic approaches have been consistent with previously reported estimates for these complex traits. MLM and polygenic modeling methods are now being applied to PGx data. MLM/GCTA analyses have been used to investigate asthma PGx traits [87], and paclitaxel-induced sensory peripheral neuropathy [88].

Table 4. Reviewed Clinical Pharmacogenetics Implementation Consortium drug/gene pairs.

Drug class	Drugs	Drug use in the clinic	Concern	Gene	Gene function	Genotype information and SNPs	Other notes	Ref.
Thiopurines	Azathioprine, mercaptopurine and thioguanine	Critical anticancer agents; immunosuppressants in inflammatory bowel disease, rheumatoid arthritis and other immune conditions	Individuals with two nonfunctional <i>TPMT</i> alleles are at 100% risk for life-threatening myelosuppression	<i>TPMT</i>	Thiopurine methyltransferase activity	Multiple SNPs in <i>TPMT</i> are detailed here [50] CPIC defines specific haplotypes and SNPs for <i>TPMT</i> [51]	Substantial ethnic differences in the frequencies of low-activity variant alleles	[52]
	Clopidogrel	Antiplatelet therapy, particularly for those undergoing percutaneous coronary intervention	Dependent on <i>CYP2C19</i> haplotype, patients have a variation in clopidogrel response. By haplotype, patients can be grouped as follows: ultrarapid metabolizer, extensive metabolizer, intermediate metabolizer or poor metabolizer	<i>CYP2C19</i>	Inhibition of platelet aggregation	The key polymorphism is <i>CYP2C19</i> *2 (c.681G>A; rs4244285), specifically studied with respect to clinical outcomes. However, multiple SNPs are under investigation and detailed here [53]	The frequencies of <i>CYP2C19</i> poor metabolizers are ~2–5% among Caucasians and Africans and ~15% in Asians	[54]
	Warfarin	Treatment and prevention of thrombotic disorders	The variant alleles <i>CYP2C9</i> *2 and *3 impair metabolism of S-warfarin by ~30–40% and ~80–90%, respectively. If a patient receives too much warfarin, or is a fast metabolizer, they may be at risk of a bleeding event. If a patient receives too little warfarin, or is a slow metabolizer, they may be at risk of a thrombotic event	<i>CYP2C9</i>	<i>CYP2C9</i> is a hepatic drug-metabolizing enzyme in the CYP450 superfamily, and is the primary metabolizer of S-warfarin	<i>CYP2C9</i> has more than 30 known variant alleles <i>CYP2C9</i> *2 (rs1799853) and <i>CYP2C9</i> *3 (rs1057910) are the two most common variants with reduced enzyme activity	The frequencies of the <i>CYP2C9</i> variant alleles differ between racial/ethnic groups. In addition, the <i>CYP2C9</i> variant alleles with reduced activity ( <i>CYP2C9</i> *5, *6, *8 and *11) contribute to dose variability among African-Americans	[55]

Table 4. Reviewed Clinical Pharmacogenetics Implementation Consortium drug/gene pairs (cont.).

Drug class	Drugs	Drug use in the clinic	Concern	Gene	Gene function	Genotype information and SNPs	Other notes	Ref.
Thiopurines (cont.)	Warfarin	Treatment and prevention of thrombotic disorders	Warfarin has a narrow therapeutic index and wide interindividual variability If a patient receives too much warfarin, or is a fast metabolizer, they may be at risk of a bleeding event If a patient receives too little warfarin, or is a slow metabolizer, they may be at risk of a thrombotic event	<i>VKORC1</i>	<i>VKORC1</i> encodes the vitamin K-epoxide reductase protein, the target enzyme of warfarin	Variation in rs9923231 is significantly associated with warfarin sensitivity and reduced dose requirements. The polymorphism alters a <i>VKORC1</i> transcription factor binding site, leading to lower protein expression There are rare nonsynonymous <i>VKORC1</i> variants that confer warfarin resistance resulting in high warfarin requirements	The variation in allele frequency of rs9923231 across ancestry largely explains the average dose requirements. The differences across white subjects, black subjects and Asians	[55]
	Codeine	Treatment for pain management	Poor metabolizers may lack any effect of codeine, warranting the use of an alternative analgesic Ultrarapid metabolizers of codeine are at risk of severe toxicity associated with a 'normal' dose of codeine	<i>CYP2D6</i>	Hepatic <i>CYP2D6</i> bioactivates codeine to morphine	More than 80 <i>CYP2D6</i> alleles have been defined, and are detailed here [56] Based on diplotype, a likely phenotypic outcome can be defined: ultrarapid metabolizer, extensive metabolizer, intermediate metabolizer and poor metabolizer	In some cases, patients have more than two copies of the <i>CYP2D6</i> gene <i>CYP2D6</i> allele frequencies differ substantially between racial and ethnic groups	[57]

Table 4. Reviewed Clinical Pharmacogenetics Implementation Consortium drug/gene pairs (cont.).

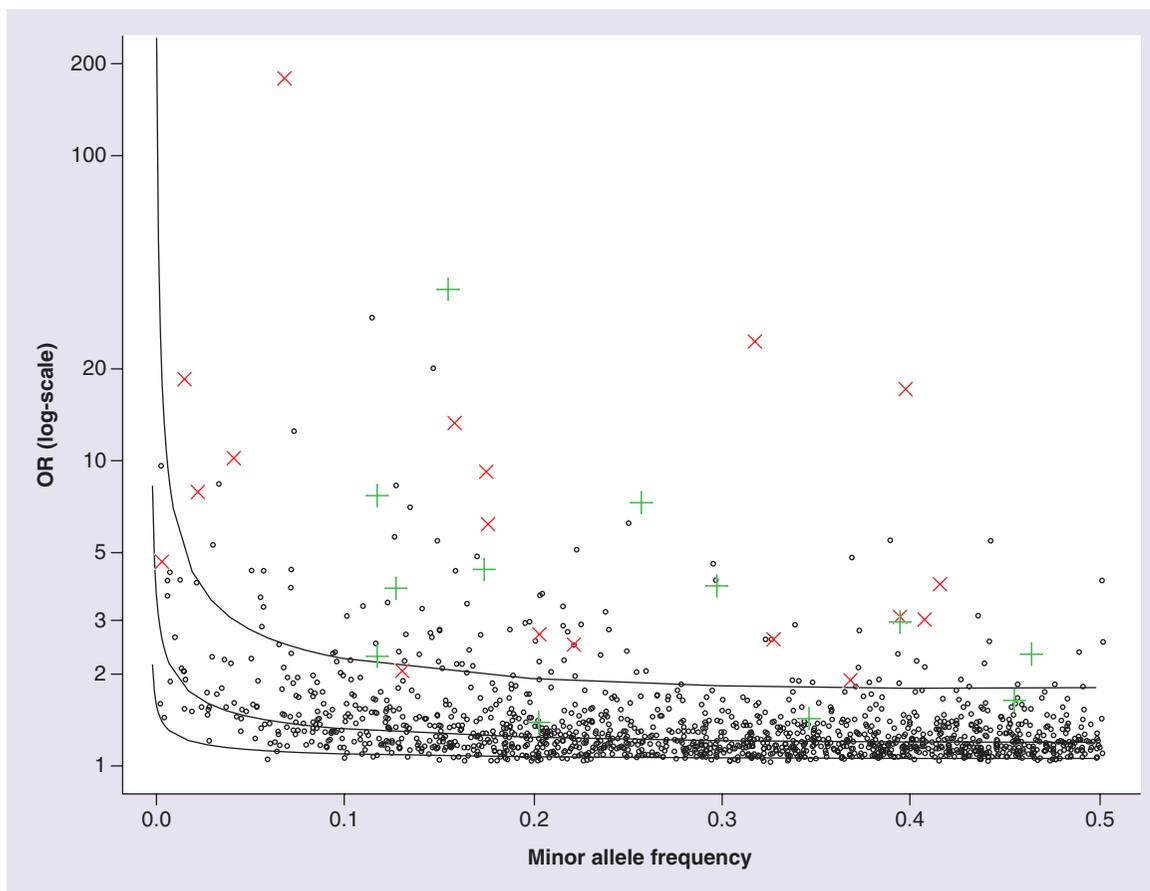
Drug class	Drugs	Drug use in the clinic	Concern	Gene	Gene function	Genotype information and SNPs	Other notes	Ref.
Thiopurines (cont.)	Abacavir	Anti-HIV treatment which suppresses HIV's ability to convert its RNA genome into DNA before insertion into a host cell's genome	Variants in HLA-B can result in an increased risk in hypersensitivity, a multiorgan illness	HLA-B	HLA-B encodes a cell surface protein for presenting intracellular antigens to the immune system	Variation in HLA-B: HLA-B*57:01 has also been shown to be over-represented in HIV long-term nonprogressors	[58]	
	Simvastatin	Used for lipid lowering	Muscle toxicity (myopathy)	SLCO1B1	The OATP1B1 protein that is encoded by SLCO1B1 facilitates the hepatic uptake of statins, and variation in SNP rs4149056 can have a damaging effect on OATP1B1	A minor allele C for SNP rs4149056 in SLCO1B1 increases systemic exposure to simvastatin and the risk of muscle toxicity	[9]	
	Allopurinol	Treatment of hyperuricemia and gout	Cutaneous adverse reactions including: drug hypersensitivity syndrome, Stevens–Johnson syndrome, toxic epidermal necrolysis	HLA-B	HLA-B encodes a cell surface protein for presenting intracellular antigens to the immune system	Variant allele of the HLA-B, HLA-B*58:01	[59]	
Tricyclics	Amitriptyline, clomipramine, doxepin, imipramine and trimipramine, desipramine and nortriptyline	Treatment for pain management and psychiatric disorders	Variation in drug metabolism based on polymorphism profile	CYP2D6	These drugs undergo hydroxylation by CYP2D6 to less active metabolites	Although likely there is a combined effect of polymorphisms in both CYP2D6 and CYP2C19, further research needs to be carried out to characterize the combined effect of polymorphisms in both genes	[60]	

Table 4. Reviewed Clinical Pharmacogenetics Implementation Consortium drug/gene pairs (cont.).

Drug class	Drugs	Drug use in the clinic	Concern	Gene	Gene function	Genotype information and SNPs	Other notes	Ref.
Tricyclics (cont.)	Amitriptyline, clomipramine, doxepin, imipramine and trimipramin	Treatment for pain management and psychiatric disorders	Variation in drug metabolism based on polymorphism profile	CYP2C19	These drugs are demethylated by CYP2C19 to pharmacologically active metabolites	Although likely there is a combined effect of polymorphisms in both CYP2D6 and CYP2C19, little information is available on how to adjust initial doses based on combined genotype information	Pharmacogenomics guidelines are specific to treatment of psychiatric disorders vs pain management	[60]
	Carbamazepine	Treatment for seizures, nerve pain and bipolar disorder	Increased risk of serious blistering cutaneous adverse drug reactions: Stevens–Johnson syndrome and toxic epidermal necrolysis	HLA-B	HLA-B encodes a cell surface protein for presenting intracellular antigens to the immune system	Considered 'positive' if one or two copies of HLA-B*15:02 are present or as 'negative' if no copies of HLA-B*15:02 are present	HLA-B*15:02 is most prevalent in Oceanian, east Asian and south/central Asian populations	[61]
Fluoro-pyrimidines	Capecitabine	Fluoropyrimidines are chemotherapeutic agents for the treatment of many types of cancers	Deficiency in DPD can result in profound toxicity, such as myelosuppression, mucositis, neurotoxicity, hand–foot syndrome and diarrhea, when taking 5-fluorouracil	DPYD-5FU	This gene encodes for an enzyme critical for detoxifying metabolism of fluoropyrimidines	Homozygotes of *2A, *13 and rs67376798 are considered deficient in DPD Heterozygotes for any combination of *2A, *13 and rs67376798 have intermediate or partial DPD activity Individuals with none of these alleles are likely to have normal activity	Weak or conflicting evidence for other alleles of DPYD	[62]

Table 4. Reviewed Clinical Pharmacogenetics Implementation Consortium drug/gene pairs (cont.).

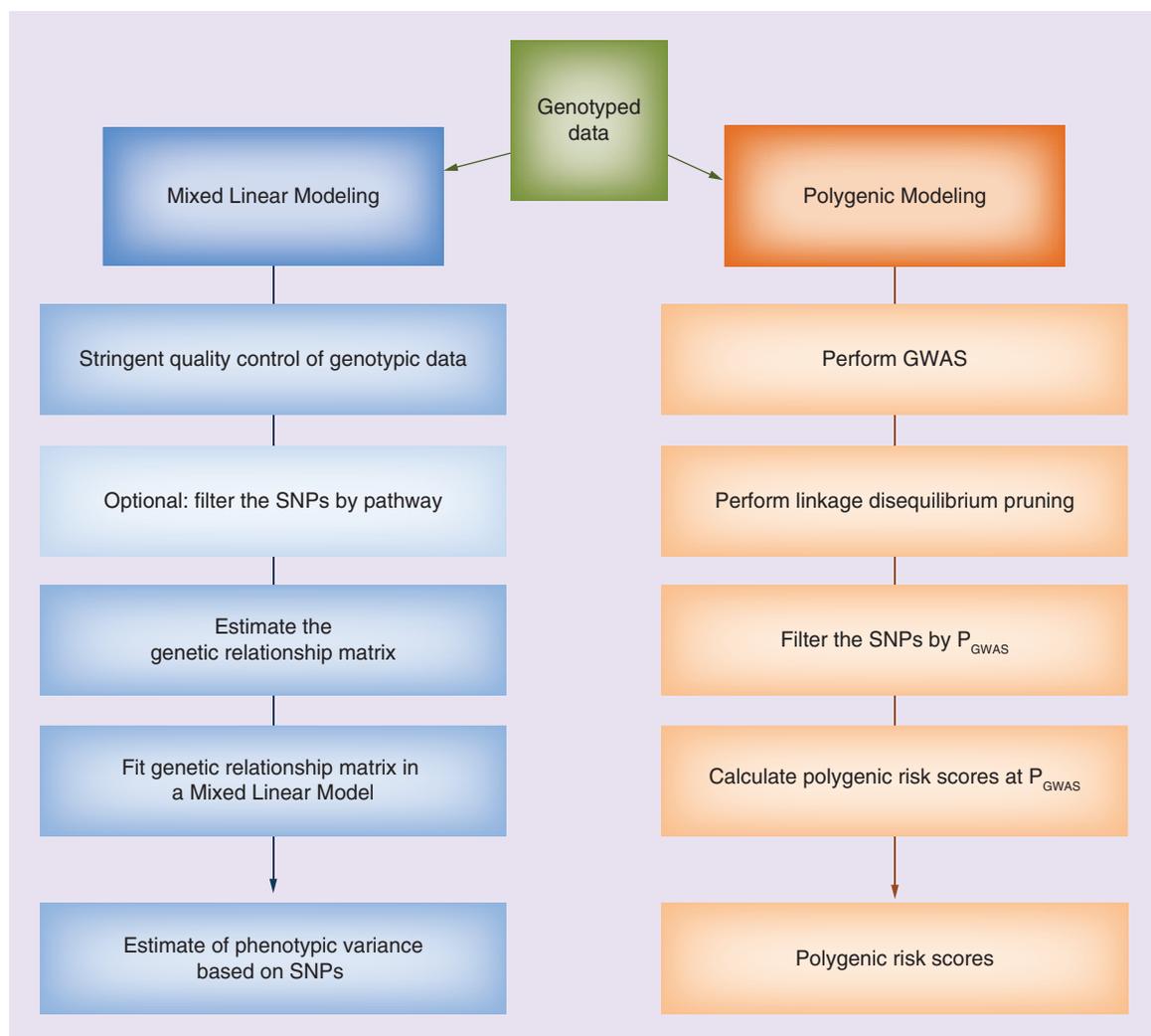
Drug class	Drugs	Drug use in the clinic	Concern	Gene	Gene function	Genotype information and SNPs	Other notes	Ref.
Pegylated IFN- $\alpha$	PEG-IFN- $\alpha$ , PEG-IFN 2a and 2b, ribavirin	Treatment of hepatitis C virus genotype 1	<i>IL28B</i> genotype strongest baseline predictor of response to PEG-IFN- $\alpha$ and RBV therapy	<i>IFNL3/IL28B</i>	The gene encodes IFN- $\lambda$ 3, a member of the type 3 IFN- $\lambda$ family with antiviral, antiproliferative and immune-modulatory activities	The two most commonly tested SNPs are rs12979860 and rs8099917	The rs12979860 allele frequency varies among different ethnic groups, explaining the differences in treatment response rates among east Asians, Caucasians, African-Americans and Hispanics with chronic hepatitis C virus infection	[63]
Ivacaftor		Treatment for cystic fibrosis		<i>CFTR</i>	Mutations within <i>CFTR</i> cause cystic fibrosis	<i>G551D-CFTR</i> variant in at least one allele, guidelines indicate ivacaftor therapy if no contraindications Ivacaftor therapy is not recommended for cystic fibrosis patients with other <i>CFTR</i> mutations	While 1900 disease-causing <i>CFTR</i> variants have been identified, they can be grouped by shared features Growing understanding of how various mutations disrupt <i>CFTR</i> protein function is beginning to help clinicians categorize cystic fibrosis patients for viable therapeutic strategies	[64]



**Figure 1. Contrasting the effect size and minor allele frequency range of pharmacogenomic variants versus variants from the NHGRI GWA catalog.** Black circles are the NHGRI GWAS catalog results, plotted by OR results in logarithmic (base10) scale versus minor allele frequency. Each green cross represents a replicated efficacy result for a pharmacogenomics study. Each red X represents a replicated toxicity result for pharmacogenomics. The solid lines represent the 80% power equivalent curves across minor allele frequency, from top to bottom for  $n = 1 \times 10^3$ ,  $10 \times 10^3$  and  $100 \times 10^3$ , respectively (assuming  $n/2$  cases and  $n/2$  controls). OR: Odds ratio.

Paclitaxel is a chemotherapeutic agent commonly prescribed to treat carcinomas of the breast, ovaries, lung, head and neck. Peripheral neuropathy is one of the most common toxicities with paclitaxel treatment, and occurs in a substantial subset of patients. Known causes of peripheral neuropathy do not completely explain the incidence of toxicity amongst patients treated with paclitaxel, suggesting a genetic basis for susceptibility to the toxicity. Small candidate gene studies have had mixed results identifying variants related to variability paclitaxel-induced peripheral neuropathy [4,89]. One study reports a high risk odds ratio (OR: 19.1) for paclitaxel neurotoxicity associated with *CYP3A4\*22* [90], as a result of reduced metabolic activity of the \*22 allele [91], but this result requires replication (*CYP3A4\*22* is not on earlier GWAS panels and cannot be readily imputed). GWAS for this PGx trait have identified some candidate SNPs, but replication has been inconsistent [2,92].

Chhibber *et al.* (2014) [88] investigated a polygenic etiology of paclitaxel-induced neuropathy. They estimated the variance explained by common SNPs (MAF >1%) for two outcomes: the maximum grade of sensory peripheral neuropathy, and the dose at first instance of peripheral neuropathy. They investigated the variance explained by all autosomal SNPs, SNPs selected based on genomic location, and SNPs in gene sets selected based on prior knowledge regarding possible mechanisms of the pathogenesis of paclitaxel-induced peripheral neuropathy using the GCTA software tool. They found whole genome estimates of heritability were not significant; however, using a pathway-based approach for filtering SNPs yielded significant results. Specifically, the Axonogenesis GO Term set (GO: 0007409) had significant estimates of heritability close to 20%, suggesting a portion of the heritability of paclitaxel-induced neuropathy is driven by genes involved in the regulation of axon extension. These results show both the utility of



**Figure 2. Overview of polygenic analysis methods.** On the left, the general workflow of using Mixed Linear Modeling pursued using the software genome-wide complex trait analysis. On the right, the general workflow of Polygenic Modeling. Both methodologies allow the user to identify multiple SNPs related to pharmacogenomics outcome, with different information resulting from each approach. GWAS: Genome-wide association study.

polygenic approaches for PGx traits, as well as the utility of exploring pathway-based expert knowledge filtering of SNPs before investigating polygenic architecture.

McGeachie *et al.* (2013) estimated the heritability of bronchodilator response (~30%), airway hyper-responsiveness (~50%) and asthma liability (~61%) due to SNPs in aggregate using the MLM/GCTA approach [87]. Linkage studies have yielded comparable heritability estimates for both bronchodilator response (~12–40%) and airway responsiveness (~67%) [20,93,94], supporting the validity of the polygenic modeling approach. In addition, the estimate obtained for the heritability of asthma corresponds to published asthma heritability from twin studies ranging from 70 to 90% [95]. With polygenic approaches, the total variance explained by a series of alleles should approach the heritability estimates by other methods, unless

there are nonadditive mechanisms or causal alleles are not well tagged in the GWAS SNP panels. This study indicates polygenic modeling can provide heritability estimates within the range of heritability measured in familial studies. Therefore, MLM/GCTA are suitable for providing narrow-sense heritability estimates for PGx traits where family or other approaches are not possible for estimating heritability.

MLM/GCTA methods were largely developed for GWAS data of complex traits, and at this point, most GWAS for non-PGx traits have very large sample size. With PGx traits, low sample size is common and this can limit the utility of polygenic approaches unless strategies are implemented to increase sample size, such as multi-institution collaborations to combine datasets. In addition, all methods have expectations of the type of phenotype that will be used, implicit in the

development of the method. Pharmacological measurements and outcome measures can be complex, such as ordinal variables or survival times subject to censoring, to which current polygenic models can be difficult to apply. Despite these limitations, polygenic analyses are showing utility in providing an additional tool for seeking information about the relationship between genetic architecture and PGx traits and estimates of heritability of these traits.

Further limitations of MLM/GCTA methods include the underlying assumptions that genotype effects are predominantly additive, thereby limiting assessment of the 'mutational burden' as a measure of genetic influence on a trait, including response to therapy. For example, this approach ignores the pervasive influence of epistatic gene–gene interactions, where the effect of one variant is contingent on the presence of another variant. Also, while this approach may yield an estimate of the trait's heritability, it remains to be determined whether mutational load of many variants can serve as clinical biomarker panels to guide therapeutic decision.

### Future methods

In addition to polygenic analyses, other approaches may provide keys to elucidating the etiology of PGx traits. Different predictive models based on genetic architecture may be necessary to explain many PGx traits that remain to be elucidated. These models may not include loci of large effect, and some of these models may not be additive and fail assumptions of linear regression. Novel approaches are being introduced and refined at a fast rate, and these may emerge as key tools for exposing further the genetic architecture underlying PGx traits. New technologies for characterizing the genome are also emerging. These technologies include large-scale high-throughput sequencing to detect comprehensive genetic variation data including low-frequency variants [96], genetic and genomic variation such as copy-number variants, new gene expression technologies and methods to detect the complex epigenetic landscape of the human genome. One can argue that drug therapy ranks among those environmental stimuli that alter the epigenetic chromatin landscape, thereby adding another dimension to PGx. Novel analysis methods include pathway [97] and prior knowledge based approaches [98], rare variant analyses [99,100] and interaction studies [101]. Integration of these diverse large-scale datasets has the potential for driving PGx discovery and clinical applications.

Pathway approaches are becoming more common, taking advantage of prior knowledge previously obtained in molecular and cellular biology studies. Diverse databases cataloging the results of countless

PGx studies include the PharmGKB database mentioned earlier. In addition, newly developed tools allow users to tap simultaneously into multiple database sources for pathway based analyses, such as Biofilter [102–104] and PARIS [102]. Furthermore, as mentioned, pathway based approaches can serve as 'input' for polygenic analyses, reducing the search space for variables by collapsing multiple genes into groups [88].

Methods are now emerging that enable exploring rare-variant data, usually defined as SNPs with allele frequencies <0.01, data of greater abundance with comprehensive sequencing data becoming available. The impact of rare variants on PGx traits are just beginning to be explored. Examples of already discovered rare-variants for PGx traits include rare variants found within the *SLCO1B1* gene, where haplotypes have been associated with reduced methotrexate clearance during treatment of childhood acute lymphoblastic leukemia. *SLCO1B1* variants accounted for 10.7% of the population variability in clearance. Of those variants, common nonsynonymous variants contributed the most to variability, but rare nonsynonymous variants contributed to 1.9% of total variation in clearance [105]. This example illustrates the promise of searching for rare variants but also cautions against optimistic expectations regarding clinical utility. In this case, the rare variants contribute a relatively small portion to the variability attributable to *SLCO1B1*, and for clinical utility in and an individual patient, the *SLCO1B1* phasing is typically unknown, adding uncertainty to any clinical recommendations.

Rare-variant collapsing strategies have now been developed for assessing their influence on traits, as the power for detecting the relationship between single low-frequency variants is limited. Collapsing approaches provide a way to identify specific patterns of genetic variation predictive of outcome variation. Several collapsing methods have been published in the past 5 years [106–114]. An example of a novel collapsing strategy is BioBin [115–117], a low-frequency variant collapsing method that considers the cumulative effect of rare variants within genetic features chosen by the users. These features can include genes but can also be pathways, or other biologically based criteria such as evolutionarily conserved regions.

### The role of epistasis in PGx traits

One of the reasons for the popularity of the GWAS and candidate gene approach is the simplicity of the regressive model for interpretation, and clear guidelines for ascertainment of significance and multiple hypothesis testing corrections. However, a variety of tools exist for the development of more complex predictive models beyond single-variant/outcome association testing for

common variants. For example, step-wise regression can be used to develop models with additional terms, instead of using single variant data. More complex models may show better outcome prediction, such as gene-by-gene (GxG) interaction models.

The overall role of dynamic GxG interactions remains a matter of debate. One can argue that a substantial portion of the 'missing heritability' of complex traits is accounted for by epistasis [118], but few studies document this in PGx. It may require identifying the interplay of more than one genetic variant to adequately predict the outcome of drug administration [119]. An example of an interaction has been found between the dopamine D2 receptor and the dopamine transporter, encoded by *DRD2* and *DAT*, respectively. Both genes harbor several common regulatory variants but only *DRD2* is associated with lethal risk resulting from cocaine abuse when each gene is studied separately. Yet, a combination of a single variants from both *DRD2* and *DAT* convey a seven- to eight-fold increased risk in a highly significant interaction model [120]. Such cases of gene-gene-environment interaction may be more prevalent than currently anticipated and need to be explored on a broader basis.

One challenge for seeking more complex models is the number of options to investigate, when investigating pairwise GxG and SNP-by-SNP (SNP×SNP) interaction models, as the number of potential interactions skyrockets as the number of variants grows. Tools exist for generating pair-wise GxG interaction models that address this. For example, Biofilter [104] is a tool that allows users to filter and annotate genetic data, as well as generate pairwise SNP×SNP models prioritized by the biological evidence supporting the genetic interaction. Multifactor dimensionality reduction (MDR) performs an exhaustive analysis of all n-wise interacting loci to generate models [101]. The Analysis Tool for Heritable and Environmental Network Associations (ATHENA) is a software tool that combines advanced filtering and machine learning analytical techniques to generate multi-variable models that can predict categorical or quantitative outcomes [121,122]. ATHENA can be used for both G×G/SNP×SNP interaction models that move beyond pairwise interactions, as well as for metadimensional analysis, where different data types of high-throughput genetic predictor variables are incorporated. However, all these methodologies require large sample cohorts, which are rarely encountered in PGx studies.

#### Clinical & regulatory decision making: moving from 'bench to bedside'

The list of drugs for which genetic information has potential utility in guiding individualized therapy is

growing. Clinical implementation is lagging behind our current knowledge in part because of multiple challenges faced in clinical practice. Recognizing the mandate to optimize drug therapy, the FDA maintains a website with current assessments of how clinicians should utilize PGx information (see Table of Pharmacogenomic Biomarkers in Drug Labels [45]). As more of the complex genetic architecture of PGx traits is uncovered, substantial challenges remain for translating PGx findings to the clinic. Key questions include: Is this an effective biomarker with clinical utility? How many individuals will be helped by genotyping a specific PGx variant? Will there be an impact on survival, recovery and/or prevention of a major adverse reaction and how much of an impact? Will a genetic variant manifest only in one population or is there evidence of consistency across multiple ancestral populations? Will the cost of genotyping for a PGx variant confer sufficient benefit to offset the cost?

A major concern for moving PGx findings to the clinic is the impact of ancestry on genetic variation. Highly significant associations between variants and PGx traits may differ considerably across ancestries, which has a direct impact on dosing decisions. For example, a significant association was found between the *HLA-B\*1502* variant and carbamazepine-induced (CBZ-induced) Stevens-Johnson syndrome in Han Chinese and Thai individuals [123–126]. However, separate studies have indicated that *HLA-B\*1502* is not a marker for all forms of CBZ-induced hypersensitivity in individuals of European descent [127,128]. In one study, the only four individuals out of 12 cases with CBZ-induced hypersensitivity had the *HLA-B\*1502* marker; these four individuals also had Asian ancestry [128].

Ancestry specific PGx differences in association of the arginine (Arg) 16 allele in the beta<sub>2</sub>-adrenergic receptor (beta<sub>2</sub>-AR) with asthma severity and bronchodilator response [129] have been found. Two admixed populations, Puerto Ricans and Mexicans, have different proportions of European, African and Native American ancestry. These two populations have the highest and lowest asthma prevalence, morbidity and mortality respectively. In the study by Choudhury *et al.* (2005), associations between bronchodilator response, asthma severity and the beta<sub>2</sub>-AR (Arg) 16 allele were found in Puerto Ricans, but not in Mexicans. These results are likely accounted for by the presence of more than one causative variant in the same gene, or in interaction genes, with distinct population distribution.

We have mentioned already *CYP2C9* variation and warfarin dosing. Polymorphisms in *CYP2C9* account for 18% of the variance in warfarin dose, and polymorphisms in *VKORC1* account for 30%, in European Americans; however, these variants account for a

smaller portion of variability in patients with Asian or African Ancestry [40,130–137]. Additional *CYP2C9* variant alleles with reduced activity (*CYP2C9*\*5, \*6, \*8 and \*11) have been found to contribute to dose variability among African–Americans [55,138].

The field of PGx already has a record of investigations in groups beyond European Americans, when contrasted with much of the initial work of GWAS that was focused on European American ancestry. CPIC guidelines usually contain statements about existing knowledge of gene/drug pair information across ancestry. Work is being done to determine repeated drug administration rGC values across ancestry [29]. These analyses incorporating multiple ancestries should continue to be an important pursuit for the field of PGx moving forward. FDA labeling should also consistently reflect what populations PGx discoveries were made in, as that may impact the utility of a biomarker for a given patient.

Finally, substantial inconsistencies exist in study design, dosing regimens, study population and analysis methods for the field of PGx. For example, three studies, with differences in ethnic background and disease state of patients, study size and methods used to measure response to treatment, have reported contradictory results on the association of the FcγRIIIA 158V/F polymorphism and response to etanercept or infliximab in patients with Rheumatoid Arthritis [139–141]. Such inconsistencies are common between PGx studies, making interpretation of results across studies challenging, even for the same PGx trait. Furthermore, huge variability exists in the information that is reported when a PGx study is published. A standardized way of reporting PGx results and more consistency in study design could assist in developing clear guidelines for what constitutes a validated and actionable PGx result, and provide the means for comparing results across studies. The CPIC-authored studies have made recommendations for evaluating PGx results and reporting information accessible to clinicians [46].

## Conclusion

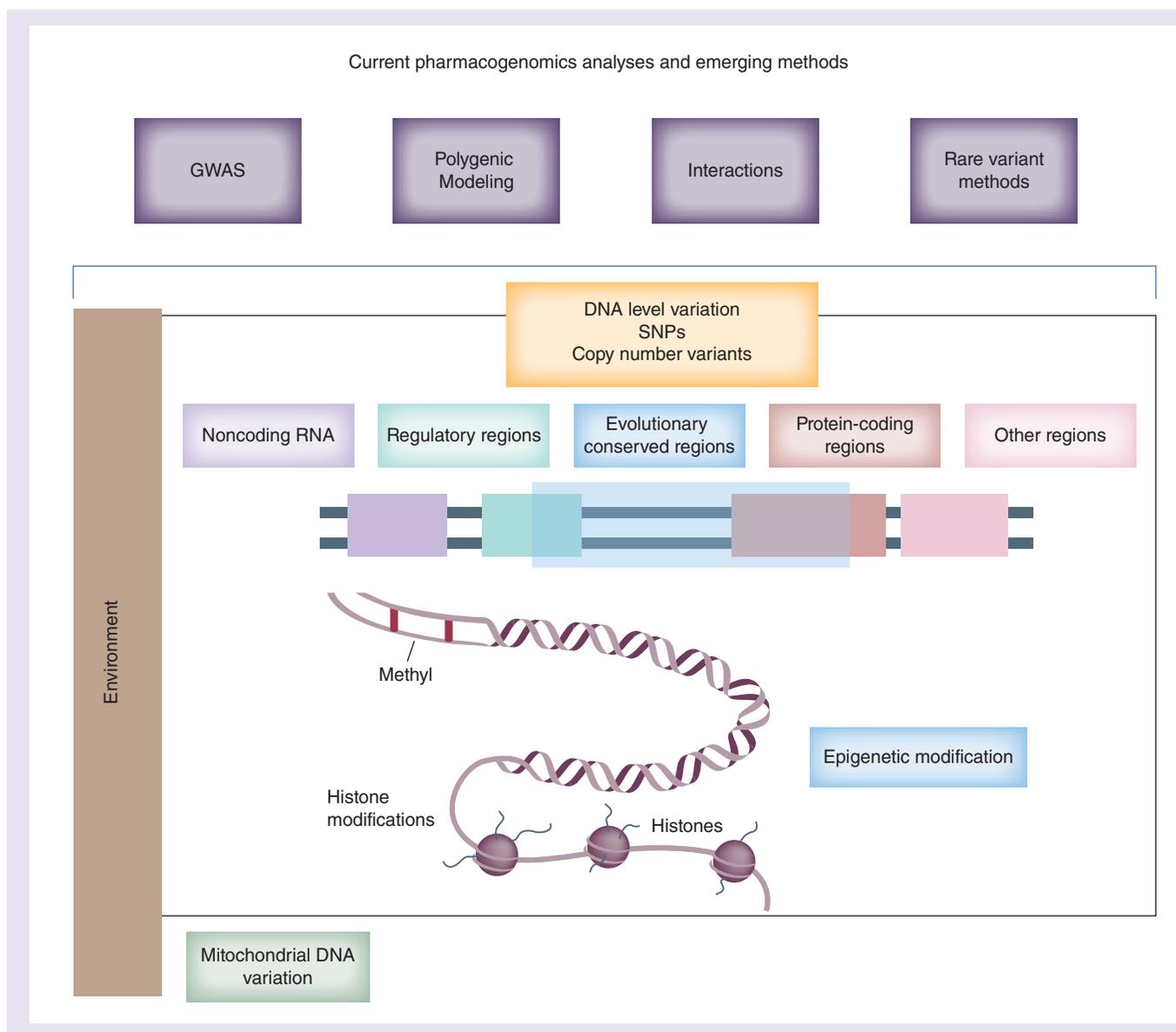
Current understanding of the genetic architecture of PGx traits presents a picture of a substantial impact of genetic variation on PK, PD, adverse events and other pharmacological outcomes. We have detailed here several examples of successes for the field of PGx. This information is being moved into the clinic for aiding decision making and results of these studies guide future drug development. While this knowledge is proving useful, we have outlined here key considerations for future PGx research and use of clinical biomarkers. **Figure 3** provides an overview of important aspects that should be integrated in PGx studies

to identify more robust markers for PGx traits and advance a more comprehensive understanding of the relationship between genetic architecture and drug response.

Improving the way the PGx field has been sharing PGx association results, and expanding what is considered ‘validation’ and ‘replication’ for PGx association results, has broad potential for improving the utility of PGx findings as robust clinical biomarkers. Standardized reporting of PGx results will assist in compiling evidence and subsequent interpretation of multiple study results. Furthermore, molecular evaluation and validation of the mechanism by which polymorphisms have an effect on outcome needs to be an important step after association studies have identified variants of interest. While seeking replication of association results over multiple studies can provide evidence for a biomarker, establishing the biological mechanistic role of a genetic variant on outcome can identify robust markers for clinical trial [8,72]. Multiple polymorphisms have known effects on protein coding genes, such as the well understood ADME genes. However, GWAS have identified numerous genetic variants outside of protein coding genes. As nearly 80% of the human genome is transcribed while only 1.2% encodes proteins, and as countless genomic regions carry epigenetic regulatory marks, our emerging understanding of the dynamic nature of nonprotein-coding regions of the genome must be leveraged for studying functionality of SNPs identified in association studies.

The field of PGx and GWAS of complex traits have focused almost exclusively on SNPs of common frequency. Rare variants, as well as other genetic variation such as copy number variation and mitochondrial variants may also prove important moving forward. Much of the original GWAS for complex traits was limited to individuals of European descent. The PGx field has stronger track record of studies across ancestry, accruing information about variation in drug response and genetic variation across multiple ancestries. Such ancestry information has clinical relevance and is being incorporated with FDA drug labeling.

An emphasis on cross-disciplinary work has become increasingly critical, with involvement of clinicians, genetic epidemiologists, statisticians, bioinformaticists and molecular and cellular biologists. The Pharmacogenomics Research Network (PGRN), and the related Pharmacogenomics Statistical Analysis Resource (P-STAR), exemplify cross-disciplinary collaborations supporting PGx discovery. Furthermore, novel computational and statistical methods will prove critical, given the explosion of data generated in recent years. Simulations will be useful for exploring models for PGx traits.



**Figure 3. Uncovering the genetic etiology of pharmacogenomic traits: methodologies and data.** Along the top of the figure: pharmacogenomics studies should incorporate multiple types of analyses, beyond GWAS moving forward. Lower part of the figure: pharmacogenomics methods need to incorporate multiple types of genomic data, and consider the importance of environment as a modifier. Combining these elements may yield improved predictions of pharmacogenomics outcomes. Furthermore, detailed molecular genetics studies following up on genomic association discovery will be important for identifying robust biomarkers for clinical decision-making.

GWAS: Genome-wide association study.

DNA/histone lower part of figure adapted with permission from [142].

Future work utilizing electronic health records will provide new dimensions for the successful development of PGx phenotypes, cohorts, studies and hypotheses, made available on a large scale through consortia such as the eMERGE network [143]. For analytical methods development, future efforts should include extension of MLM/GCTA/polygenic methods for better handling non-normal PGx traits, investigating sensitivity of heritability estimates with model specification and covariate selection and the development of integrated

analysis methods for simultaneously incorporating different types of genomic features (genetic and epigenetic) and prior knowledge (pathways, gene sets, etc.) in each PGx study. Going beyond additive models, future studies should also focus on dynamic (epistatic) gene–gene interactions, and the impact of environmental influence.

### Future perspective

PGx research has already yielded numerous examples of the pervasive effect of genetic factors on drug response.

These advances demonstrate that clinical applications of pharmacogenomic biomarker tests have outstanding potential to enhance efficacy and reduce adverse effects, considered a main cause of morbidity and mortality – thereby showing promise for advancing the NIH mandate for the future of genomics. However, much of the genetic influence on treatment outcomes remains hidden, leaving uncertain how many genes and genetic variants contribute to pharmacological traits, how common and rare variants affect response and whether gene–gene interactions play a role. These relationships form the ‘pharmacogenomics architecture’ that still needs to be elucidated, presaging a profound evolution of the field of PGx, as is occurring in genomics studies of complex disorders. New approaches and studies across multiple human populations will prove critical

for the characterization of the genetic architecture of pharmacogenomic traits required for realizing the full potential of PGx in guiding the development of optimal individualized therapies. With these advances realized over the next 5–10 years, the findings of PGx will dramatically increase use of genotypic data by clinicians in decisions on individual therapies, with substantially improved health outcomes.

#### Financial & competing interests disclosure

The authors thank the Pharmacogenomics Research Network (PGRN) and its trans-network project on ‘Polygenic Modeling’: P-STAR (PGRN Statistical Analysis Resource (funded as part of PAT – HL065962, XGEN (Expression Genetics in Drug Therapy) PMT GM092655, PAAR4Kids (Pharmacogenomics of Anticancer Agents Research in Children) GM092666,

### Executive summary

#### What have we learned?

- Heritability of pharmacogenomic traits: challenges & successes
  - Defining the heritability of pharmacogenomic (PGx) traits encounters hurdles distinct from those found in the analysis of complex disorders.
  - Heritability estimates in PGx: methods and current estimates.
- The PGx landscape: drug efficacy & adverse events
  - PGx in the clinic: warfarin, clopidogrel.
  - Dosing guidelines: US FDA Labels and Clinical Pharmacogenetics Implementation Consortium (CPIC).
  - Larger relative strength of effect size for PGx traits compared with the range of effect sizes observed with complex-trait genome-wide association studies.
- Functional role of genomic architecture in PGx traits
  - Focus of interpretation of the relationship between genetic variability and outcome for PGx has been centered on protein-coding regions.
  - Much more to discover.
    - Mechanistic etiology of genetic variation on PGx traits is a critical focus for future PGx studies.

#### Beyond PGx genome-wide association studies: polygenic analyses

- Polygenic genetic architecture
  - Contribution of multiple common SNPs to phenotypic variance in aggregate.
- Two methods have already been used for a variety of complex outcomes for non-PGx traits:
  - Mixed linear modeling.
  - Polygenic modeling.
- Overview of current method use, discovery and limitations.

#### Future methods

- Summary of other methods of utility for future PGx research.
- The role of epistasis in PGx traits.

#### Clinical & regulatory decision-making: moving from ‘bench to bedside’

- As more of the complex genetic architecture of PGx traits is uncovered, substantial challenges remain for translating PGx findings to the clinic.
- Highly significant associations between variants and PGx traits may differ considerably across ancestries, which has a direct impact on dosing decisions.
- Substantial inconsistencies exist in study design, dosing regimens, study population and analysis methods for the field of PGx that should be addressed.

#### Conclusion

- Much has been learned in the PGx field about the genetic architecture of PGx; however, there is more to be understood.
- A variety of additional methods and approaches should be included in future PGx research.
- There are many challenges still faced by the field, but ultimately promise for incorporating understanding of individuals genetic architecture for personalized/precision medicine.

PEAR (Pharmacogenomic Evaluation of Antihypertensive Responses) GM074492, PARC (Pharmacogenomics and Risk of Cardiovascular Disease) HL069757, PHAT (Pharmacogenetics of Asthma Treatment) HL065899, PAPI (Pharmacogenomics of Anti-Platelet Intervention) HL105198, PHRAT (Pharmacogenomics of Rheumatoid Arthritis Therapy) GM092691. The

authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

## References

Papers of special note have been highlighted as:

• of interest; •• of considerable interest

- Green ED, Guyer MS. Charting a course for genomic medicine from base pairs to bedside. *Nature* 470(7333), 204–213 (2011).
- Bergmann TK, Vach W, Feddersen S *et al.* GWAS-based association between RWDD3 and TECTA variants and paclitaxel induced neuropathy could not be confirmed in Scandinavian ovarian cancer patients. *Acta Oncol.* 52(4), 871–874 (2013).
- PGRN.  
www.pgrn.org/display/pgrnwebsite/PGRN+Home
- Ofverholm A, Einbeigi Z, Manouchehrpour S, Albertsson P, Skrtic S, Enerbäck C. The ABCB1 3435 T allele does not increase the risk of paclitaxel-induced neurotoxicity. *Oncol. Lett.* 1(1), 151–154 (2010).
- PharmGKB.  
www.pharmgkb.org/
- Maher B. Personal genomes: the case of the missing heritability. *Nature* 456(7218), 18–21 (2008).
- **After many genome-wide association studies for common traits, very little of the heritability of traits has been explained. This commentary outlines several ways more can be elucidated about the genomic architecture underlying traits and outcomes. These ideas can also be applied to future work in pharmacogenomics (PGx), as the PGx field moves forward.**
- Ioannidis JPA. Biomarker Failures. *Clin. Chem.* 59(1), 202–204 (2013).
- Sadee W. Pharmacogenomic biomarkers: validation needed for both the molecular genetic mechanism and clinical effect. *Pharmacogenomics* 12(5), 675–680 (2011).
- **Discussion of the importance of laboratory research into the molecular mechanisms of PGx findings.**
- Wilke RA, Ramsey LB, Johnson SG *et al.* The clinical pharmacogenomics implementation consortium: CPIC guideline for SLCO1B1 and simvastatin-induced myopathy. *Clin. Pharmacol. Ther.* 92(1), 112–117 (2012).
- Shuldiner AR, O'Connell JR, Bliden KP *et al.* Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. *JAMA* 302(8), 849–857 (2009).
- Vesell ES, Page JG. Genetic control of dicumarol levels in man. *J. Clin. Invest.* 47(12), 2657–2663 (1968).
- Faraday N, Yanek LR, Mathias R *et al.* Heritability of platelet responsiveness to aspirin in activation pathways directly and indirectly related to cyclooxygenase-1. *Circulation* 115(19), 2490–2496 (2007).
- Stark AL, Zhang W, Mi S *et al.* Heritable and non-genetic factors as variables of pharmacologic phenotypes in lymphoblastoid cell lines. *Pharmacogenomics J.* 10(6), 505–512 (2010).
- **Research into considerations for the use of lymphoblastoid cell lines for PGx research.**
- Dolan ME, Newbold KG, Nagasubramanian R *et al.* Heritability and linkage analysis of sensitivity to cisplatin-induced cytotoxicity. *Cancer Res.* 64(12), 4353–4356 (2004).
- Peters EJ, Motsinger-Reif A, Havener TM *et al.* Pharmacogenomic characterization of US FDA-approved cytotoxic drugs. *Pharmacogenomics* 12(10), 1407–1415 (2011).
- **High-throughput evaluation of the cytotoxic effect of 29 commonly prescribed chemotherapeutics across 125 lymphoblastoid cell lines derived from 14 extended CEPH families.**
- Watters JW, Kraja A, Meucci MA, Province MA, McLeod HL. Genome-wide discovery of loci influencing chemotherapy cytotoxicity. *Proc. Natl Acad. Sci. USA* 101(32), 11809–11814 (2004).
- Duan S, Bleibel WK, Huang RS *et al.* Mapping genes that contribute to daunorubicin-induced cytotoxicity. *Cancer Res.* 67(11), 5425–5433 (2007).
- Lamba JK, Crews K, Pounds S *et al.* Pharmacogenetics of deoxycytidine kinase: identification and characterization of novel genetic variants. *J. Pharmacol. Exp. Ther.* 323(3), 935–945 (2007).
- Kalow W, Tang BK, Endrenyi L. Hypothesis: comparisons of inter- and intra-individual variations can substitute for twin studies in drug research. *Pharmacogenetics.* 8(4), 283–289 (1998).
- **Introduction and explanation of the use of repeated drug administration for estimation of heritability.**
- Wu AC, Tantisira K, Li L, Schuemann B, Weiss S, Childhood Asthma Management Program Research Group. Repeatability of response to asthma medications. *J. Allergy Clin. Immunol.* 123(2), 385–390 (2009).
- Leabman MK, Giacomini KM. Estimating the contribution of genes and environment to variation in renal drug clearance. *Pharmacogenetics* 13(9), 581–584 (2003).
- Pentikäinen PJ, Neuvonen PJ, Penttilä A. Pharmacokinetics of metformin after intravenous and oral administration to man. *Eur. J. Clin. Pharmacol.* 16(3), 195–202 (1979).
- Sjövall J, Alván G, Huitfeldt B. Intra- and inter-individual variation in pharmacokinetics of intravenously infused amoxicillin and ampicillin to elderly volunteers. *Br. J. Clin. Pharmacol.* 21(2), 171–181 (1986).
- Hallén B, Guilbaud O, Strömberg S, Lindeke B. Single-dose pharmacokinetics of terodiline, including a stable

- isotope technique for improvement of statistical evaluations. *Biopharm. Drug Dispos.* 9(3), 229–250 (1988).
- 25 Arvidsson A, Hedman A. Plasma and renal clearance of iohexol – a study on the reproducibility of a method for the glomerular filtration rate. *Scand. J. Clin. Lab. Invest.* 50(7), 757–761 (1990).
  - 26 Wagner JG. Inter- and intrasubject variation of digoxin renal clearance in normal adult males. *Drug Intell. Clin. Pharm.* 22(7–8), 562–567 (1988).
  - 27 Kalow W, Endrenyi L, Tang B. Repeat administration of drugs as a means to assess the genetic component in pharmacological variability. *Pharmacology* 58(6), 281–284 (1999).
  - 28 Ozdemir V, Kalow W, Tang BK *et al.* Evaluation of the genetic component of variability in CYP3A4 activity: a repeated drug administration method. *Pharmacogenetics* 10(5), 373–388 (2000).
  - 29 Micheli JE, Chinn LW, Shugarts SB *et al.* Measuring the overall genetic component of nevirapine pharmacokinetics and the role of selected polymorphisms: towards addressing the missing heritability in pharmacogenetic phenotypes? *Pharmacogenet. Genomics* 23(11), 591–596 (2013).
  - 30 Birkenfeld AL, Jordan J, Hofmann U *et al.* Genetic influences on the pharmacokinetics of orally and intravenously administered digoxin as exhibited by monozygotic twins. *Clin. Pharmacol. Ther.* 86(6), 605–608 (2009).
  - 31 Kroetz DL, Nguyen T, Giang T *et al.* Heritability of digoxin pharmacokinetics. *Clin. Pharmacol. Ther.* 77(2), P21–P21 (2005).
  - 32 Leabman M, Brown C, Chung J *et al.* Heritability of metformin renal clearance. *Clin. Pharmacol. Ther.* 77(2), P61–P61 (2005).
  - 33 Gage BF, Lesko LJ. Pharmacogenetics of warfarin: regulatory, scientific, and clinical issues. *J. Thromb. Thrombolysis* 25(1), 45–51 (2008).
  - 34 Schwarz UI, Ritchie MD, Bradford Y *et al.* Genetic determinants of response to warfarin during initial anticoagulation. *N. Engl. J. Med.* 358(10), 999–1008 (2008).
  - 35 Taube J, Halsall D, Baglin T. Influence of cytochrome P-450 CYP2C9 polymorphisms on warfarin sensitivity and risk of over-anticoagulation in patients on long-term treatment. *Blood* 96(5), 1816–1819 (2000).
  - 36 Rost S, Fregin A, Ivaskevicius V *et al.* Mutations in VKORC1 cause warfarin resistance and multiple coagulation factor deficiency type 2. *Nature* 427(6974), 537–541 (2004).
  - 37 Rieder MJ, Reiner AP, Gage BF *et al.* Effect of VKORC1 haplotypes on transcriptional regulation and warfarin dose. *N. Engl. J. Med.* 352(22), 2285–2293 (2005).
  - 38 Scott SA, Edelmann L, Kornreich R, Desnick RJ. Warfarin pharmacogenetics: CYP2C9 and VKORC1 genotypes predict different sensitivity and resistance frequencies in the Ashkenazi and Sephardi Jewish populations. *Am. J. Hum. Genet.* 82(2), 495–500 (2008).
  - 39 Veenstra DL, You JHS, Rieder MJ *et al.* Association of Vitamin K epoxide reductase complex 1 (VKORC1) variants with warfarin dose in a Hong Kong Chinese patient population. *Pharmacogenet. Genomics* 15(10), 687–691 (2005).
  - 40 International Warfarin Pharmacogenetics Consortium, Klein TE, Altman RB *et al.* Estimation of the warfarin dose with clinical and pharmacogenetic data. *N. Engl. J. Med.* 360(8), 753–764 (2009).
  - 41 Kimmel SE, French B, Kasner SE *et al.* A pharmacogenetic versus a clinical algorithm for warfarin dosing. *N. Engl. J. Med.* 369(24), 2283–2293 (2013).
  - 42 Pirmohamed M, Burnside G, Eriksson N *et al.* A randomized trial of genotype-guided dosing of warfarin. *N. Engl. J. Med.* 369(24), 2294–2303 (2013).
  - 43 Scott SA, Sangkuhl K, Gardner EE *et al.* Clinical pharmacogenetics implementation consortium guidelines for cytochrome P450–2C19 (CYP2C19) genotype and clopidogrel therapy. *Clin. Pharmacol. Ther.* 90(2), 328–332 (2011).
  - 44 Collet J-P, Hulot J-S, Pena A *et al.* Cytochrome P450 2C19 polymorphism in young patients treated with clopidogrel after myocardial infarction: a cohort study. *Lancet* 373(9660), 309–317 (2009).
  - 45 Table of Pharmacogenomic Biomarkers in Drug Labeling. [www.fda.gov/drugs/scienceresearch/researchareas/pharmacogenetics/ucm083378.htm](http://www.fda.gov/drugs/scienceresearch/researchareas/pharmacogenetics/ucm083378.htm)
  - 46 Relling MV, Klein TE. CPIC: Clinical Pharmacogenetics Implementation Consortium of the Pharmacogenomics Research Network. *Clin. Pharmacol. Ther.* 89(3), 464–467 (2011).
- **Explanation of the development and goals of Clinical Pharmacogenetics Implementation Consortium, and discussion of important considerations for the clinical community for moving PGx findings from ‘bench to bedside.’**
  - 47 Swen JJ, Nijenhuis M, de Boer A *et al.* Pharmacogenetics: from bench to byte – an update of guidelines. *Clin. Pharmacol. Ther.* 89(5), 662–673 (2011).
  - 48 PharmGKB: Dosing Guidelines – CPIC. [www.pharmgkb.org/view/dosing-guidelines.do?source=CPIC](http://www.pharmgkb.org/view/dosing-guidelines.do?source=CPIC)
  - 49 PharmGKB: CPIC pairs. [www.pharmgkb.org/page/cpicGeneDrugPairs](http://www.pharmgkb.org/page/cpicGeneDrugPairs)
  - 50 TPMT nomenclature committee. [www.imh.liu.se/tpmtalleles](http://www.imh.liu.se/tpmtalleles)
  - 51 PharmGKB: TPMT. [www.pharmgkb.org/gene/PA356#tabview=tab4&subtab=31](http://www.pharmgkb.org/gene/PA356#tabview=tab4&subtab=31)
  - 52 Relling MV, Gardner EE, Sandborn WJ *et al.* Clinical Pharmacogenetics Implementation Consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing. *Clin. Pharmacol. Ther.* 89(3), 387–391 (2011).
  - 53 CYP2C19 allele nomenclature. [www.cypalleles.ki.se/cyp2c19.htm](http://www.cypalleles.ki.se/cyp2c19.htm)
  - 54 Scott SA, Sangkuhl K, Stein CM *et al.* Clinical Pharmacogenetics Implementation Consortium guidelines for CYP2C19 genotype and clopidogrel therapy: 2013 update. *Clin. Pharmacol. Ther.* 94(3), 317–323 (2013).

- 55 Johnson JA, Gong L, Whirl-Carrillo M *et al.* Clinical Pharmacogenetics Implementation Consortium Guidelines for CYP2C9 and VKORC1 genotypes and warfarin dosing. *Clin. Pharmacol. Ther.* 90(4), 625–629 (2011).
- 56 The Human Cytochrome P450 (CYP) Allele Nomenclature Database. [www.cypalleles.ki.se](http://www.cypalleles.ki.se)
- 57 Crews KR, Gaedigk A, Dunnenberger HM *et al.* Clinical Pharmacogenetics Implementation Consortium Guidelines for Cytochrome P450 2D6 Genotype and Codeine Therapy: 2014 Update. *Clin. Pharmacol. Ther.* 95(4), 376–382 (2014).
- 58 Martin MA, Hoffman JM, Freimuth RR *et al.* Clinical Pharmacogenetics Implementation Consortium Guidelines for HLA-B Genotype and Abacavir Dosing: 2014 Update. *Clin. Pharmacol. Ther.* 95(5), 499–500 (2014).
- 59 Hershfield MS, Callaghan JT, Tassaneeyakul W *et al.* Clinical Pharmacogenetics Implementation Consortium guidelines for human leukocyte antigen-B genotype and allopurinol dosing. *Clin. Pharmacol. Ther.* 93(2), 153–158 (2013).
- 60 Hicks JK, Swen JJ, Thorn CF *et al.* Clinical Pharmacogenetics Implementation Consortium guideline for CYP2D6 and CYP2C19 genotypes and dosing of tricyclic antidepressants. *Clin. Pharmacol. Ther.* 93(5), 402–408 (2013).
- 61 Leckband SG, Kelsoe JR, Dunnenberger HM *et al.* Clinical Pharmacogenetics Implementation Consortium guidelines for HLA-B genotype and carbamazepine dosing. *Clin. Pharmacol. Ther.* 94(3), 324–328 (2013).
- 62 Caudle KE, Thorn CF, Klein TE *et al.* Clinical Pharmacogenetics Implementation Consortium guidelines for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing. *Clin. Pharmacol. Ther.* 94(6), 640–645 (2013).
- 63 Muir AJ, Gong L, Johnson SG *et al.* Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for IFNL3 (IL28B) genotype and PEG interferon- $\alpha$ -based regimens. *Clin. Pharmacol. Ther.* 95(2), 141–146 (2014).
- 64 Clancy JP, Johnson SG, Yee SW *et al.* Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for ivacaftor therapy in the context of CFTR genotype. *Clin. Pharmacol. Ther.* (2014).
- 65 Hindorf LA, Sethupathy P, Junkins HA *et al.* Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. *Proc. Natl Acad. Sci. USA* 106(23), 9362–9367 (2009).
- 66 Zhou S-F. Polymorphism of human cytochrome P450 2D6 and its clinical significance: Part I. *Clin. Pharmacokinet.* 48(11), 689–723 (2009).
- 67 Thorn CF, Klein TE, Altman RB. PharmGKB: The Pharmacogenomics Knowledge Base. *Methods Mol. Biol.* 1015, 311–320 (2013).
- The Pharmacogenomics Knowledge Base, through the Pharmacogenomics Research Network (PGRN). They describe the PharmGKB website ([www.pharmgkb.org](http://www.pharmgkb.org)), a knowledgebase including genotypic, molecular and clinical PGx knowledge with links to additional external resources for PGx research.
- 68 Wang D, Poi MJ, Sun X, Gaedigk A, Leeder JS, Sadee W. Common CYP2D6 polymorphisms affecting alternative splicing and transcription: long-range haplotypes with two regulatory variants modulate CYP2D6 activity. *Hum. Mol. Genet.* 23(1), 268–278 (2014).
- 69 Daly AK. Genome-wide association studies in pharmacogenomics. *Nat. Rev. Genet.* 11(4), 241–246 (2010).
- 70 Daly AK. Pharmacogenomics of adverse drug reactions. *Genome Med.* 5(1), 5 (2013).
- 71 Pompeu YA, Stewart JD, Mallal S, Phillips E, Peters B, Ostrov DA. The structural basis of HLA-associated drug hypersensitivity syndromes. *Immunol. Rev.* 250(1), 158–166 (2012).
- 72 Sadee W, Wang D, Papp AC *et al.* Pharmacogenomics of the RNA world: structural RNA polymorphisms in drug therapy. *Clin. Pharmacol. Ther.* 89(3), 355–365 (2011).
- 73 ENCODE Project Consortium. A user's guide to the encyclopedia of DNA elements (ENCODE). *PLoS Biol.* 9(4), e1001046 (2011).
- 74 Ward LD, Kellis M. HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. *Nucl. Acids Res.* 40(Database issue), D930–D934 (2011).
- 75 Boyle AP, Hong EL, Hariharan M *et al.* Annotation of functional variation in personal genomes using RegulomeDB. *Genome Res.* 22(9), 1790–1797 (2012).
- 76 Gamazon ER, Zhang W, Konkashbaev A *et al.* SCAN: SNP and copy number annotation. *Bioinformatics* 26(2), 259–262 (2010).
- 77 Yang J, Lee SH, Goddard ME, Visscher PM. GCTA: a tool for genome-wide complex trait analysis. *Am. J. Hum. Genet.* 88(1), 76–82 (2011).
- 78 Yang J, Benyamin B, McEvoy BP *et al.* Common SNPs explain a large proportion of the heritability for human height. *Nat. Genet.* 42(7), 565–569 (2010).
- 79 Lee SH, Wray NR, Goddard ME, Visscher PM. Estimating missing heritability for disease from genome-wide association studies. *Am. J. Hum. Genet.* 88(3), 294–305 (2011).
- 80 Yang J, Manolio TA, Pasquale LR *et al.* Genome partitioning of genetic variation for complex traits using common SNPs. *Nat. Genet.* 43(6), 519–525 (2011).
- 81 Yang J, Ferreira T, Morris AP *et al.* Conditional and joint multiple-SNP analysis of GWAS summary statistics identifies additional variants influencing complex traits. *Nat. Genet.* 44(4), 369–375, S1–S3 (2012).
- 82 Purcell SM, Wray NR, Stone JL *et al.* Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature* 460(7256), 748–752 (2009).
- 83 Bush WS, Sawcer SJ, de Jager PL *et al.* Evidence for polygenic susceptibility to multiple sclerosis – the shape of things to come. *Am. J. Hum. Genet.* 86(4), 621–625 (2010).
- 84 Lango Allen H, Estrada K, Lettre G *et al.* Hundreds of variants clustered in genomic loci and biological pathways affect human height. *Nature* 467(7317), 832–838 (2010).

- 85 Speliotes EK, Willer CJ, Berndt SI *et al.* Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nat. Genet.* 42(11), 937–948 (2010).
- 86 Stahl EA, Wegmann D, Trynka G *et al.* Bayesian inference analyses of the polygenic architecture of rheumatoid arthritis. *Nat. Genet.* 44(5), 483–489 (2012).
- 87 McGeachie MJ, Stahl EA, Himes BE *et al.* Polygenic heritability estimates in pharmacogenetics: focus on asthma and related phenotypes. *Pharmacogenet. Genomics* 23(6), 324–328 (2013).
- 88 Chhibber A, Mefford J, Stahl EA *et al.* Polygenic inheritance of paclitaxel-induced sensory peripheral neuropathy driven by axon outgrowth gene sets in CALGB 40101 (Alliance). *Pharmacogenomics J.* 14(4), 336–342 (2014).
- 89 Bergmann TK, Gréen H, Brasch-Andersen C *et al.* Retrospective study of the impact of pharmacogenetic variants on paclitaxel toxicity and survival in patients with ovarian cancer. *Eur. J. Clin. Pharmacol.* 67(7), 693–700 (2011).
- 90 De Graan A-JM, Elens L, Sprowl JA *et al.* CYP3A4\*22 genotype and systemic exposure affect paclitaxel-induced neurotoxicity. *Clin. Cancer Res.* 19(12), 3316–3324 (2013).
- 91 Wang D, Guo Y, Wrighton SA, Cooke GE, Sadee W. Intronic polymorphism in CYP3A4 affects hepatic expression and response to statin drugs. *Pharmacogenomics J.* 11(4), 274–286 (2011).
- 92 Baldwin RM, Owzar K, Zembutsu H *et al.* A genome-wide association study identifies novel loci for paclitaxel-induced sensory peripheral neuropathy in CALGB 40101. *Clin. Cancer Res.* 18(18), 5099–5109 (2012).
- 93 Rijcken B, Schouten JP, Weiss ST, Rosner B, De Vries K, Van der Lende R. Long-term variability of bronchial responsiveness to histamine in a random population sample of adults. *Am. Rev. Respir. Dis.* 148(4 Pt 1), 944–949 (1993).
- 94 Moffatt MF, Gut IG, Demenais F *et al.* A large-scale, consortium-based genome-wide association study of asthma. *N. Engl. J. Med.* 363(13), 1211–1221 (2010).
- 95 Thomsen SF, van der Sluis S, Kyvik KO, Skytthe A, Backer V. Estimates of asthma heritability in a large twin sample. *Clin. Exp. Allergy* 40(7), 1054–1061 (2010).
- 96 Van Dijk EL, Auger H, Jaszczyszyn Y, Thermes C. Ten years of next-generation sequencing technology. *Trends Genet.* 30(9), 418–426 (2014).
- 97 Khatri P, Sirota M, Butte AJ. Ten Years of Pathway Analysis: Current Approaches and Outstanding Challenges. *PLoS Comput. Biol.* 8(2), e1002375 (2012).
- 98 Petersen A, Spratt J, Tintle NL. Incorporating prior knowledge to increase the power of genome-wide association studies. In: *Genome-Wide Association Studies and Genomic Prediction*. Gondro C, van der Werf J, Hayes B (Eds). Humana Press, NJ, USA, 519–541 (2013).
- 99 Worthey EA. Analysis and annotation of whole-genome or whole-exome sequencing-derived variants for clinical diagnosis. *Curr. Protoc. Hum. Genet.* 79, Unit 9.24 (2013).
- 100 Wagner MJ. Rare-variant genome-wide association studies: a new frontier in genetic analysis of complex traits. *Pharmacogenomics* 14(4), 413–424 (2013).
- 101 Cordell HJ. Genome-wide association studies: detecting gene-gene interactions that underlie human diseases. *Nat. Rev. Genet.* 10(6), 392–404 (2009).
- 102 Yaspan BL, Bush WS, Torstenson ES *et al.* Genetic analysis of biological pathway data through genomic randomization. *Hum. Genet.* 129(5), 563–571 (2011).
- 103 Pendergrass SA, Frase A, Wallace J *et al.* Genomic analyses with biofilter 2.0: knowledge driven filtering, annotation, and model development. *BioData Min.* 6(1), 25 (2013).
- 104 Bush WS, Dudek SM, Ritchie MD. Biofilter: a knowledge-integration system for the multi-locus analysis of genome-wide association studies. *Pac. Symp. Biocomput.*, 368–379 (2009).
- 105 Ramsey LB, Bruun GH, Yang W *et al.* Rare versus common variants in pharmacogenetics: SLCO1B1 variation and methotrexate disposition. *Genome Res.* 22(1), 1–8 (2012).
- 106 Bhatia G, Bansal V, Harismendy O *et al.* A covering method for detecting genetic associations between rare variants and common phenotypes. *PLoS Comput. Biol.* 6(10), e1000954 (2010).
- 107 Morgenthaler S, Thilly WG. A strategy to discover genes that carry multi-allelic or mono-allelic risk for common diseases: a cohort allelic sums test (CAST). *Mutat. Res.* 615(1–2), 28–56 (2007).
- 108 Li B, Leal SM. Methods for detecting associations with rare variants for common diseases: application to analysis of sequence data. *Am. J. Hum. Genet.* 83(3), 311–321 (2008).
- 109 Madsen BE, Browning SR. A groupwise association test for rare mutations using a weighted sum statistic. *PLoS Genet.* 5(2), e1000384 (2009).
- 110 Han F, Pan W. A data-adaptive sum test for disease association with multiple common or rare variants. *Hum. Hered.* 70(1), 42–54 (2010).
- 111 Price AL, Kryukov GV, de Bakker PIW *et al.* Pooled association tests for rare variants in exon-resequencing studies. *Am. J. Hum. Genet.* 86(6), 832–838 (2010).
- 112 Hoffmann TJ, Marini NJ, Witte JS. Comprehensive approach to analyzing rare genetic variants. *PLoS ONE* 5(11), e13584 (2010).
- 113 Wu MC, Lee S, Cai T, Li Y, Boehnke M, Lin X. Rare-variant association testing for sequencing data with the sequence kernel association test. *Am. J. Hum. Genet.* 89(1), 82–93 (2011).
- 114 Yandell M, Huff C, Hu H *et al.* A probabilistic disease-gene finder for personal genomes. *Genome Res.* 21(9), 1529–1542 (2011).
- 115 Moore CB, Wallace JR, Frase AT, Pendergrass SA, Ritchie MD. BioBin: a bioinformatics tool for automating the binning of rare variants using publicly available biological knowledge. *BMC Med. Genomics* 6(Suppl. 2), S6 (2013).
- 116 Moore CB, Wallace JR, Wolfe DJ *et al.* Low frequency variants, collapsed based on biological knowledge, uncover complexity of population stratification in 1000 genomes project data. *PLoS Genet.* 9(12), e1003959 (2013).
- 117 Moore CB, Wallace JR, Frase AT, Pendergrass SA, Ritchie MD. Using biobin to explore rare variant population stratification. *Pac. Symp. Biocomput.* 332–343 (2013).

- 118 Sadee W. The relevance of “missing heritability” in pharmacogenomics. *Clin. Pharmacol. Ther.* 92(4), 428–430 (2012).
- Discussion of moving beyond single SNP associations for PGx research to gene–gene and gene–environment explorations, as well as considering regulatory variants.
- 119 Evans WE, Relling MV. Pharmacogenomics: translating functional genomics into rational therapeutics. *Science* 286(5439), 487–491 (1999).
- Discussion of how the effect of medications are not monogenic in nature, describing how they are actually determined by the interplay of several genes encoding proteins involved in multiple pathways of drug metabolism, disposition and effects. Discussion of other important considerations for PGx research.
- 120 Sullivan D, Pinsonneault JK, Papp AC et al. Dopamine transporter DAT and receptor DRD2 variants affect risk of lethal cocaine abuse: a gene–gene–environment interaction. *Transl. Psychiatry* 3, e222 (2013).
- 121 Holzinger ER, Dudek SM, Frase AT, Krauss RM, Medina MW, Ritchie MD. Athena: a tool for meta-dimensional analysis applied to genotypes and gene expression data to predict hdl cholesterol levels. *Pac. Symp. Biocomput.* 385–396 (2013).
- 122 Holzinger ER, Buchanan CC, Dudek SM, Torstenson EC, Turner SD, Ritchie MD. Initialization parameter sweep in ATHENA: optimizing neural networks for detecting gene–gene interactions in the presence of small main effects. *Genet. Evol. Comput. Conf.* 12, 203–210 (2010).
- 123 Chen P, Lin J-J, Lu C-S et al. Carbamazepine-induced toxic effects and HLA-B\*1502 screening in Taiwan. *N. Engl. J. Med.* 364(12), 1126–1133 (2011).
- 124 Man CBL, Kwan P, Baum L et al. Association between HLA-B\*1502 allele and antiepileptic drug-induced cutaneous reactions in Han Chinese. *Epilepsia* 48(5), 1015–1018 (2007).
- 125 Hung S-I, Chung W-H, Liu Z-S et al. Common risk allele in aromatic antiepileptic-drug induced Stevens-Johnson syndrome and toxic epidermal necrolysis in Han Chinese. *Pharmacogenomics* 11(3), 349–356 (2010).
- 126 Locharernkul C, Loplumert J, Limotai C et al. Carbamazepine and phenytoin induced Stevens-Johnson syndrome is associated with HLA-B\*1502 allele in Thai population. *Epilepsia* 49(12), 2087–2091 (2008).
- 127 Alfirevic A, Jorgensen AL, Williamson PR, Chadwick DW, Park BK, Pirmohamed M. HLA-B locus in Caucasian patients with carbamazepine hypersensitivity. *Pharmacogenomics* 7(6), 813–818 (2006).
- 128 Lonjou C, Thomas L, Borot N et al. A marker for Stevens-Johnson syndrome ...: ethnicity matters. *Pharmacogenomics J.* 6(4), 265–268 (2006).
- 129 Choudhry S, Ung N, Avila PC et al. Pharmacogenetic differences in response to albuterol between Puerto Ricans and Mexicans with asthma. *Am. J. Respir. Crit. Care Med.* 171(6), 563–570 (2005).
- 130 Limdi N, Goldstein J, Blaisdell J, Beasley T, Rivers C, Acton R. Influence of CYP2C9 genotype on warfarin dose among African American and European Americans. *Per. Med.* 4(2), 157–169 (2007).
- 131 Scott SA, Jaremko M, Lubitz SA, Kornreich R, Halperin JL, Desnick RJ. CYP2C9\*8 is prevalent among African-Americans: implications for pharmacogenetic dosing. *Pharmacogenomics* 10(8), 1243–1255 (2009).
- 132 Tai G, Farin F, Rieder MJ et al. *In-vitro* and *in-vivo* effects of the CYP2C9\*11 polymorphism on warfarin metabolism and dose. *Pharmacogenet. Genomics* 15(7), 475–481 (2005).
- 133 Redman AR, Dickmann LJ, Kidd RS, Goldstein JA, Ritchie DM, Hon YY. CYP2C9 genetic polymorphisms and warfarin. *Clin. Appl. Thromb. Hemost.* 10(2), 149–154 (2004).
- 134 Cavallari LH, Langaee TY, Momary KM et al. Genetic and clinical predictors of warfarin dose requirements in African Americans. *Clin. Pharmacol. Ther.* 87(4), 459–464 (2010).
- 135 Wadelius M, Chen LY, Lindh JD et al. The largest prospective warfarin-treated cohort supports genetic forecasting. *Blood* 113(4), 784–792 (2009).
- 136 Gage BF, Eby C, Johnson JA et al. Use of pharmacogenetic and clinical factors to predict the therapeutic dose of warfarin. *Clin. Pharmacol. Ther.* 84(3), 326–331 (2008).
- 137 Limdi NA, Wadelius M, Cavallari L et al. Warfarin pharmacogenetics: a single VKORC1 polymorphism is predictive of dose across 3 racial groups. *Blood* 115(18), 3827–3834 (2010).
- 138 Suarez-Kurtz G, Botton MR. Pharmacogenomics of warfarin in populations of African descent. *Br. J. Clin. Pharmacol.* 75(2), 334–346 (2013).
- 139 Kastbom A, Bratt J, Ernestam S et al. Fcγ receptor type IIIA genotype and response to tumor necrosis factor alpha-blocking agents in patients with rheumatoid arthritis. *Arthritis Rheum.* 56(2), 448–452 (2007).
- 140 Tutuncu Z, Kavanaugh A, Zvaifler N, Corr M, Deutsch R, Boyle D. Fcγ receptor type IIIA polymorphisms influence treatment outcomes in patients with inflammatory arthritis treated with tumor necrosis factor alpha-blocking agents. *Arthritis Rheum.* 52(9), 2693–2696 (2005).
- 141 Cañete JD, Suárez B, Hernández MV et al. Influence of variants of Fcγ receptors IIA and IIIA on the American College of Rheumatology and European League Against Rheumatism responses to anti-tumour necrosis factor alpha therapy in rheumatoid arthritis. *Ann. Rheum. Dis.* 68(10), 1547–1552 (2009).
- 142 Walker DM, Gore AC. Transgenerational neuroendocrine disruption of reproduction. *Nat. Rev. Endocrinol.* 7(4), 197–207 (2011).
- 143 Gottesman O, Kuivaniemi H, Tromp G et al. The Electronic Medical Records and Genomics (eMERGE) Network: past, present, and future. *Genet. Med.* 15(10), 761–771 (2013).