Published in final edited form as:

Wiley Interdiscip Rev Syst Biol Med. 2013 January ; 5(1): 1–9. doi:10.1002/wsbm.1199.

Pharmacogenomics Discovery and Implementation in GWAS Era

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Abstract

Clinical response to therapeutic treatments often varies among individual patients, ranging from beneficial effect to even fatal adverse reaction. Pharmacogenomics holds the promise of personalized medicine through elucidating genetic determinants responsible for pharmacological outcomes (e.g., cytotoxicities to anti-cancer drugs) and therefore guide the prescription decision prior to drug treatment. Besides traditional candidate gene-based approaches, technical advances have begun to allow application of whole-genome approaches to pharmacogenomic discovery. In particular, comprehensive understanding of human genetic variation provides the basis for applying GWAS (genome-wide association studies) in pharmacogenomic research to identify genomic loci associated with pharmacological phenotypes (e.g., individual dose requirement for warfarin). We therefore briefly reviewed the background for pharmacogenetic/pharmacogenomic research, with statins and warfarin as examples for the GWAS discovery and their clinical implementation. In conclusion, with some challenges, whole-genome approaches such as GWAS have allowed unprecedented progress in identifying genetic variants associated with pharmacological phenotypes, as well as provided foundation for the next wave of pharmacogenomic discovery utilizing sequencing-based approaches. Furthermore, investigation of the complex interactions among genetic, and epigenetic factors on the whole-genome scale will become the post-GWAS research focus for pharmacologic complex traits.

Keywords

Pharmacogenomics; GWAS

The clinical response to prescribed drugs is often not consistent across individual patients or populations. At the extreme ends of the drug response spectrum can be severe adverse drug reactions, which have been shown to be one of major health problems responsible for hospital admissions, as well as over 100,000 annual deaths in the United States¹. Understandably, this kind of variation in drug response is of particular concern for those drugs with narrow therapeutic indexes. For example, warfarin, a widely prescribed anticoagulant, exhibits substantial inter-individual variability of dose requirement.

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Conflicts of interest: None.

Therefore, if not properly monitored, inappropriate warfarin doses may increase patients' risks for either thrombosis (i.e., dose too low) or serious bleeding (i.e., dose too high). Ideally, being able to identify patients that will respond well, badly, or not at all prior to treatment could greatly improve health care, by potentially maximizing the beneficial effect and minimizing the adverse effect of prescribed medicine.

Previous studies have shown that drug response is likely a complex phenotype or trait that is affected by both genetic and non-genetic factors (e.g., dosage, diet, environment), as well as patient-specific factors such as age, health condition, and socioeconomic status. Genetics (e.g., through regulating drug-metabolizing enzymes), however, has been demonstrated to contribute a significant proportion of drug response variability. For example, genetic variants in *CYP2C9* (cytochrome P450, family 2, subfamily C, polypeptide 9) and *VKORC1* (vitamin K epoxide reductase complex, subunit 1) were found to contribute significantly to the variability of warfarin dose requirement, independent of other non-genetic factors². In oncology, using a cell-based model, the genetic contribution (e.g., through single nucleotide polymorphisms [SNPs] regulating drug response associated gene expression phenotypes, i.e., expression quantitative trait loci [eQTLs]) have also been clearly demonstrated for several chemotherapeutic agents^{3, 4} (e.g., etoposide, carboplatin, cisplatin, daunorubicin, cytarabine)⁵. Pharmacogenetics and pharmacogenomics, therefore, hold the promise for the ultimate aim of personalized medicine by investigating the relationships between a patient's genetic make-up and various therapeutic phenotypes.

The Genetic Basis for Pharmacogenomic/Pharmacogenetic Research

The completion of the first human genome sequences represented the beginning of the genomic era of biomedical discovery⁶. Human genetic variation refers to the observed differences in DNA sequences (i.e., polymorphisms or genetic variants) both within and across populations. With the advances of sequencing and genotyping technologies, it is now feasible to obtain comprehensive genetic variation data of multiple genes, even at a genome-wide scale, in multiple samples efficiently (e.g., using whole-genome genotyping arrays and the next-generation sequencing technologies).

During the past decade, the general patterns of human genetic variation and the genetic architectures of a variety of complex traits and phenotypes such as gene expression, susceptibilities to common diseases, and response to therapeutic treatment have begun to be elucidated with the availability of comprehensive population-based genetic variation data for large genetic variation cataloging efforts including the International HapMap Project^{7, 8} and the 1000 Genomes Project⁹. For example, significant variation in quantitative gene expression, and gene regulation through genetic variants in the forms of SNPs and copy number variants (CNVs) have been investigated both within and across human populations using these models and genetic variation data¹⁰⁻¹⁷. Similar to quantitative gene expression, drug response is likely to be a heritable phenotype, which may be at least partially attributed to genetic diversity. For example, a previous study estimated that genetic factors could determine more than 40% of the susceptibility to cytotoxicity induced by cisplatin¹⁸, a platinating agent used in treating various types of human cancers including lung cancer, lymphoma, thus indicating that sensitivity to the cytotoxic effects of chemotherapeutic agents could be under substantial genetic influence.

General Approaches in Pharmacogenomic/Pharmacogenetic Research

For investigating the relationships between genes/genetic variants and therapeutic phenotypes, in general, there are two categories of research approaches: i.e., the candidate gene approach and the whole-genome approach. Briefly, studies applying the candidate gene approach focus on a limited number of candidate genes based on *a priori* knowledge for a

therapeutic phenotype of interest. Therefore, a successful candidate gene study relies on the selection of candidate genes and polymorphisms that may be potentially relevant to the therapeutic phenotype of interest. In general, candidate genes and polymorphisms can be selected based on the relevance of a known pathway or evidence from previous studies. For example, in a previous study using the candidate gene approach, genetic polymorphisms in *TPMT* (encoding thiopurine S-methyltransferase) were successfully identified to decrease *TPMT* enzyme activity and subsequently increase 6-mercaptopurine toxicity²³. In this case, the choice of candidate gene *TPMT* was based on the key role of thiopurine methyltransferase plays in converting the active drug to its inactive metabolite, 6-methylmercaptopurine²⁴. As mentioned above, another successful example for the candidate gene approach is the identification of polymorphisms in *CYP2C9* and *VKORC1* associated with inter-individual variability in the dose response of warfarin²⁵. Warfarin is metabolized primarily via oxidation in the liver by *CYP2C9*, and exerts its anticoagulant effect by inhibiting the *VKORC1* protein.

Though the candidate gene approach focuses on potential genes with functional relevance (e.g., drug-metabolizing enzymes for a pharmacokinetic phenotype), there still can be unknown genes or unidentified genetic variants that may help determine or modify the phenotypes of interest, e.g., through interactions with those known candidate genes. As a matter of fact, drug response phenotypes are often not explained sufficiently with known genes, this suggests that there could be novel genes, pathways and genetic variants unidentified in previous studies also responsible significantly for therapeutic phenotypes.

Furthermore, epigenetic markers including microRNAs¹⁹ and DNA methylation²⁰⁻²² have emerged to be crucial regulators of quantitative gene expression phenotype, thus providing the foundation for investigating the complex interactions among genetic, epigenetic factors and complex traits or phenotypes. Indeed, various genome-wide molecular profiling platforms are available for this purpose, including platforms for whole-genome gene expression, microRNA, as well as cytosine modification levels quantification. Therefore, the whole-genome approach could be used to expand and complement the candidate gene approach to interrogate the entire human genome, transcriptome, or epigenome for the relationships between genes/genomic loci and therapeutic phenotypes. For example, a SNP in *NRG3* gene (a gene that has never been linked to cancer) was found to be associated with cellular sensitivity to platinum agents through its effect on genome-wide gene expression and this association was replicated in ovarian cancer patients who underwent platinum-based therapy²⁶. More recently, the next-generation sequencing technologies²⁷ further opened up the possibility to identify all genetic variants and mRNA transcripts (i.e., through RNA-sequencing), thus potentially allowing direct mapping of the causal genetic variants or transcripts contributing to therapeutic phenotypes of interest without relying on linkage disequilibrium between genotyped variants and causal variants or sequences of known transcripts.

Interpretation of GWAS pharmacogenomic findings

GWAS in theory provided an unbiased way to demonstrate the relationship between genome-wide genetic variants and the phenotypic traits of interest. A Manhattan plot or QQ plot are often employed to illustrate these relationships. With the evolution of the genomic technology, GWAS gradually becomes a common method employed to study the relationship between human genome and drug response. Indeed, GWAS has been performed to evaluate genetic contribution to the treatment effect of various commonly used medications in the recent 5 years; for example, response to anti-TNF treatment in rheumatoid arthritis²⁸, flucloxacillin-induced liver injury²⁹, QT prolongation³⁰ and response to³¹ iloperidone in treatment of schizophrenia, metabolic side effects³² and

parkinsonism severity³³ induced by antipsychotic drugs, treatment response to antipsychotics³⁴, antidepressant response with escitalopram and nortriptyline³⁵, response to citalopram in major depressive disorder³⁶, antihypertensive response to thiazide diuretic³⁷, angiotensin II receptor blocker (candesartan) for antihypertensive response³⁸, glycemic response to metformin in type 2 diabetes³⁹, statin-mediated reduction in total and LDL-cholesterol⁴⁰, smoking cessation⁴¹, bisphosphonate-induced osteonecrosis of the jaw⁴², methotrexate disposition in acute lymphoblastic leukemia (ALL) children⁴³, overall survival of advanced non-small cell lung cancer patients treated with carboplatin and paclitaxel⁴⁴, glucocorticoid response in asthma patients⁴⁵, and short-acting $\beta(2)$ -agonist bronchodilator response⁴⁶.

These examples demonstrated the improvement of genomic technology where genomic coverage increased significantly from the initial 100,000 SNPs microarray to those that covered 1 million SNPs. They also showed the diversity of phenotypic traits studied, where both therapeutic efficacy and toxicity were of interest. Changes in technology, phenotypic diversity as well as increased sophistication in analytical methods in GWAS highlight the needs to properly interpret the GWAS findings.

From a phenotypic point of view, individual sensitivity to a given drug can be assessed through wide arrays of methods, from PK end points (e.g., amount of active drug in the blood stream) to disease symptom control (e.g., heart rate, blood pressure, tumor size reduction) to survival outcome and to patient quality of life. Various technologies can be employed to quantify each of these measurements. Therefore, it is extremely important when interpret GWAS findings to be within the context of the exact phenotypic traits studied. For example, when significant associations were observed between a set of genetic variants and blood level of a drug, it is incorrect to extrapolate to a conclusion that the genetic variants are affecting drug effect in patients. In addition, when conducting pharmacogenomic GWAS in clinical patient populations, the phenotypic heterogeneity can become a major barrier in proper identification of genetic contributors for the trait of interest.

Early GWASs tend to focus on the top associated relationship(s) as the so called “low hanging fruit”, for which the strongest genotypic-phenotypic relationship was seen for a given trait. Furthermore, the terms of genetic variants and genes are often used interchangeably in these early GWASs. In another word, when a genetic variant was found to be significantly associated with a phenotypic trait, the gene where the variants reside in or near was often considered to be the cause of the observed genotype-phenotype relationship. The method used to identify these signals is straight forward (e.g., case control association test) and the interpretation of the results is often straight forward as well. This type of findings usually has larger effect size and therefore is clinically relevant and can be adapted into clinical practice easily (see the statin example). Note that these early pharmacogenomics GWAS successes where genetic variants concentrated in a few genes/genomic loci and show very significant association, tend to relate to more rare clinical phenotypes, for example flucloxacillin-induced liver injury happens in less than 1 out of 5000 individuals²⁹ and statin-induced myopathy at regular statin dosage occurs at a rate of 0.01%⁴⁷.

Despite the success of these GWASs, the issue of missing heritability dampened the enthusiasm of using GWAS to improve our understanding of genetic links to human complex traits⁴⁸. While considerable efforts are ongoing looking for rare but functional important genetic variants; more people started to evaluate those moderate genetic associations that could act together to affect phenotypic traits. For example, the development of various polygenic models that combine/integrate individual genetic signals together to

predict phenotypic traits. The methods used to integrate moderate genetic signals include but not limit to location based approach (e.g., SNP located in transcription factor or microRNA binding sites) and functional based approach e.g., eQTL studies^{5, 19, 49}. Instead of focusing on only association p value, which could be moderate in these cases, these studies applied system biology tools which integrate various functional relevant endophenotypes (e.g., gene expression in the form of mRNA or protein, microRNA expression and DNA methylation) to depict potential biological mechanism behind the observed genotype-phenotype association. These new integrative approaches facilitate the filtering of true genetic signals from potential “noise” from a GWAS and expand our understanding of genotypic-phenotypic relationship from simple association to a biological functional network.

Implementation of GWAS pharmacogenomic findings

From the implementation point of view, the field of medicine has always strived to improve the therapeutic outcomes by providing the right drug to the right person based on the knowledge available. Prior to the genomic era, the medication choice is often based on individual physiological (e.g., allergic history) and pathological characteristics (e.g., specific disease); while dose adjustment is made based on patients' age, organ function, and sometimes body weight, body surface area, or gender. These long validated parameters are and will continue to be used in Medicine to guide drug therapy. Some of these clinical parameters could be driven by genetic factors and therefore served as an endophenotype in guiding medication choices. In the post genomic era, multi-disciplinary teams that consist of laboratory, computational and clinical research communities are working together to further improve drug therapy by incorporating individual's genetic information (both germline and somatic) into the existing prescribing models⁵⁰.

Ideally, for a genetic variant to be considered important and get incorporated into clinical therapeutic decision, it needs to 1) robustly predict clinical treatment outcomes; 2) clinical consequence is relevant and significant; 3) added value to existing prescribing method; 4) cost-effective; and 5) actionable with existing alternative dosage or therapy. Furthermore, there are many practice hurdles, for example, the knowledge dissemination (e.g., prescriber education), the reimbursement, and the technical consistency⁵¹. In the next section, we will provide examples of a few pharmacogenomic studies that employed the whole-genome approach and their current status of clinical implementation.

Examples

Statins

Statins (namely simvastatin, lovastatin, pravastatin, fluvastatin, atorvastatin, rosuvastatin and pitavastatin) are among the top 10 prescribed drugs (atorvastatin, simvastatin and rosuvastatin) in the US (<http://www.pharmacytimes.com/publications/issue/2012/July2012/Top-200-Drugs-of-2011>). They are commonly used to lower low-density lipoprotein cholesterol by inhibiting cholesterol synthesis pathway. Statins are relatively safe with the most common side effect being skeletal muscle toxicity. Depend on the severity, this muscle toxicity can be classified as incipient myopathy, myopathy, or rhabdomyolysis, which is life threatening but rare with a frequency of 1 in 100,000⁵². Statin induced myopathy is dosage dependent⁴⁷. Therefore, in 2011 the FDA announced an update to the simvastatin product label recommending against initiation of the 80-mg dose and cautioning against continuation of an 80-mg dose unless the patient has already tolerated it without muscle problems for more than 1 year (<http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm258338.htm>).

The first GWAS for statin-induced myopathy was conducted using approximately 300,000 markers in 85 subjects with definite or incipient myopathy and 90 controls, all of whom were taking 80 mg of simvastatin daily as a part of a trial involving 12,000 participants. Genetic variants within *SLCO1B1* [solute carrier organic anion transporter family, member 1B1], were found to be significantly associated with the risk of developing myopathy⁴⁷. Resequencing indicated rs4149056 is the putative causative allele with OR of 4.5 per minor C allele⁴⁷. This finding has since been replicated in several independent studies including the Heart Protection study⁵³, the STRENGTH (Statin Response Examined by Genetic Haplotype Markers) study⁵⁴ and a retrospective case-control study⁵⁵. Note that although the association between rs4149056 and simvastatin induced adverse event is strong in STRENGTH trial, there is rather modest relationship between this SNP and atorvastatin and no significant association observed for pravastatin⁵⁴. This lack of association between rs4149056 and pravastatin was also confirmed elsewhere⁵⁵.

Based on these strong scientific evidence, the Clinical Pharmacogenomics Implementation Consortium (CPIC) recently published a Guideline for *SLCO1B1* and Simvastatin-Induced Myopathy⁵². This guideline focused on reducing muscle toxicity and optimize patient adherence with simvastatin treatment. To date, the pharmacogenetic testing of *SLCO1B1* variants is not mandated by either the US FDA or European Medicine Agency (EMA)⁵⁶.

Warfarin

Warfarin is the most commonly prescribed oral anticoagulant worldwide⁵⁷. It is used for the treatment and prevention of thrombotic disorders by inhibiting the vitamin K-dependent clotting pathway. Despite its efficacy, warfarin has a narrow therapeutic index and individual response to warfarin is highly variable. Too much of the drug will result in hemorrhage, (e.g., cranial hemorrhage which could be deadly); while too little of the drug will result in undesired coagulation, (e.g., deep vein thrombosis (DVT)). Not surprisingly, adverse events related to inappropriate warfarin dosing is one of the most common reasons for emergency room visits⁵⁸. Therefore, considerable efforts are invested to dose warfarin correctly based on patients' age, size, other medications, diet, and more recently genetics. For a GWAS with warfarin, many phenotypic traits can be studied (e.g., incidence of cranial hemorrhage, or INR). Each has its pros and cons. For cranial hemorrhage, although it is extremely important, due to the low incidence of such event, it is very difficult to recruit sufficient number of patients for a GWAS. To date, GWASs conducted for warfarin have been mainly focusing on identification of genetic variants that could be used to predict stabilized warfarin dose, which has a direct translational importance.

Prior to GWAS era, candidate gene studies have been focusing on *CYP2C9*, an enzyme predominantly responsible for metabolizing S-warfarin (the more potent enantiomer as compared to R-warfarin) and *VKORC1*, the target of warfarin. *CYP2C9* variant alleles were found to associate with low warfarin dose requirement⁵⁹, with as high as 6.21 odds ratio⁶⁰. *VKORC1* haplotypes was also found to link to warfarin dose⁶¹.

Initial GWAS was performed in 181 White warfarin treated patients, followed by independent replication in 374 patients for stabilized warfarin dose⁶². Approximately 550,000 polymorphisms were evaluated and the most significant independent effect was found for *VKORC1* polymorphism ($p = 6.2 \times 10^{-13}$) with moderate association observed for *CYP2C9* variants (rs1057910 and rs4917639, p approximately 10^{-4})⁶². Subsequent GWAS was performed on a larger scale with a sample size of 1,053⁶³. Univariate regression analysis found the strongest statistical signals ($p < 10^{-78}$) at SNPs clustering near *VKORC1* and the second lowest p-values ($p < 10^{-31}$) emanating from *CYP2C9*. Multiple regression adjusting for known influences on warfarin dose (*VKORC1*, *CYP2C9*, age, gender) identified a single SNP (rs2108622) with genome-wide significance ($p = 8.3 \times 10^{-10}$) that

alters protein coding of the *CYP4F2* gene⁶³. The role of variants in *CYP4F2* gene was also confirmed by a separate GWAS conducted in Japanese⁶⁴.

Based on these scientific findings, the US FDA modified the warfarin label, stating that *CYP2C9* and *VKORC1* genotypes may be useful in determining the optimal initial dose of warfarin in 2007⁶⁵. The label was further updated in 2010 to include a table describing recommendations for dosing ranges for patients with different combinations of *CYP2C9* and *VKORC1* genotype. To date, several dosing algorithms have been developed that incorporate genetics information along with nongenetic clinical algorithms^{66, 67}.

Several small scale randomized prospective studies have been conducted to examine the role of pharmacogenetics in guiding initiation of warfarin^{68, 69}. Although limited in sample size and study design, they tend to support the role of pharmacogenetic testing in warfarin dosing. Furthermore, there are several large scale ongoing randomized controlled trials to evaluate the role of genetic in warfarin dosing, for example, the Clarification of Optimal Anticoagulation through Genetics (COAG), the European Pharmacogenetics of AntiCoagulant Therapy (EU-PACT), the Genetic InFormatics Trial (GIFT), Pharmacogenetic dosing of warfarin: a controlled randomized trial by the Taiwan Warfarin Consortium, and Warfarin Adverse Events Reduction for Adults Receiving Genetic Testing at Therapy Initiation (WARFARIN)⁵⁷.

Recently, the CPIC developed a peer-reviewed gene-drug guideline to assist in the interpretation and usage of *CYP2C9* and *VKORC1* genotype data for estimating therapeutic warfarin dose to achieve an INR of 2-3, should genotype results be available to the clinicians⁵⁷. The guideline states “the greatest benefit of genetics in warfarin dosing is likely to be in patients requiring less than 21 mg/week or more than 49 mg/week (constituting >40% of all patients). Genetics-based algorithms also better predict warfarin dose than the FDA-approved warfarin label table. The best way to estimate the expected stable dose of warfarin is to use the algorithms available on <http://www.warfarindosing.org> and the dosing algorithm published by the International Warfarin Pharmacogenetics Consortium at <http://www.pharmgkb.org/do/serve?objId=PA162372936&objCls=Dataset#tabview=tab2>.

Conclusion

In summary, technical advancement in high throughput genotyping has allowed the application of GWAS in pharmacogenomic discovery. Compared to the traditional candidate gene approach in pharmacogenetics, GWAS can provide an unbiased, comprehensive scan of the human genome for potential genomic loci responsible for therapeutic outcome phenotypes. Given that pharmacological phenotypes are likely to be complex and influenced by a variety of genetic and non-genetic factors, future genome-wide approaches integrating molecular data (e.g., epigenetic markers) and genotypes could enhance our understanding of the complex networks, including gene-gene and gene-environment interactions, underlying pharmacological phenotypes. Furthermore, the next-generation sequencing technologies may advance the next wave of pharmacogenomic discovery to the unprecedented depth beyond the current GWAS efforts.

Acknowledgments

WZ is supported by a grant (R21 HG006367) from the National Institutes of Health/National Human Genome Research Institute.

RSH received support from National Institute of General Medical Science K08 [GM089941], the National Cancer Institute R21 [CA139278], National Institutes of Health/National Institute of General Medical Science

[Pharmacogenomics of Anticancer Agents grant U01GM61393], University of Chicago Cancer Center Support Grant P30 [CA14599], the National Center for Advancing Translational Sciences of the National Institutes of Health [UL1RR024999] and the University of Chicago Breast Cancer SPORE grant P50 [CA125183].

References

1. Lazarou J, Pomeranz BH, Corey PN. Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies. *Jama*. 1998; 279:1200–1205. [PubMed: 9555760]
2. Kamali F, Wynne H. Pharmacogenetics of warfarin. *Annu Rev Med*. 2010; 61:63–75. [PubMed: 19686083]
3. Welsh M, Mangravite L, Medina MW, Tantisira K, Zhang W, Huang RS, McLeod H, Dolan ME. Pharmacogenomic discovery using cell-based models. *Pharmacol Rev*. 2009; 61:413–429. [PubMed: 20038569]
4. Zhang W, Dolan ME. Use of cell lines in the investigation of pharmacogenetic loci. *Curr Pharm Des*. 2009; 15:3782–3795. [PubMed: 19925429]
5. Gamazon E, Huang RS, Cox NJ, Dolan ME. Chemotherapeutic Drug Susceptibility Associated SNPs are Enriched in Expression Quantitative Trait Loci. *Proc Natl Acad Sci USA*. 2010; 107:9287–9292. [PubMed: 20442332]
6. Collins F, McKusick V. Implications of the Human Genome Project for medical science. *JAMA*. 2001; 285:540–544. [PubMed: 11176855]
7. The International HapMap Project. *Nature*. 2003; 426:789–796. [PubMed: 14685227]
8. A haplotype map of the human genome. *Nature*. 2005; 437:1299–1320. [PubMed: 16255080]
9. A map of human genome variation from population-scale sequencing. *Nature*. 2010; 467:1061–1073. [PubMed: 20981092]
10. Spielman RS, Bastone LA, Burdick JT, Morley M, Ewens WJ, Cheung VG. Common genetic variants account for differences in gene expression among ethnic groups. *Nat Genet*. 2007; 39:226–231. [PubMed: 17206142]
11. Zhang W, Duan S, Kistner EO, Bleibel WK, Huang RS, Clark TA, Chen TX, Schweitzer AC, Blume JE, Cox NJ, et al. Evaluation of genetic variation contributing to differences in gene expression between populations. *Am J Hum Genet*. 2008; 82:631–640. [PubMed: 18313023]
12. Duan S, Huang RS, Zhang W, Bleibel WK, Roe CA, Clark TA, Chen TX, Schweitzer AC, Blume JE, Cox NJ, et al. Genetic architecture of transcript-level variation in humans. *Am J Hum Genet*. 2008; 82:1101–1113. [PubMed: 18439551]
13. Storey JD, Madeoy J, Strout JL, Wurfel M, Ronald J, Akey JM. Gene-expression variation within and among human populations. *Am J Hum Genet*. 2007; 80:502–509. [PubMed: 17273971]
14. Stranger BE, Nica AC, Forrest MS, Dimas A, Bird CP, Beazley C, Ingle CE, Dunning M, Flicek P, Koller D, et al. Population genomics of human gene expression. *Nat Genet*. 2007; 39:1217–1224. [PubMed: 17873874]
15. Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, Gibbs RA, Belmont JW, Boudreau A, Hardenbol P, Leal SM, et al. A second generation human haplotype map of over 3.1 million SNPs. *Nature*. 2007; 449:851–861. [PubMed: 17943122]
16. Cheung VG, Conlin LK, Weber TM, Arcaro M, Jen KY, Morley M, Spielman RS. Natural variation in human gene expression assessed in lymphoblastoid cells. *Nat Genet*. 2003; 33:422–425. [PubMed: 12567189]
17. Morley M, Molony CM, Weber TM, Devlin JL, Ewens KG, Spielman RS, Cheung VG. Genetic analysis of genome-wide variation in human gene expression. *Nature*. 2004; 430:743–747. [PubMed: 15269782]
18. Dolan ME, Newbold KG, Nagasubramanian R, Wu X, Ratain MJ, Cook EH Jr, Badner JA. Heritability and linkage analysis of sensitivity to cisplatin-induced cytotoxicity. *Cancer Res*. 2004; 64:4353–4356. [PubMed: 15205351]
19. Gamazon Eric R, Ziliak D, Im Hae K, LaCroix B, Park Danny S, Cox Nancy J, Huang RS. Genetic Architecture of MicroRNA Expression: Implications for the Transcriptome and Complex Traits. *The American Journal of Human Genetics*. 2012; 90:1046–1063.

20. Fraser HB, Lam LL, Neumann SM, Kobor MS. Population-specificity of human DNA methylation. *Genome Biol.* 2012; 13:R8. [PubMed: 22322129]
21. Bell JT, Pai AA, Pickrell JK, Gaffney DJ, Pique-Regi R, Degner JF, Gilad Y, Pritchard JK. DNA methylation patterns associate with genetic and gene expression variation in HapMap cell lines. *Genome Biol.* 2011; 12:R10. [PubMed: 21251332]
22. Moen LE, Mu W, Delaney S, Wing C, McQuade J, Godley LA, Dolan ME, Zhang W. Differences in DNA methylation between the African and European HapMap populations. *Proc Am Assoc Cancer Res.* 2012; 5010
23. Zhou S. Clinical pharmacogenomics of thiopurine S-methyltransferase. *Curr Clin Pharmacol.* 2006; 1:119–128. [PubMed: 18666383]
24. Jones TS, Yang W, Evans WE, Relling MV. Using HapMap Tools in Pharmacogenomic Discovery: The Thiopurine Methyltransferase Polymorphism. *Clin Pharmacol Ther.* 2007; 81:729–734. [PubMed: 17329987]
25. Manolopoulos VG, Ragia G, Tavridou A. Pharmacogenetics of coumarinic oral anticoagulants. *Pharmacogenomics.* 2010; 11:493–496. [PubMed: 20350128]
26. Huang R, Johnatty S, Gamazon E, Im H, Ziliak D, Duan S, Zhang W, Kistner E, Chen P, Beesley J, et al. Platinum sensitivity-related germline polymorphism discovered via a cell-based approach and analysis of its association with outcome in ovarian cancer patients. *Clin Cancer Res.* 2011; 17:5490–5500. [PubMed: 21705454]
27. Mardis ER. Next-generation DNA sequencing methods. *Annu Rev Genomics Hum Genet.* 2008; 9:387–402. [PubMed: 18576944]
28. Liu C, Batliwalla F, Li W, Lee A, Roubenoff R, Beckman E, Khalili H, Damle A, Kern M, Furie R, et al. Genome-wide association scan identifies candidate polymorphisms associated with differential response to anti-TNF treatment in rheumatoid arthritis. *Mol Med.* 2008; 14:575–581. [PubMed: 18615156]
29. Daly AK, Donaldson PT, Bhatnagar P, Shen Y, Pe'er I, Floratos A, Daly MJ, Goldstein DB, John S, Nelson MR, et al. HLA-B*57:01 genotype is a major determinant of drug-induced liver injury due to flucloxacillin. *Nat Genet.* 2009; 41:816–819. [PubMed: 19483685]
30. Volpi S, Heaton C, Mack K, Hamilton JB, Lannan R, Wolfgang CD, Licamele L, Polymeropoulos MH, Lavedan C. Whole genome association study identifies polymorphisms associated with QT prolongation during iloperidone treatment of schizophrenia. *Mol Psychiatry.* 2008; 14:1024–1031. [PubMed: 18521091]
31. Lavedan C, Licamele L, Volpi S, Hamilton J, Heaton C, Mack K, Lannan R, Thompson A, Wolfgang CD, Polymeropoulos MH. Association of the NPAS3 gene and five other loci with response to the antipsychotic iloperidone identified in a whole genome association study. *Mol Psychiatry.* 2008; 14:804–819. [PubMed: 18521090]
32. Adkins DE, Aberg K, McClay JL, Bukszar J, Zhao Z, Jia P, Stroup TS, Perkins D, McEvoy JP, Lieberman JA, et al. Genomewide pharmacogenomic study of metabolic side effects to antipsychotic drugs. *Mol Psychiatry.* 2011; 16:321–332. [PubMed: 20195266]
33. Alkelai A, Greenbaum L, Rigbi A, Kanyas K, Lerer B. Genome-wide association study of antipsychotic-induced parkinsonism severity among schizophrenia patients. *Psychopharmacology.* 2009; 206:491–499. [PubMed: 19680635]
34. McClay JL, Adkins DE, Aberg K, Stroup S, Perkins DO, Vladimirov VI, Lieberman JA, Sullivan PF, van den Oord EJCG. Genome-wide pharmacogenomic analysis of response to treatment with antipsychotics. *Mol Psychiatry.* 2011; 16:76–85. [PubMed: 19721433]
35. Uher R, Perroud N, Ng M, Hauser J, Henigsberg N, Maier W, Mors O, Placentino A, Rietschel M, Souery D, et al. Genome-wide pharmacogenetics of antidepressant response in the GENDEP project. *Am J Psychiatry.* 2010; 167:555–564. [PubMed: 20360315]
36. Garriock H, Kraft J, Shyn S, Peters E, Yokoyama J, Jenkins G, Reinalda M, Slager S, McGrath P, Hamilton S. A genomewide association study of citalopram response in major depressive disorder. *Biol Psychiatry.* 2010; 67:133–138. [PubMed: 19846067]
37. Turner ST, Bailey KR, Fridley BL, Chapman AB, Schwartz GL, Chai HS, Sicotte H, Kocher J-P, Rodin AS, Boerwinkle E. Genomic Association Analysis Suggests Chromosome 12 Locus

- Influencing Antihypertensive Response to Thiazide Diuretic. *Hypertension*. 2008; 52:359–365. [PubMed: 18591461]
38. Turner ST, Bailey KR, Schwartz GL, Chapman AB, Chai HS, Boerwinkle E. Genomic Association Analysis Identifies Multiple Loci Influencing Antihypertensive Response to an Angiotensin II Receptor Blocker. *Hypertension*. 2012; 59:1204–1211. [PubMed: 22566498]
 39. Common variants near ATM are associated with glycemic response to metformin in type 2 diabetes. *Nat Genet*. 2011; 43:117–120. [PubMed: 21186350]
 40. Barber M, Mangravite L, Hyde C, Chasman D, Smith J, McCarty C, Li X, Wilke R, Rieder M, Williams P, et al. Genome-wide association of lipid-lowering response to statins in combined study populations. *PLoS One*. 2010; 5:e9763. [PubMed: 20339536]
 41. Uhl G, Drgon T, Johnson C, Walther D, David S, Aveyard P, Murphy M, Johnstone E, Munafò M. Genome-wide association for smoking cessation success: participants in the Patch in Practice trial of nicotine replacement. *Pharmacogenomics*. 2010; 11:357–367. [PubMed: 20235792]
 42. Nicoletti P, Carstos VM, Palaska PK, Shen Y, Floratos A, Zavras AI. Genomewide Pharmacogenetics of Bisphosphonate-Induced Osteonecrosis of the Jaw: The Role of RBMS3. *The Oncologist*. 2012; 17:279–287. [PubMed: 22267851]
 43. Ramsey LB, Bruun GH, Yang W, Treviño LR, Vattathil S, Scheet P, Cheng C, Rosner GL, Giacomini KM, Fan Y, et al. Rare versus common variants in pharmacogenetics: SLCO1B1 variation and methotrexate disposition. *Genome Research*. 2012; 22:1–8. [PubMed: 22147369]
 44. Sato Y, Yamamoto N, Kunitoh H, Ohe Y, Minami H, Laird N, Katori N, Saito Y, Ohnami S, Sakamoto H, et al. Genome-wide association study on overall survival of advanced non-small cell lung cancer patients treated with carboplatin and paclitaxel. *J Thorac Oncol*. 2011; 6:132–138. [PubMed: 21079520]
 45. Tantisira K, Lasky-Su J, Harada M, Murphy A, Litonjua A, Himes B, Lange C, Lazarus R, Sylvia J, Klanderman B, et al. Genomewide association between GLCCI1 and response to glucocorticoid therapy in asthma. *N Engl J Med*. 2011; 365:1173–1183. [PubMed: 21991891]
 46. Himes B, Jiang X, Hu R, Wu A, Lasky-Su J, Klanderman B, Ziniti J, Senter-Sylvia J, Lima J, Irvin C, et al. Genome-Wide Association Analysis in Asthma Subjects Identifies SPATS2L as a Novel Bronchodilator Response Gene. *PLoS Genet*. 2012; 8:e1002824. [PubMed: 22792082]
 47. SEARCH Collaborative Group. Link E, Parish S, Armitage J, Bowman L, Heath S, Matsuda F, Gut I, Lathrop M, Collins R. SLCO1B1 variants and statin-induced myopathy—a genomewide study. *N Engl J Med*. 2008; 359:789–799. [PubMed: 18650507]
 48. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, McCarthy MI, Ramos EM, Cardon LR, Chakravarti A, et al. Finding the missing heritability of complex diseases. *Nature*. 2009; 461:747–753. [PubMed: 19812666]
 49. Nicolae D, Gamazon E, Zhang W, Duan S, Dolan M, Cox N. Trait-associated SNPs are more likely to be eQTLs: annotation to enhance discovery from GWAS. *PLoS Genet*. 2010; 6:e1000888. [PubMed: 20369019]
 50. Manolio T, Green E. Genomics reaches the clinic: from basic discoveries to clinical impact. *Cell*. 2011; 147:14–16. [PubMed: 21962499]
 51. Huang R, Ratain M. Pharmacogenetics and pharmacogenomics of anticancer agents. *CA Cancer J Clin*. 2009; 59:42–55. [PubMed: 19147868]
 52. Wilke RA, Ramsey LB, Johnson SG, Maxwell WD, McLeod HL, Voora D, Krauss RM, Roden DM, Feng Q, Cooper-DeHoff RM, et al. The Clinical Pharmacogenomics Implementation Consortium: CPIC Guideline for SLCO1B1 and Simvastatin-Induced Myopathy. *Clin Pharmacol Ther*. 2012; 92:112–117. [PubMed: 22617227]
 53. Heart Protection Study Collaborative G. Effects on 11-year mortality and morbidity of lowering LDL cholesterol with simvastatin for about 5 years in 20 536 high-risk individuals: a randomised controlled trial. *The Lancet*. 2011; 378:2013–2020.
 54. Voora D, Shah SH, Spasojevic I, Ali S, Reed CR, Salisbury BA, Ginsburg GS. The SLCO1B1*5 Genetic Variant Is Associated With Statin-Induced Side Effects. *Journal of the American College of Cardiology*. 2009; 54:1609–1616. [PubMed: 19833260]
 55. Brunham LR, Lansberg PJ, Zhang L, Miao F, Carter C, Hovingh GK, Visscher H, Jukema JW, Stalenhoef AF, Ross CJD, et al. Differential effect of the rs4149056 variant in SLCO1B1 on

- myopathy associated with simvastatin and atorvastatin. *Pharmacogenomics J.* 2012; 12:233–237. [PubMed: 21243006]
56. Becquemont L, Alfirevic A, Amstutz U, Brauch H, Jacqz-Aigrain E, Laurent-Puig P, Molina M, Niemi M, Schwab M, Somogyi A, et al. Practical recommendations for pharmacogenomics-based prescription: 2010 ESF-UB Conference on Pharmacogenetics and Pharmacogenomics. *Pharmacogenomics.* 2011; 12:113–124. [PubMed: 21174626]
 57. Johnson JA, Gong L, Whirl-Carrillo M, Gage BF, Scott SA, Stein CM, Anderson JL, Kimmel SE, Lee MTM, Pirmohamed M, et al. Clinical Pharmacogenetics Implementation Consortium Guidelines for CYP2C9 and VKORC1 Genotypes and Warfarin Dosing. *Clin Pharmacol Ther.* 2011; 90:625–629. [PubMed: 21900891]
 58. Shehab N, Sperling L, Kegler S, Budnitz D. National estimates of emergency department visits for hemorrhage-related adverse events from clopidogrel plus aspirin and from warfarin. *Arch Intern Med.* 2010; 170:1926–1933. [PubMed: 21098354]
 59. Higashi M, Veenstra D, Kondo L, Wittkowsky A, Srinouanprachanh S, Farin F, Rettie A. Association between CYP2C9 genetic variants and anticoagulation-related outcomes during warfarin therapy. *JAMA.* 2002; 287:1690–1698. [PubMed: 11926893]
 60. Aithal GP, Day CP, Kesteven P, Daly AK. Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. *The Lancet.* 1999; 353:717–719.
 61. Rieder M, Reiner A, Gage B, Nickerson D, Eby C, McLeod H, Blough D, Thummel K, Veenstra D, Rettie A. Effect of VKORC1 haplotypes on transcriptional regulation and warfarin dose. *N Engl J Med.* 2005; 352:2285–2293. [PubMed: 15930419]
 62. Cooper GM, Johnson JA, Langae TY, Feng H, Stanaway IB, Schwarz UI, Ritchie MD, Stein CM, Roden DM, Smith JD, et al. A genome-wide scan for common genetic variants with a large influence on warfarin maintenance dose. *Blood.* 2008; 112:1022–1027. [PubMed: 18535201]
 63. Takeuchi F, McGinnis R, Bourgeois S, Barnes C, Eriksson N, Soranzo N, Whittaker P, Ranganath V, Kumanduri V, McLaren W, et al. A genome-wide association study confirms VKORC1, CYP2C9, and CYP4F2 as principal genetic determinants of warfarin dose. *PLoS Genet.* 2009; 5:e1000433. [PubMed: 19300499]
 64. Cha P-C, Mushiroda T, Takahashi A, Kubo M, Minami S, Kamatani N, Nakamura Y. Genome-wide association study identifies genetic determinants of warfarin responsiveness for Japanese. *Human Molecular Genetics.* 2010; 19:4735–4744. [PubMed: 20833655]
 65. Gage B, Lesko L. Pharmacogenetics of warfarin: regulatory, scientific, and clinical issues. *J Thromb Thrombolysis.* 2008; 25:45–51. [PubMed: 17906972]
 66. Gage BF, Eby C, Johnson JA, Deych E, Rieder MJ, Ridker PM, Milligan PE, Grice G, Lenzini P, Rettie AE, et al. Use of Pharmacogenetic and Clinical Factors to Predict the Therapeutic Dose of Warfarin. *Clin Pharmacol Ther.* 2008; 84:326–331. [PubMed: 18305455]
 67. International Warfarin Pharmacogenetics Consortium. Klein T, Altman R, Eriksson N, Gage B, Kimmel S, Lee M, Limdi N, Page D, Roden D, et al. Estimation of the warfarin dose with clinical and pharmacogenetic data. *N Engl J Med.* 2009; 360:753–764. [PubMed: 19228618]
 68. Anderson JL, Horne BD, Stevens SM, Grove AS, Barton S, Nicholas ZP, Kahn SFS, May HT, Samuelson KM, Muhlestein JB, et al. Randomized Trial of Genotype-Guided Versus Standard Warfarin Dosing in Patients Initiating Oral Anticoagulation. *Circulation.* 2007; 116:2563–2570. [PubMed: 17989110]
 69. Epstein RS, Moyer TP, Aubert RE, Okane DJ, Xia F, Verbrugge RR, Gage BF, Teagarden JR. Warfarin Genotyping Reduces Hospitalization Rates: Results From the MM-WES (Medco-Mayo Warfarin Effectiveness Study). *Journal of the American College of Cardiology.* 2010; 55:2804–2812. [PubMed: 20381283]