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GENOME-WIDE ASSOCIATION STUDIES IN PHARMACOGENOMICS

Aniya, Z. P.

Addis Ababa University, College of Health Sciences, Department of Pharmacology, P.O.Box 9086, Addis Ababa, Ethiopia

*zelalemp@yahoo.com

Abstract

The aim of this study is to review the applications of genome-wide association studies (GWAS) in pharmacogenomics. GWAS have matured into a powerful tool to identify single nucleotide polymorphisms (SNPs) that are associated with various phenotypes. GWAS in pharmacogenomics are increasingly being performed to identify variants that affect therapeutic response and susceptibility to adverse drug reactions (ADRs). Such studies not only confirm previous findings but also identify novel variants. GWAS-identified and replication-confirmed variants for therapeutic response could be exemplified with SNPs in VKORC1 gene for coumarin anticoagulants, CYP2C19 gene for clopidogrel, and IL28B gene for interferon-alpha. For serious ADRs, significantly associated SNPs have been reported in human leukocyte antigen (HLA)-A*31:01 for carbamazepine-induced skin rash, SLCO1B1 gene for simvastatin-induced myopathy, and HLA-B*57:01 for flucloxacillin and HLA-DRB1*15:01 for lumiracoxib-induced liver injuries among others. Subsequent GWAS using larger sample sizes, and genotyping platforms with better marker SNP density could enhance the discovery of genetic variants on pharmacogenomic traits to advance clinical care.

Key words: GWAS, pharmacogenomics, SNP

Introduction

Genetics is the study of how traits are being transmitted over generations and how variations in the information for these traits will influence the outcome. All the information regarding heritable traits is stored by and carried from generation to generation in the chromosomes in the form of genes. The human genome is composed of about twenty thousand genes, and it is variable among individuals [1]. The most common type of variation is single-nucleotide polymorphism (SNP), which is a single nucleotide variation at a specific location in the genome found in more than 1% of the population [2]. SNPs are biallelic; and more than ten million SNPs have been reported in the human genome [3]. Non-synonymous SNPs in gene-encoding regions have a higher probability of affecting the structure and function of proteins, leading to development of disease or altered response to drug therapy [2]. Different individuals with the same diagnosis could respond differently to the same drug administered at the same dose, with diminished or excessive response [4]. The person-to-person variability of a drug response is a problem in clinical practice, and can lead to therapeutic failure or adverse effects of drugs [5]. Potential risk factors for drug inefficacy or toxicity include patient's age and gender, nutritional status, co-morbidities, drug interactions, chronic diseases, and lifestyle variables such as alcohol consumption [6]. For some drugs genetic factors that affect the kinetics and dynamics of drugs are equally important in the determination individual variability in the efficacy and toxicity of drugs [4]. Generally, genetic factors are estimated to account for 15–30% of inter-individual differences in drug response, but for certain drugs, this can even be higher [7]. Genetic variations in the form of SNPs are partly responsible for the clinical variation seen in response to pharmacotherapy [6]. This could affect the likelihood of achieving therapeutic success, changing the maintenances dosage of treatment, or experiencing adverse drug reactions (ADRs). The term pharmacogenomics is often described as the study of genetic factors affecting drug response, investigating the effect of several genes on a particular phenotype [6]. It focuses on understanding how genetic variants that encode for drug-metabolizing enzymes, drug transporters, drug targets, and proteins involved in disease biology influence individual differences of treatment efficacy and adverse effects. Pharmacogenomics offers the promise to reduce ADRs and enhance drug efficacy by facilitating selection of patients able to respond to specific agents. The development of pharmacogenomics will ultimately enable clinicians to identify patients who are likely to benefit from a drug with minimal adverse effects [8].

Recently, pharmacogenomic studies have advanced the discovery of genes associated with individual differences in drug response [9]. Based on these discoveries, the U.S. Food and Drug Administration (FDA) has relabeled more than hundred of drugs to include genetic information so that routine genetic testing, on clinically acceptable specific and sensitive markers for susceptible populations to be done prior to prescribing the drugs [9]. Approaches to map genes that underlie common diseases and complex traits mainly falls into two categories: candidate-gene and genome-wide association studies [10]. Until recently, genetic risk factors for diseases were primarily studied in candidate gene association studies (CGAS) [11]. In such studies, DNA samples from cases and controls will be genotyped for SNPs in a specific gene for which prior knowledge suggested a role in the pathogenesis of the disease of interest or has functional relevance [12].

The CGAS have provided valuable data in the area of pharmacogenomics [13]. It has been possible to study phenotype-genotype relationship for genes encoding enzymes of drug metabolism like cytochrome P450 (CYP) oxidases, drug transporters and various drug targets [13, 14]. For example, polymorphisms in CYP2C9 for warfarin and CYP2C19 for clopedogrel drug responses [15]; azathioprine-induced bone marrow suppression associated with polymorphism in thiopurine S-methyltransferase (TPMT), irinotecan-induced neutropenia association with polymorphism in uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1), and abacavir-induced hypersensitivity reactions associated with polymorphism in human leukocyte antigen (HLA) region [9] among others.

Although CGAS have been able to identify genetic risk variants that are known to contribute for susceptibility to common diseases and pharmacogenetic traits, such studies have a number of limitations [11]. The major limitation is that genetic variants that are related to unknown mechanisms, which might have relevance to disease susceptibility, will not be detected. Another limitation is that many CGAS relied on the analysis of limited number of SNPs and did not consider regions that regulate gene expression. In addition to these, in some CGAS, consistent results were not obtained with the associations reported [14]. The reasons for this include small sample sizes, concentration on single variant in a gene rather than evaluation of the

genetic diversity of the whole gene, and incomplete knowledge of the drug's mechanism of action in terms of both efficacy and safety [16]. In this sense, genome-wide study approach could have a great potential to understand common diseases [10], and it is increasingly being applied in the areas of pharmacogenomics [13].

Genome wide association studies

Genome wide association studies (GWAS) are genetic studies in which dense array of genetic markers that capture substantial proportion of common variations in the genome sequence are typed in a set of DNA samples which are informative for a trait of interest [17]. The goal of GWAS is to identify DNA sequence variants that affect an individual's risk to a disease or response to drug treatment through detection of association between genotype frequencies and trait status.

GWAS investigate the possible association of genetic variations throughout the entire human genome [18]. This approach therefore represents comprehensive and unbiased scan of the genome even in the absence of evidence regarding the function or location of the causal genes [19]. In contrast to CGAS, GWAS allow the identification of novel susceptibility variants in previously unrecognized biological pathways that may provide better understanding of phenotypes [20, 21]. GWAS are suitable for simultaneous identification of several common-risk variants in a single study and thus relevant for complex traits where concerted action of many risk factors contributes to the disease [11]. The availability of comprehensive data on human genetic variation from Human Genome and International Haplotype Mapping (HapMap) projects [22] together with high-throughput genotyping technologies with very low error rates that allow large numbers of SNPs to be genotyped simultaneously [23], have made GWAS technically feasible.

The International HapMap Project has genotyped millions of SNPs on samples representing European, African, and Asian populations; and the data characterizes the patterns of linkage disequilibrium (LD) across the genome in these populations [22]. SNPs in the genome have groups of neighbors that are nearly in perfect correlation with each other [24]. Once the patterns of LD are known for a given region of the genome, a minimal set of correctly chosen variants (tag SNPs) can thereby serve as a proxy for many others and provide adequate information about most of the common variation within the genomic region [10].

The most frequently used GWAS design to date has been the case-control study design in which genotype frequencies in patients with the disease of interest are compared to those in a disease-free group [25]. The GWAS can be divided into four major steps: (i) careful selection of cases and controls from the same population; (ii) isolation of DNA, genotyping and quality control measures to enrich the dataset; (iii) appropriate statistical tests to identify differences in allele and genotype frequencies between cases and controls for the SNPs that passed the quality filter; and (iv) then, replication of the GWAS findings with an independent set of cases and controls. In GWAS systematic stepwise standard quality filtering processes will be performed on the samples as well as on the raw genotype data [25]. These include checking the reported sex of each individual against that predicted by the genetic data, missing call rate of the samples as well as the SNPs, sample relatedness, population stratification, minor allele frequency, and Hardy-Weinberg equilibrium [26, 27]. After the quality filtering, the test for associations should be undertaken using statistical software usually PLINK [28]. PLINK is a popular and computationally efficient soft-ware program that offers a comprehensive and well-documented set of automated GWAS quality filter and analysis tools. For each SNP, chi-square test using the different genetic inheritance models (dominant, recessive, multiplicative) should be carried out to compare allele and genotype frequencies between cases and controls. Logistic regression analysis adjusted for covariates, could also be implemented.

Since GWAS involve multiple hypothesis-testing, large test statistics are expected just by chance along with the genuine disease associated alleles [10]. Thus, appropriate statistical considerations to avoid false positive results are essential [29]. The conventional and still the most widely applied approach is correcting P-values by Bonferroni correction, whereby the threshold for genome-wide significance is computed by dividing the level of significance ($\alpha = 0.05$) by the number of marker SNPs tested [18]. A marker that is significant after Bonferroni correction is declared genome-wide significant. The currently used strategies for the identification of genetic risk factors involve multi-stage testing for associations whereby the initial GWAS findings will be confirmed in a replication cohort in order to reduce false positive results [17]. The aim of the replication study is to determine which of the findings arising from the GWAS reflect true reproducible associations [17].

Replication study is carried out for only a small fraction of the genetic markers that were contained in the initial GWAS using a different SNP genotyping method [2]. This will minimize the chance of false positive associations that arise as a result of technical genotyping artifacts [10]. Replication of association results in an independent population has become the 'gold standard' for validation of results [30]. Credibility increases when multiple investigative groups find the same association in independent samples [17].

Genetic risk variants identified through GWAS, and confirmed by replication studies can have impacts on clinical medicine through prediction of outcomes or through elucidation of underlying biology [17, 31]. Much of the immediate focus is for genetic testing that utilizes common variants as biomarkers to predict disease, to monitor disease progression and treatment response, or to avoid serious ADRs, and thus advance clinical care through personalized medicine [17]. On the other hand, identification of genetic loci and the relevant genes at those loci can help to describe new biological pathways and therapeutic targets, which can in turn provide clues for the development of novel preventive and treatment approaches [31].

It is now possible and indeed the norm to conduct GWAS to find associations between disease phenotypes and genetic variants that may predispose to the diseases [32], and many of such studies have been quite successful [33]. The successful applications in identifying novel susceptibility genes for complex diseases showed an interest in applying GWAS to identify genetic variants for pharmacogenomic traits [6].

An important advantage of GWAS in pharmacogenomics is that multiple response phenotypes are often collected within the same study, such as efficacy and adverse events, allowing a broader dissection of trait genetics in a single study [8]. In addition, larger genetic effect sizes may exist for pharmacogenomic traits providing greater statistical power for GWAS [21]. This could also allow pharmacogenomic associations to be identified with a relatively moderate sample sizes [8]. Another advantage to pharmacogenomics is the ability to rule out contributions by unidentified genes to a drug response phenotype [21]. As pharmacogenomic GWAS can directly investigate the role of genetic variation on clinical outcomes, the findings can be more rapidly translated to clinical practice. The objective of this literature review is to provide an overview on GWAS and their applications in identifying biomarker SNPs for pharmacogenomic traits.

Materials and methods

Search strategy and selection criteria: Published reports were systematically searched in electronic databases of PUBMED. The search terms used include "GWAS in pharmacogenomics", "GWAS for drug response" and "GWAS for serious ADRs" to identify published articles that included data for GWAS in pharmacogenomics. Methodological restrictions for the inclusion criteria include studies published as original articles that focused on drug response and ADRs, studies investigating both monotherapy and combination treatments, and studies with subsequent replications. There were no restrictions on doses and routes of drug administration. The search yielded 145 articles; of these, 39 were included in the GWAS pharmacogenomics in this review.

Results and discussion

GWAS on therapeutic responses

Until now, GWAS on drug response have been mainly concerned with drugs for which the dose needs to be individualized or for which failure to respond presents an important clinical problem [13]. The prominent GWAS findings on drug response confirmed with replication studies include those for therapeutic response to coumarin anticoagulants [34, 35], interferon-alpha [36-38] and clopidogrel [39].

Response to coumarin anticoagulants

Warfarin is the most commonly prescribed anticoagulant for the treatment and prevention of thrombotic diseases including myocardial infarction, ischemic stroke and venous thrombosis. However, it poses significant challenges because of narrow therapeutic index and large inter-individual variability in response [40]. Current knowledge concerning the pharmacogenomics of warfarin indicates that genetic factors can explain inter-individual variability of warfarin response and dose requirement [41]. Up to one-third of this variation is related to polymorphisms in two genes involved in warfarin pharmacogenetics: CYP2C9 that encodes the main warfarin metabolizing enzyme; and vitamin-K epoxide reductase complex 1 (VKORC1) which encodes drug target [13]. GWAS have been performed with the objective of identifying genetic variants that may explain inter-individual variability in dose requirement for coumarin anticoagulants. GWAS on warfarin involving 1,053 patients (Swedish subjects) showed SNPs in VKORC1 and CYP2C9 genes

with genome-wide significance [34]. The strongest signal was at rs9923231 (P = 5.4E-78) near VKORC1 with a second signal at rs1057910 (P = 4.5E-17) for CYP2C9*3 and rs1799853 (P = 8.8E-13) for CYP2C9*2. The study confirmed the earlier CGAS findings on these genes [13]. This study also found genome-wide significance for rs2108622 (P = 8.3E-10) in CYP4F2 gene after adjustment for all known genetic and non-genetic factors [34]. The CYP4F2 iso-enzyme metabolizes vitamin-K, and polymorphism in this gene has been associated with reduced vitamin-K metabolism and the need for a higher warfarin maintenance dosage [42]. The GWAS showed that SNPs in VKORC1 and CYP2C9 predict about 40% of dose variance [34]. The strong and widely replicated associations of warfarin dose with VKORC1 and CYP2C9 have provided one of the most successful applications of pharmacogenetics to date and offer promise for genetic predication of required dose in a clinical setting [6].

Similarly, a GWAS for another coumarin anticoagulant, acenocoumarol, was conducted using 1451 Caucasian subjects and the results were replicated in 287 subjects [35]. SNP rs10871454 with genome wide significance (P = 2.0E-123), which is in LD to SNPs within VKORC1 gene, was identified. Another SNP, rs4086116 (P = 3.3E-24) was also obtained within CYP2C9 gene. After adjustment for these SNPs, rs2108622 polymorphism within CYP4F2 gene reached genome-wide significance (P = 2.0E-08). Another SNP rs1998591 (P = 1.9E-09) that contributes to the variation in acenocoumarol dosage was also identified in CYP2C18 gene. The study showed that polymorphism within VKORC1, CYP2C9, CYP4F2 and CYP2C18 genes could explain about half of acenocoumarol dosage variation [35].

Response to interferon-alpha

The most effective current standard of care in patients with chronic hepatitis C virus (HCV) infection, which is a combination of pegylated interferon-α (PegIFN-α) with ribavirin (RBV), does not produce sustained virologic response in all patients treated [13]. GWAS for null virological response to PegIFN-α/RBV treatment on Japanese patients showed two SNPs near the gene interleukin 28B (IL28B) to have strong associations (rs12980275, P = 1.9E-13; and rs8099917, P = 3.1E-15) [36]. The results were replicated in an independent cohort, combined P = 2.8E-27, odds ratio (OR) = 17.7, and P = 2.68E-32, OR = 27.1, respectively. In another GWAS of sustained virological response to PegIFN-α/RBV therapy in 293 Australian individuals with chronic HCV, and validation study in an independent replication cohort consisting of 555 individuals showed an association within the gene region encoding IL28B (rs8099917, P = 9.3E-09, OR = 2.0) [37]. A third study on patients of European ancestry also showed genome-wide significant (P = 1.1E-25) polymorphism near the IL28B gene associated with an approximately two-fold change in response to PegIFN-α/RBV treatment [38]. The three independent studies had genome-wide significant associations with IL28B gene, and were validated further by the fact that the studies involved different ethnic groups. These findings provide interesting new insights into the disease process and may be valuable in determining treatment. These studies are other examples of the successful application of GWAS for drug response.

Response to clopidogrel

GWAS for a response to an anti-platelet drug clopidogrel, which is widely used in the treatment of cardiovascular disease (transient ischemic attack) and in the prevention of myocardial infarction, has been investigated [39]. The most significant SNP was rs12777823 (P = 1.5E-13) in strong LD with CYP2C19*2 variant that accounts for diminished clopidogrel response. The relation between CYP2C19*2 genotype and platelet aggregation was also replicated. The GWAS provided confirmation of earlier CGAS of clopidogrel response with CYP2C19 polymorphism [43].

GWAS on serious ADRs

GWAS that focus on idiosyncratic adverse effects of drugs may allow the identification of variants that could be used as markers for genetic testing [17]. Relatively a few GWAS in pharmacogenomics of ADRs have been conducted in comparison to the large number of disease risk GWAS [19] probably because many serious ADRs are rare, and therefore it is a difficult area to investigate. However, from those published, it is evident that the potential for gaining insight into the genetic aspects of serious ADRs, and hence for developing tools to minimize ADRs is very large. The prominent GWAS findings on serious ADRs confirmed with replication studies include those for carbamazepine-induced skin rash [44, 45], simvastatin-induced myopathy [46], and flucloxacillin, lumiracoxib and amoxicillin-clavulanate-induced hepatotoxicities [47-49].

Carbamazepine-induced skin rash

Carbamazepine (CBZ), a widely used anticonvulsant and mood-stabilizing drug for the treatment of

epilepsy and bipolar disorder, causes idiosyncratic skin rash in up to 10% of patients that may progress to blistering cutaneous ADRs (cADRs) such as Stevens–Johnson syndrome and toxic epidermal necrolysis [50, 51]. Recently, GWAS on CBZ-induced severe skin rash were conducted in different populations [44, 45]. The first study involved 53 Japanese cases of CBZ-induced cADRs [45]. The top SNP was rs1633021 (P = 1.2E-13) which is in a complete LD with HLA-A*31:01. The finding was confirmed in a replication cohort of 61 cases. In a second GWAS of 65 cases of European ethnic origin [44], strong signal in HLA region were identified, with several SNPs around HLA-A reaching genome-wide significance (P = 3.5E-08). HLA-A*31:01 association was confirmed with a replication study of 145 cases. The study showed that the presence of HLA-A*31:01 allele increased the risk of developing CBZ-induced cADRs from 5% to 26.0%. The relatively high specificity and sensitivity estimated in both studies suggest that genotyping for HLA-A*31:01 prior to prescribing CBZ would be valuable and cost effective in preventing CBZ-induced serious cADRs.

Simvastatin-induced myopathy

HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme-A) reductase inhibitors (statins) are the most widely prescribed therapeutic class of drugs with established clinical benefits both in terms of lowering serum lipid profiles and reducing cardiovascular events and mortality [52].

Although statins have a favorable risk-to-benefit ratio, they have the potential to cause serious ADRs which can result in muscular inflammation (myositis) and muscle breakdown (rhabdomyolysis) that can be potentially fatal in its extreme form [53]. Factors that affect the pharmacokinetics of statins such as a decreased hepatic uptake, which can lead to an elevated plasma levels, would potentially induce the muscle toxicity [53]. The main carrier proteins involved in statin transport is the organic anion-transporting polypeptide 1B1 (OATP1B1), encoded by solute carrier organic anion transporter 1B1 (SLCO1B1) [54]. This transporter is located on the basolateral membrane of the hepatocytes. It regulates the influx of statins from the portal circulation to the hepatocytes, the extent of the drug hepatic uptake and its serum concentration. Polymorphism in the SLCO1B1 gene decreases the activity of the carrier, resulting in marked increase in plasma concentrations of statins, particularly simvastatin [54, 55]. The SEARCH (Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine) collaborative group conducted a GWAS by analyzing about 300,000 SNP markers in 85 cases of simvastatin-induced myopathy and 90 matched controls [46]. Genome-wide significant association (P = 4.1E-09, OR = 4.3) was seen for rs4363657 that is in nearly complete LD with a non-synonymous SNP rs4149056 (C/T) located within SLCO1B1 gene. The association was replicated in a trial of simvastatin involving 20,000 participants [46]

Flucloxacillin-induced hepatotoxicity

An antimicrobial drug flucloxacillin, which is widely used in Europe for the treatment of staphylococcal infections, has been associated with cholestatic hepatitis [13]. In a GWAS of 51 cases and 282 controls, a strong association in HLA region was obtained with rs2395029 (P = 8.7E-33) that is in a complete LD with HLA-B*57:01 allele [47]. Possession of HLA-B*57:01 allele was associated with an eighty-fold increased risk of developing hepatotoxicity. This finding was replicated in a second cohort [47]. Another SNP rs10937275 in ST6 β-galactosamide α-2, 6-sialyltranferase-1 (ST6GAL1) also showed genome-wide significance (P = 1.4E-08). This gene is believed to have a possible role in B-cell immune response [47](Daly et al., 2009). Both these findings provide insight for the immune-mediated mechanism of flucloxacillin-induced hepatotoxicity, and have the potential to improve susceptibility prediction [47].

Lumiracoxib-induced hepatotoxicity

Lumiracoxib is a selective cyclooxygenase-2 inhibitor useful for symptomatic relief of acute pain and osteoarthritis [56]. Concerns over hepatotoxicity had contributed to the withdrawal of lumiracoxib in many drug markets world-wide. GWAS to identify risk variants for lumiracoxib-induced hepatotoxicity was performed in 41 cases and 176 treatment-tolerant controls in about 700,000 SNPs [48]. A number of SNPs in HLA region showed strong evidence of association (top SNP was rs9270986 with P = 2.8E-10). The findings were replicated in an independent set of 98 cases and 405 controls (rs3129900, P = 4.4E-12; rs9270986, P = 1.0E-09) [48]. Further study with high resolution HLA genotyping was performed, and an association with HLA-DRB1*15:01 was detected (P = 6.8E-25). Genotyping the HLA-DRB1*15:01 can serve as means of genetic testing. The results of the study showed the potential to improve the safety profile of lumiracoxib by identifying individuals at high risk for hepatotoxicity. This interesting approach is being considered as a

possible means of re-introducing lumiracoxib to the market [6, 19].

Amoxicillin-clavulanate induced hepatotoxicity

Amoxicillin-clavulanate (AC) is among the most commonly prescribed antimicrobials world-wide. Although it is generally well tolerated and the overall risk benefit is favorable, hepatotoxicity can occur rarely that appear to be due to the clavulanate component [49]. The largest GWAS on susceptibility to AC-induced hepatotoxicity with 201 cases and 532 controls showed association with many loci in the HLA region [49]. The strongest effect was with rs9274407 (P = 4.8E-14), which is correlated with rs3135388, a tag SNP of HLA-DRB1*15:01-DQB1*06:02. An independent association was also observed at rs2523822 (P = 1.8E-10) in HLA region related to HLA-A*02:01. High-resolution HLA genotyping in 177 cases and 219 controls confirmed previously shown associations of HLA-DQB1*06:02 (P = 5.0E-10) [49, 57]. There are other GWAS reported on serious ADRs that discovered SNPs with genome-wide significance in combined analysis of the initial GWAS and replication studies. These include polymorphisms for epirubicin-induced leucopenia [58], ribavirin-induced anemia [59] and nevirapine-induced skin rash [60]. There were also studies that focused on important drug toxicities that discovered SNPs with suggestive genome-wide significance, but follow-up studies with larger cohort will be required to prove the association. These include ximelagatran-induced hepatotoxicity [61], bisphosphonates-induced osteonecrosis of the jaw [62], allopurinol-induced severe skin reactions [63], antipsychotic drugs-induced extra-pyramidal side effects [64] and aromatase inhibitors-induced musculoskeletal adverse events [65].

Limitations of GWAS in pharmacogenomic studies

In general, the possible false-positive results, requirement for large sample sizes, genotyping cost and insensitivity to rare variants are important limitations of GWAS [17]. Many of the design and analysis features of GWAS deal with minimizing the false-positive rates while maintaining power to identify true positive associations. This can be achieved to some extent if sufficient numbers of SNPs are carried over from the initial scan into replication studies involving adequate sample sizes [25]. An important short-coming for pharmacogenomic GWAS is the limited sample sizes for the initial GWAS and replication cohorts [21]. Large sample sets are generally not possible for pharmacogenomic outcomes as they usually include by definition both a trait (often with low prevalence) and a drug response phenotype (which further reduces the available study population). To address sample size limitations, researchers are currently combining resources and establishing global collaboration that supports large-scale GWAS [21]. By combining cohorts from multiple sites, pharmacogenomic GWAS could have higher power to detect and validate the risk variants. GWAS approach is well powered to detect common variants with modest effect, but it is less effective in testing rare variants, a problem confounded by the DNA microarrays used in such studies that have been designed to capture common variations. If the risk variants are poorly interrogated on the genome-wide platforms, it is likely to miss important associations [66]. As the number of SNPs and diversity of population represented on genotyping platforms increase, the coverage of genetic variations in the human genome will become more complete, providing greater confidence that clinically important variants on pharmacogenomic traits will not be missed [21]. Since the cost of genome-wide genotyping continues to fall [67], the availability of low-cost genotyping platforms could make it feasible for undertaking more pharmacogenomic GWAS.

Conclusion

GWAS in the area of pharmacogenomics are increasing, and interesting discoveries have been emerged. The findings from some of these studies may potentially be used to develop genetic tests to predict therapeutic outcome or identify susceptible individuals for serious ADRs. Subsequent larger scale studies could increase the understanding of genetic factors that affect drug response.

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References

1. Seng, K.C., Seng, C.K., The success of the genome-wide association approach: a brief story of a long struggle. Eur J Hum Genet 2008;16:554–564.
2. Kim, S., Misra, A., SNP genotyping: technologies and biomedical applications. Annu Rev Biomed Eng 2007;9:289-320.
3. Wang, W.Y.S., Barratt, B.J., Clayton, D.G., et al., Genome-wide association studies: theoretical and practical concerns. Nat Rev Genet 2005;2:109-118.
4. Meyer, U.A., Pharmacogenetics and adverse drug reactions. Lancet 2000;356:1667-1671.
5. Blakey, J.D., Hall, I.P., Current progress in

pharmacogenetics. Br J Clin Pharmacol 2011;71(6):824-831.
6. Stankov, K., Sabo, A., Mikov, M., Pharmacogenetic Biomarkers as Tools for Pharmacoepidemiology of Severe Adverse Drug Reactions. Drug Development Research 2013;74(1):1-14.
7. Evans, W.E., Relling, M.V., Moving towards individualized medicine with pharmacogenomics. Nature 2004;429(6990):464-468.
8. Ritchie, M.D., The success of pharmacogenomics in moving genetic association studies from bench to bedside: study design and implementation of precision medicine in the post-GWAS era. Hum Genet 2012;131(10):1615-1626.
9. Wei, C.Y., Lee, M.T., Chen, Y.T., Pharmacogenomics of adverse drug reactions: implementing personalized medicine. Hum Mol Genet 2012; 21(R1):R58-65.
10. Hirschhorn, J.N., Daly, M.J., Genome-wide association studies for common diseases and complex traits. Nat Rev Genet. 2005;6(2):95-108.
11. Russmann, S., Jetter, A., Kullak-Ublick, G.A., Pharmacogenetics of drug-induced liver injury. Hepatology 2010;52(2):748-761.
12. Hirschhorn, J.N., Lohmueller, K., Byrne, E., et al., Comprehensive review of genetic association studies. Genet Med 2002;4(2):45-61.
13. Daly, A.K., Pharmacogenetics and human genetic polymorphisms. Biochem J. 2010;429(3):435-449.
14. Daly, A.K., Day, C.P., Candidate gene case-control association studies: advantages and potential pitfalls. Br J Clin Pharmacol 2001;52(5):489-499.
15. Daly, A.K., Drug-induced liver injury: past, present and future. Pharmacogenomics 2010;11(5):607-611.
16. Pirmohamed, M., Personalized Pharmacogenomics: Predicting Efficacy and Adverse Drug Reactions. Annu Rev Genomics Hum Genet 2014;15:349–370.
17. McCarthy, M.I., Abecasis, G.R., Cardon, L.R., et al., Genome-wide association studies for complex traits: consensus, uncertainty and challenges. Nat Rev Genet 2008;9(5):356-369.
18. Karlsen, T.H., Melum, E., Franke, A., The utility of genome-wide association studies in hepatology. Hepatology 2010;51(5):1833-1842.
19. Daly, A.K., Using genome-wide association studies to identify genes important in serious adverse drug reactions. Annu Rev Pharmacol Toxicol 2012;52:21-35.
20. Manolio, T.A., Bringing genome-wide association findings into clinical use. Nat Rev Genet 2013;14(8):549-558.
21. Motsinger-Reif, A.A., Jorgenson, E., Relling, M.V., et al., Genome-wide association studies in pharmacogenomics: successes and lessons. Pharmacogenet Genomics 2013;23(8):383-394.
22. International HapMap Consortium. A haplotype map of the human genome. Nature 2005;437(7063):1299-1320.
23. Browning, B.L., Browning, S.R., Haplotypic Analysis of Wellcome Trust Case Control Consortium. Hum Genet 2008;123(3):273-280.
24. Gabriel, S.B., Schaffner, S.F., Nguyen, H., et al., The structure of haplotype blocks in the human genome. Science 2002;296(5576):2225-2229.
25. Pearson, T., Manolio, T., How to Interpret a Genome-wide Association Study. Journal of American Medical Association 2008;299(11):1335-1344.
26. Anderson, C., Pettersson, F., Clarke, G., et al., Data quality control in genetic case-control association studies. Nat Protoc 2010;5(9):1564–1573.
27. Turner, S., Armstrong, L., Bradford, Y., et al., Quality Control Procedures for Genome-Wide Association Studies. Curr Protoc Hum Genet 2011;1.19.11-11.19.18.
28. Purcell, S., Neale, B., Todd-Brown, K., et al., PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81(3):559-575.
29. Rice, T.K., Schork, N.J., Rao, D.C., Methods for handling multiple testing. Adv Genet. 2008;60:293-308.
30. Chanock, S.J., Manolio, T., Boehnke, M., et al., Replicating genotype-phenotype associations. Nature 2007;447(7145):655-660.
31. Hirschhorn, J.N., Gajdos, Z.K., Genome-wide association studies: results from the first few years and potential implications for clinical medicine. Annu Rev Med 2011;62:11-24.
32. Manolio, T., Brooks, L., Collins, F., A HapMap harvest of insights into the genetics of common disease. J Clin Invest 2008;118(5):1590-1605.
33. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;447(7145):661-678.
34. Takeuchi, F., McGinnis, R., Bourgeois, S., et al., A genome-wide association study confirms VKORC1, CYP2C9, and CYP4F2 as principal genetic determinants of warfarin dose. PLoS Genet 2009;5(3):e1000433.
35. Teichert, M., Eijgelsheim, M., Rivadeneira, F., Uitterlinden A, Van Schaik R, Hofman A, et al. A genome-wide association study of acenocoumarol maintenance dosage. Hum Mol Genet 2009;18(19):3758-3768.
36. Tanaka, Y., Nishida, N., Sugiyama, M., et al., Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Hum Genet 2009;41(10):1105-1109.
37. Suppiah, V., Moldovan, M., Ahlenstiel, G., et al., IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 2009;41(10):1100-1104.
38. Ge, D., Fellay, J., Thompson, A.J., et al., Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 2009;461(7262):399-401.
39. Shuldiner, A.R., O'Connell, J.R., Bliden, K.P., et al., Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. Journal of American Medical Association 2009;302(8):849-857.
40. Kimmel, S.E., Warfarin therapy: in need of improvement after all these years. Expert Opin Pharmacother 2008;9(5):677-686.
41. Becquemont, L., Alfirevic, A., Amstutz, U., et al., Ctical recommendations for pharmacogenomics-based prescription: 2010 ESF-UB Conference on Pharmacogenetics and Pharmacogenomics. Pharmacogenomics 2011;12(1):113-124.
42. Bejarano-Achache, I., Levy, L., Mlynarsky, L., et al., Effects of CYP4F2 polymorphism on response to warfarin during induction phase: a prospective, open-label, observational cohort study. Clinical Therapeutics 2012;34(4):811-823.
43. 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 2009;373(9660):309-317.
44. McCormack, M., Alfirevic, A., Bourgeois, S., et al., HLA-A*3101 and carbamazepine-induced hypersensitivity reactions in Europeans. N Engl J Med 2011;364(12):1134-1143.
45. Ozeki, T., Mushiroda, T., Yowang, A., et al., Genome-wide association study identifies HLA-A*3101 allele as a genetic

risk factor for carbamazepine-induced cutaneous adverse drug reactions in Japanese population. Hum Mol Genet 2011;20(5):1034-1041.
46. Link, E., Parish, S., Armitage, J., et al., SLCO1B1 variants and statin-induced myopathy- A genomewide study. N Engl J Med 2008;359(8):789-799.
47. Daly, A.K., Donaldson, P.T., Bhatnagar, P., et al., HLA-B*5701 genotype is a major determinant of drug-induced liver injury due to flucloxacillin. Nat Genet 2009;41(7):816-819.
48. Singer, J.B., Lewitzky, S., Leroy, E., et al., A genome-wide study identifies HLA alleles associated with lumiracoxib-related liver injury. Nat Genet 2010;42(8):711-714.
49. Lucena, M.I., Molokhia, M., Shen, Y., et al., Susceptibility to amoxicillin-clavulanate-induced liver injury is influenced by multiple HLA class I and II alleles. Gastroenterology 2011;141(1):338-347.
50. Vittorio, C.C., Muglia, J.J., Anticonvulsant hypersensitivity syndrome. Arch Intern Med 1995;155(21):2285-2290.
51. Leeder, J.S., Mechanisms of idiosyncratic hypersensitivity reactions to antiepileptic drugs. Epilepsia 1998;39 (Suppl 7):S8-16.
52. Alberton, M., Wu, P., Druyts, E., et al., Adverse events associated with individual statin treatments for cardiovascular disease: an indirect comparison meta-analysis. QJM : Monthly Journal of the Association of Physicians 2012;105(2):145-157.
53. Catapano, A., Statin-induced myotoxicity: pharmacokinetic differences among statins and the risk of rhabdomyolysis, with particular reference to pitavastatin. Curr Vasc Pharmacol 2012;10(2):257-267.
54. Niemi, M., Transporter pharmacogenetics and statin toxicity. Clin Pharmacol Ther 2010;87(1):130-133.
55. Giorgi, M.A., Caroli, C., Arazi, H.C., et al., Pharmacogenomics and adverse drug reactions: the case of statins. Expert Opin Pharmacother 2011;12(10):1499-1509.
56. Bannwarth, B., Berenbaum, F., Lumiracoxib in the management of osteoarthritis and acute pain. Expert Opin Pharmacother 2007;8(10):1551-1564.
57. Andrade, R.J., Lucena, M.I., Alonso, A., et al., HLA class II genotype influences the type of liver injury in drug-induced idiosyncratic liver disease. Hepatology 2004;39(6):1603-1612.
58. Srinivasan, Y., Sasa, M., Honda, J., et al., Genome-wide association study of epirubicin-induced leukopenia in Japanese patients. Pharmacogenet Genomics 2011;21(9):552-558.
59. Ochi, H., Maekawa, T., Abe, H., et al., ITPA polymorphism affects ribavirin-induced anemia and outcomes of therapy A genome-wide study of Japanese HCV virus patients. Gastroenterology 2010;139(4):1190-1197.
60. Chantarangsu, S., Mushiroda, T., Mahasirimongkol, S., et al., Genome-wide association study identifies variations in 6p21.3 associated with nevirapine-induced rash. Clin Infect Dis 2011;53(4):341-348.
61. Kindmark, A., Jawaid, A., Harbron, C.G., et al., Genome-wide pharmacogenetic investigation of a hepatic adverse event without clinical signs of immunopathology suggests an underlying immune pathogenesis. Pharmacogenomics J 2008;8(3):186-195.
62. Sarasquete, M., García-Sanz, R., Marín, L., et al., Bisphosphonate-related osteonecrosis of the jaw is associated with polymorphisms of the cytochrome P450 CYP2C8 in multiple myeloma: A genome-wide single nucleotide polymorphism analysis. Blood 2008;112(7):2709-2712.
63. Tohkin, M., Kaniwa, N., Saito, Y., et al., A whole-genome association study of major determinants for allopurinol-related Stevens-Johnson syndrome and toxic epidermal necrolysis in Japanese patients. Pharmacogenomics J 2013;13(1):60-69.
64. Aberg, K., Adkins, D., Bukszár, J., et al., Genomewide association study of movement-related adverse antipsychotic effects. Biol Psychiatry 2010;67(3):279-282.
65. Ingle, J., Schaid, D., Goss, P., et al., Genome-Wide Associations and Functional Genomic Studies of Musculoskeletal Adverse Events in Women Receiving Aromatase Inhibitors. J Clin Oncol 2010;28:4674-4682.
66. Gamazon, E., Skol, A., Perera, M., The limits of genome-wide methods for pharmacogenomic testing. Pharmacogenet Genomics 2012;22:261–272.
67. David, G., Howard, L.M., Genome-wide association studies: powerful tools for improving drug safety and efficacy. Pharmacogenomics 2009;10(2):157-159.