Review

Pharmacogenomics and personalized medicine: a review focused on their application in the Chinese population

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The field of pharmacogenomics was initiated in the 1950s and began to thrive after the completion of the human genome project 10 years ago. Thus far, more than 100 drug labels and clinical guidelines referring to pharmacogenomic biomarkers have been published, and several key pharmacogenomic markers for either drug safety or efficacy have been identified and subsequently adopted in clinical practice as pre-treatment genetic tests. However, a tremendous variation of genetic backgrounds exists between different ethnic groups. The application of pharmacogenomics in the Chinese population is still a long way off, since the published guidelines issued by the organizations such as US Food and Drug Administration require further confirmation in the Chinese population. This review highlights important pharmacogenomic discoveries in the Chinese population and compares the Chinese population with other nations regarding the pharmacogenomics of five most commonly used drugs, *ie*, tacrolimus, cyclosporine A, warfarin, cyclophosphamide and azathioprine.

Keywords: pharmacogenomics; personalized medicine; ethnic difference; Chinese population; tacrolimus; cyclosporine A; warfarin; cyclophosphamide; azathioprine

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Introduction

The field of pharmacogenomics originated in the late 1950s when the term “pharmacogenetics” was first published. However, the true breakthrough in this field began 10 years ago with the completion of the human genome project. To date, many pharmacogenomic biomarkers have moved from discovery to clinical implementation. There are currently 121 Food and Drug Administration (FDA) drug labels that refer to pharmacogenomic biomarkers of drug safety or efficacy (<http://www.fda.gov/drugs/scienceresearch/researchareas/pharmacogenetics/ucm083378.htm>). Moreover, a large number of testing guideline position papers have been published by the Clinical Pharmacogenomics Implementation Consortium (CPIC)^[1]. Initially, most pharmacogenomic studies focused on human cytochrome P450 enzyme (CYP) systems. There are more than 50 known human CYPs and variant alleles in these genes are being discovered continuously in various populations. Other notable enzymes with known variants and poten-

tial clinical utility include thiopurine S-methyltransferase (TPMT), which is involved in thiopurine toxicity^[2]. At the end of 2011 it has been estimated that, over 150 000 papers reporting pharmacogenomic biomarkers have been published, with over 100 biomarkers having demonstrated clinical utility^[3]. A vast number of discoveries relating to genomic variability and drug responses have been made in the last 10 years. This review aims to summarize the current state of pharmacogenomic studies and personalized medicine, with an emphasis on progress in research focusing on the Chinese population.

Tacrolimus

Transplantation followed by immunosuppressive therapy is the typical treatment for patients with end-stage organ diseases. Tacrolimus (also known as FK506) was first approved by the US FDA in 1994 and it has remarkably improved patient survival after organ transplant. However, tacrolimus treatment has many drawbacks, including a narrow therapeutic window, high inter- and intraindividual variability in pharmacokinetics/pharmacodynamics and severe adverse effects^[4–6]. Therefore, a large number of studies have been devoted to exploring personalized determination for tacroli-

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mus treatment to achieve efficacy as well as safety.

Cytochrome P450 3A4 and 3A5 (CYP3A4 and CYP3A5) are the major cytochrome P450 subtypes involved in catalyzing the phase I metabolism of tacrolimus. CYP3A4 is the major cytochrome P450 subtype in the human liver, and there is high interindividual variation in its activity. The most studied CYP3A4 mutation is CYP3A4*1B (-392A>G)^[7]. Hesselink *et al* reported that CYP3A4*1B allele carriers had lower tacrolimus dose-adjusted trough levels (C_0) than those in patients with the wild-type (*1/*1) genotype^[8], but this rare single nucleotide polymorphism (SNP)^[9] may not be the major factor in the large interindividual variance of tacrolimus pharmacokinetics. CYP3A4*1G (20230G>A) is a newly identified SNP with the highest occurrence in Asian populations, and it was reported that dose-adjusted C_0 of the patients with CYP3A4 *1/*1 was higher than the genetic groups of CYP3A4*1/*1G and CYP3A4*1G/*1G in Han Chinese of South China^[10]. However, the definite function of CYP3A4*1G is not yet clear. A very frequent polymorphism within intron 3 of CYP3A5 (6986A>G, CYP3A5*3 allele), which causes a splicing defect that results in the absence of functional CYP3A5 protein in homozygous carriers (CYP3A5*3/*3, CYP3A5 nonexpressers), is the primary cause of its polymorphic expression^[11, 12]. The presence of the CYP3A5*3 allele was associated with higher dose-adjusted tacrolimus blood concentrations and lower tacrolimus requirements^[13, 14], which is the only consistent conclusion among pharmacogenetic/pharmacogenomics studies of tacrolimus.

The absorption of tacrolimus is affected by P-glycoprotein (P-gp, also known as ABCB1) when administered orally. Variability of P-gp activity among individuals could influence the efficacy and toxicities of tacrolimus by changing its oral bioavailability^[15–18]. In particular, the presence of P-gp in the intestine can limit tacrolimus absorption. P-gp in the liver and kidney also promotes tacrolimus efflux into the bile and urine, respectively. The P-gp efflux pump is encoded by the multidrug resistance-1 (MDR-1) gene; polymorphisms in this gene lead to polymorphic expression of P-gp in many tissues and organs (*eg*, liver, kidney, and small intestines). The most commonly studied ABCB1 polymorphisms include a C to T substitution at position 1236 on exon 12, a G to T/A mutation at position 2677 on exon 21, and a C to T substitution at position 3435 on exon 26^[15]. Despite the fact that tacrolimus is a substrate of P-gp, the association between ABCB1 genotype and tacrolimus pharmacokinetics is unclear^[19–27]. Even the two studies conducted by two Chinese hospitals—one in Southwest China^[21] (Chengdu, Sichuan Province) and one in East China^[25] (Hangzhou, Zhejiang Province)—presented conflicting results. This lack of consistency might be caused by the different ethnic groups included in these studies given that the ethnic situation of the patients in the two studies was not mentioned. Southwest China contains over 30 ethnic minorities, whereas the residents of East China are mainly Han Chinese. In our previous study, we found that both MDR1 genotype distribution and haplotype profiles were significantly different between the Chinese Han, Bai, Wa and Tibetan ethnic groups in Yunnan Province in Southwest China^[28]. We believe that

the importance of studying ABCB1 genetics as an independent factor is minimal.

Recent studies have investigated not only CYP3A4, CYP3A5 and ABCB1 genetic polymorphisms but also factors that could affect the activities of these three proteins. The pregnane X receptor (PXR, encoded by NR1I2) is reported to be the key nuclear receptor regulating the expression of CYP3A4, CYP3A5, and ABCB1^[29]. Several PXR SNPs have been reported to be associated with the CYP3A4 or P-gp/MDR1 expression level or activity *in vitro*^[29–31]. Differences in the distribution of PXR SNPs were found when comparing the Han Chinese of South China and Caucasian Americans^[31]. PXR -25385C>T, an SNP located in the PXR promoter and found to be correlated with CYP3A4 phenotype, was identified as a significant covariate for apparent oral clearance (CL/F) of tacrolimus by Benkali *et al*^[32], but this result could not be replicated in another study^[33]. Cytochrome P450 oxidoreductase (POR) is known as the obligatory electron donor in the metabolism of drugs by CYP enzymes in humans, and the correlation between POR genetic polymorphisms and CYP-catalyzed drug metabolism has become a research hot spot. A503V (POR*28), the most studied and most common SNP in the POR coding region, has been reported to contribute more to the variation of CYP3A activity *in vivo* than the functional genetic variants at the CYP3A gene locus in a white population^[34]. POR*28 was associated with lower tacrolimus exposure in CYP3A5 expressers in healthy male volunteers in East China (Suzhou, Jiangsu Province)^[35]. However, the effect of this SNP on tacrolimus pharmacokinetics remains unclear based on the existing data^[10, 35, 36].

Cyclosporine A

Cyclosporine A (also known as cyclosporin, cyclosporine or cyclosporin A) is an immunosuppressant drug that has dramatically increased graft and patient survival since it was approved in the 1970s^[37]. However, its use is limited by significant adverse side effects, in particular, acute and chronic calcineurin inhibitor nephrotoxicity^[38, 39] and high variability in bioavailability, which can range from 5% to 89%^[40]. Because cyclosporine A has a narrow therapeutic index and marked interindividual pharmacokinetic variation, its usage requires monitoring the drug concentration to ensure that it is within the recommended therapeutic range. Like tacrolimus discussed above, cyclosporine A is a substrate of P-gp, and it is processed by CYP3A4 and CYP3A5 into metabolites before elimination.

Several studies have reported that renal transplant patients who were homozygous for the CYP3A5*3 genotype were exposed to a higher cyclosporine A C_0 /dose^[41–43], whereas other studies have reported a negative correlation^[44]. Notably, it was reported that CYP3A5*3 is associated with a higher cyclosporine A C_0 /dose in Han Chinese in North China^[41]. Moreover, several studies of the CYP3A4*1G allele in intron 10 revealed that the CYP3A4*1G genotype had lower cyclosporine A clearance than CYP3A4*1/*1 in Han Chinese of East China^[45]. To date, an association between CYP3A4*1G and

cyclosporine A pharmacokinetics has not been established in patients after transplantation^[42, 46, 47]. Interestingly, two separate studies conducted by two hospitals located in different regions of China presented conflicting results, although both included Han Chinese^[45, 46]. One study^[46] included subjects from Northeast China (Shenyang, Liaoning Province), a region in which ethnic minorities account for approximately 5% of the total population. The other study^[45] was conducted in South China (Shanghai), where the majority of the population is Han Chinese. These conflicting results might be attributable to linkage disequilibrium (LD) between CYP3A4*1G and CYP3A5*3 in Asian populations^[47-49], including Han Chinese in South China^[47].

Because cyclosporine A is a substrate of P-gp, ABCB1 genetic polymorphisms might influence the amount of the drug pumped out of cells^[50]. To date, researchers have focused primarily on the associations between three polymorphisms (1236 C>T in exon 12, 2677 G>T/A in exon 21, and 3435 C>T in exon 26) and cyclosporine A pharmacokinetics, but the results have been inconsistent. Several studies have suggested that the non-synonymous variant 2677 G>T/A and synonymous variant 3435 C>T might contribute to the differences in cyclosporine A pharmacokinetics^[41, 42], but others have reported that neither polymorphism affected the cyclosporine A C_0 ^[51, 52]. Furthermore, the three SNPs were in LD with one another^[53]. Carriers of the 1236TT-2677TT-3435TT haplotype had an obviously higher dose-adjusted C_0 than carriers of the other genotypes; moreover, a more obvious influence was found for the 1236TT-2677TT-3435TT haplotype compared to the individual SNP, indicating that the use of the ABCB1 haplotype is superior to SNP analysis for predicting the concentrations of cyclosporine A in Han Chinese of South China^[54, 55].

Pharmacogenomic studies of cyclosporine A have focused on the effect of CYP3A and ABCB1 SNPs but yielded conflicting results^[56]. Therefore, in addition to CYP3A4, CYP3A5 and ABCB1 genetic polymorphisms, factors that could affect the activities of these three proteins have also been considered, such as the pregnane X receptor and NF- κ B. However, no association between PXR genetic polymorphisms and cyclosporine A has been found^[55].

NF- κ B, a protein complex found in almost all animal cell types, is a transcription factor that is critical for inflammatory responses. It has been reported that NF- κ B competitively binds to the retinoid X receptor (RXR), thus preventing the PXR-RXR complex from binding to consensus DNA sequences in the regulatory regions of downstream genes, including CYP3A4 and ABCB1^[57]. The NFKB1 gene encodes the p50 subunit of NF- κ B, which complexes with p65 to produce the major form of NF- κ B, and possesses a functional common insertion/deletion (-94 ins/del ATTG) mutation in its promoter region. The del ATTG allele may result in decreased NFKB1 transcript levels and therefore decreased p50 protein production^[58]. Among non-carriers of the ABCB1 2677 TT and 3435 TT genotypes, patients with the NFKB1 6194 ATTG ins/ins genotype had a significantly higher dose-adjusted C_0 than those with the 6194 ATTG del/del genotype^[55].

Warfarin

Since its introduction in the 1950s, warfarin has become the most commonly used oral anticoagulant for the prevention of thromboembolism in patients with deep vein thrombosis, atrial fibrillation or prosthetic heart valve replacement^[59]. It is difficult to achieve the desired anticoagulation because of warfarin's narrow therapeutic window and highly variable response among individuals. The search for genetic determinants influencing warfarin response began in the 1990s. The first target genetic factor was the warfarin-metabolizing enzyme cytochrome P450 2C9 (CYP2C9)^[60]. The most common variants in Caucasians are CYP2C9*2 (rs1799853), which has an Arg144Cys substitution, and CYP2C9*3 (rs1057920), which has an Ile359Thr substitution^[61, 62]. The mutant genes produce metabolically impaired enzymes with activities reduced by 30% (CYP2C9*2) and 80% (CYP2C9*3)^[63]. However, CYP2C9*2 is almost absent in the Han Chinese population. Individuals who carry CYP2C9*2 or *3 require lower doses of warfarin, especially those with two copies of the *3 allele^[64, 65]. Additional CYP2C9 variants with reduced metabolic capacity (CYP2C9*5, *6, *8, and *11) have also been identified, and these variants contribute to warfarin dose variation in African Americans^[66, 67].

Except for CYP2C9, the effects of genetic polymorphisms in other metabolizing enzymes make minimal contributions to personalized medication with warfarin. For instance, polymorphisms in CYP1A1^[68] and CYP3A5^[69], enzymes that metabolize (R)-warfarin, are clinically insignificant because of the minimal effects of (R)-warfarin on anticoagulation. Genes in the vitamin K regeneration cycle and vitamin K-dependent clotting factors have also been studied, including CYP2C18, CYP2C19, PROC (Protein C), ABCB1, APOE (apolipoprotein E), EPHX1 (epoxide hydrolase 1 gene), CALU (calumenin), GGCX (gamma-glutamyl carboxylase), ORM1 (orosomucoid 1), ORM2, vitamin K-dependent clotting factor II (prothrombin), VII, IX, and X and PXR^[67, 69-74]. In addition to vitamin K epoxide reductase subunit 1 (VKORC1) and CYP2C9*3, CYP2C18, PROC, and EPHX1 have small but significant associations with warfarin dose in Han Chinese in Taiwan, China^[72]. However, the effects of these genes were too small to have any significant clinical use.

The second major genetic determinant of warfarin processing is VKORC1^[75, 76]. The most common VKORC1 variant is a noncoding variant (VKORC1 -1639 G>A, rs9923231) that lies in the promoter region of VKORC1. The -1639 G allele has decreased enzyme activity, so individuals who carry the G allele require higher warfarin doses than those with the A allele^[77, 78]. CYP4F2 is another genetic factor in warfarin processing. CYP4F2 contributes to vitamin K1 oxidation, and some variants could result in reduced enzyme activity^[79]. An association between the CYP4F2 variant (rs2108622) and warfarin dose was subsequently confirmed in a meta-analysis involving more than 9000 participants from 30 studies^[80]. Although the association of CYP4F2 is significant, the effect size is much lower than those contributed of VKORC1 and CYP2C9 in Han Chinese in South China^[81], and its clinical use

needs to be studied further.

Cyclophosphamide

Cyclophosphamide is a widely used immunosuppressant drug. It is a prodrug whose activity depends on bioactivation to 4-hydroxycyclophosphamide. Bioactivation of cyclophosphamide is highly variable among patients and has been attributed primarily to CYP2B6, CYP2C19, and CYP3A4^[82, 83]. Additionally, cyclophosphamide and all its metabolites undergo phase II metabolism catalyzed by glutathione S-transferase (GST) or aldehyde dehydrogenase (ALDH). The most common variant allele of CYP2B6 in all populations contains two amino acid changes—Q172H and K262R—and is called CYP2B6*6. This haplotype occurs in approximately 15% to over 60% of individuals, depending on ethnicity^[84]. Cyclophosphamide bioactivation was reported to be enhanced in the CYP2B6*6/*6 genotype *in vitro* and *in vivo*^[85–87]. However, contradictory or negative results were presented in other studies of the association between CYP2B6 and pharmacokinetics/clinical outcome^[88–91]. Notably, remarkable interindividual variety in the pharmacokinetics of cyclophosphamide and 4-hydroxycyclophosphamide had been reported in Han Chinese in South China^[91]. Moreover, several studies associated other variants including CYP2B6*4, *5, *8, and *9 with lower 4-OH cyclophosphamide formation *in vivo* or with poor outcome^[92–94]. Taken together, the *in vivo* and *in vitro* data concerning cyclophosphamide indicate that CYP2B6 polymorphism plays a role in cyclophosphamide processing, although the studies are not yet conclusive. This may be explained by different study size, design and co-medication in cancer therapy or autoimmune disease treatment. The phase II drug-metabolizing enzymes, including GST or ALDH, have also been studied in regard to personalized medication with cyclophosphamide. Several investigations of Hodgkin's lymphoma, ovarian cancer, colorectal cancer, and breast cancer have found improved survival rates associated with polymorphic forms of GSTT1, GSTM1, and GSTP1^[95–103]. The activity and genetic polymorphisms of GSTP1 were associated with clinical outcomes and toxicities of cyclophosphamide in Han Chinese (South China) with systemic lupus erythematosus^[95].

Azathioprine

Azathioprine is a cytotoxic and immunosuppressive drug that is widely used to treat autoimmune disorders, inflammatory bowel disease and acute lymphoblastic leukemia^[104]. It is an inactive prodrug that has to be activated into 6-thioguanine nucleosides (6-TGN) and 6-methylmercaptopurine nucleotides (6-MMPN). Production of 6-TGN occurs through the hypoxanthine guanine phosphoribosyl-transferase (HPRT) pathway, whereas formation of 6-MMPN occurs in the TPMT pathway. Although clearly effective, azathioprine is characterized by considerable interindividual variability in clinical response, with approximately half of patients failing to achieve clinical remission and 15%–28% experiencing adverse effects due to overdose^[105, 106]. Genetic polymorphisms of enzymes involved in thiopurine metabolism, such as TPMT, inosine triphosphate

pyrophosphatase (ITPase) or xanthine oxidase (XO) could affect clinical response to thiopurines and be related to side effects^[107].

TPMT is a cytosolic methylating enzyme whose physiological role remains unclear despite extensive investigation. It is reported that a reduction in TPMT activity, caused by genetic polymorphisms results in severe and hematological toxicity in patients treated with standard doses of thiopurines^[108, 109]. Approximately 0.3% of individuals in a general population had low levels of TPMT activity, with approximately 10% of individuals expressing intermediate levels^[108]. The TPMT gene is highly polymorphic; more than 25 variants have been identified. Four alleles (TPMT*2, *3A, *3B, and *3C) account for approximately 95% of inherited TPMT deficiency^[109–111]. The wild-type allele, TPMT*1, encodes the fully active enzyme, and TPMT*2, TPMT*3A and TPMT*3C are the most prevalent genotypes in Caucasians, together accounting for 80% to 95% of the polymorphic alleles that lead to a significant reduction in enzyme activity due to enhanced rates of proteolysis of the mutant proteins^[112]. The frequency of TPMT*3C in Chinese people has been estimated to be 2%–3.2% making it the major mutant allele in the Chinese population^[113, 114]. However, the most common low-activity allele in the Caucasian population is TPMT*3A (~5%). This allele is also found in individuals who originate from India and Pakistan, but less frequently^[115, 116]. The US FDA states that if a patient has clinical or laboratory evidence of severe toxicity, particularly myelosuppression, TPMT testing should be considered. Substantial dose reductions of mercaptopurine are generally required for patients homozygous for TPMT deficiency (two non-functional alleles) to avoid the development of life-threatening bone marrow suppression. Although heterozygous patients with intermediate TPMT activity may have increased mercaptopurine toxicity, this condition is variable, and the majority of patients tolerate normal doses of mercaptopurine. The CPIC guidelines describe a complete strategy for adjusting the starting dose according to TPMT activity and genotypes (Table 1)^[110].

Inosine triphosphate pyrophosphatase (ITPA) is one of several enzymes whose job is to cleanse the nucleotide pool^[117]. ITPase also acts as a “cleaning” enzyme by degrading other “rogue” purine nucleotides in cells, *eg*, endogenous deoxyinosine triphosphate (dITP) and deoxy-xanthosine triphosphate (dXTP)^[106, 118, 119]. Five SNPs in the human ITPA gene have been identified, two of which are associated with loss of ITPase activity (94C>A in exon-2, and IVS2+21A>C). These interact and affect splicing branch points resulting in the missplicing of exons 2 and 3, leading to shortening of polypeptide stretches in the enzyme^[120]. The other three coding-region ITPA polymorphic sequence variants are silent mutations (138G>A, 561G>A, and 708G>A)^[119]. Approximately 1 in 15 (6.0%) people are carriers in Caucasian populations, and these individuals have an average of approximately 22% of normal red cell ITPase activity. This allele is more common in Asian populations, with a frequency of 11%–15%^[121]. It has been reported that carriers of ITPA IVS2+21A>C variants had sig-

Table 1. Dose adjustment of azathioprine recommended by CPIC guidelines.

Genotype of TPMT	Phenotype of TPMT	Dose adjustment
Carrying two or more functional alleles (*1)	High activity	Start with normal starting dose (eg, 2–3 mg/kg ¹ ·d ⁻¹); allow 2 weeks to reach steady state.
Carrying one nonfunctional allele (*2, *3A, *3B, *3C, or *4)	Intermediate activity	Start with 30%–70% of target dose; allow 2–4 weeks to reach steady state.
Carrying two nonfunctional alleles	Low or deficient activity	Consider alternative agents or reduce daily dose by 10-fold; allow 4–6 weeks to reach steady state. Special attention is required for myelosuppression.

nificantly higher concentrations of 6-TGN^[122].

Xanthine oxidase (XO) oxidizes 6-MP into 6-thioxanthine (6-TX) and subsequently into 6-thiouric acid (6-TU)^[123]. This enzyme, which is predominantly expressed in the intestinal mucosa and liver, converts 6-MP to 6-TX and subsequently to the inactive metabolite 6-TU^[124, 125], a principal pathway in 6-MP catabolism. However, research on XO genetic polymorphisms and azathioprine toxicities is sparse and no significant correlation has been established^[122, 126].

Conclusion and future directions

Although the enthusiasm about their potential pharmacogenetic utility has prompted a number of clinical practice guidelines for pharmacogenetic results, the guidelines or conclusions specific for pharmacogenomics practice in the Chinese population are still lacking. However, most pharmacogenomics studies in Chinese subjects are conducted in individual hospitals or research centers. The results of these single hospital or center studies are often hard to reproduce or even conflict with one another, which is likely due to the differences in clinical practice in research centers or institutions. Multi-center, larger-scale and long-term studies will thus become particularly important to obtain accurate pharmacogenomic data for the Chinese population. In addition, nation-wide electronic healthcare databases through which the long-term follow-up and detailed medical records of recruited patients can be assessed by medical researchers are required for further development of personalized medicine in the Chinese population.

Another obstacle in the development of pharmacogenomics in China is the cost of genetic testing; it has not been determined whether insurance should cover the expenses. Genetic tests can suggest optimal therapy regimens, predict the likelihood of drug response/toxicities and avoid exposing patients to ineffective or overly toxic regimens. Although new technologies for genetic testing have been developing rapidly, personalized genetic testing is still too expensive for most Chinese citizens. Genomic medicine has the capacity to revolutionize clinical practice, but if insurance companies and the government are unwilling to pay for genetic testing, this important progress will be stalled. However, some have pointed out a potential disadvantage of insurance paying for the testing;

the results can affect a person's health insurance coverage or increase premium. The solution first depends on privacy protection legislation to prevent genetic discrimination. Second, extensive data evaluating the economics of genetic testing is required in China.

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