Pharmacogenetics Biomarkers Predictive of Drug Pharmacodynamics as an Additional Tool to Therapeutic Drug Monitoring

Vincent Haufroid, PharmD, PhD† and Nicolas Picard, PharmD, PhD‡*

Abstract: Conventional therapeutic drug monitoring refers to the individualization of drug dosage by maintaining plasma or blood drug concentrations within a targeted therapeutic range. Accordingly, an individualized dose is proposed to the clinician according to the drug plasma or blood concentration using an a posteriori approach. Pharmacogenetics (PGx) has recently emerged as an additional tool to refine dose selection or, more interestingly to select, a priori, the first dose to administer. To date, the vast majority of genes explored in the context of PGx are those coding for metabolizing enzymes or membrane drug transporters, which mainly influence drug pharmacokinetics parameters. Indeed, among the 94 PGx-based drug dosing guidelines currently published by the Clinical Pharmacogenetics Implementation Consortium and the Dutch Pharmacogenetics Working Group on PharmGKB web site, 81 (86%) are associated with the genotype determination of either a metabolizing enzyme or a membrane drug transporter, whereas only 13 (14%) are associated with the genotype determination of a pharmacodynamics (PD)-associated gene. In this article, we describe selected PGx biomarkers that predict or could predict PD (both in terms of efficacy and toxicity). First, the most relevant clinical applications already subject to validated international guidelines (Clinical Pharmacogenetics Implementation Consortium and Dutch Pharmacogenetics Working Group), and ready to be implemented in routine clinical settings, are discussed to illustrate the clinical potential of PD-associated PGx biomarkers (*G6PD*, *HLA-B*57:01*, *HLA-B*15:02*, and *VKORC1*). Then, to illustrate not only the research potential of such biomarkers but also the complexity of PGx–PD relationships, the case of immunosuppressive drugs (for which conventional therapeutic drug monitoring is widely accepted) is extensively described with the potential to include some of these PGx biomarkers in future PGx dosing guidelines.

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INTRODUCTION

Many drugs currently available on the market are "one size fits all," but daily use demonstrates that the clinical response may vary largely between patients;[1] this sometimes requires the use of personalized approaches such as therapeutic drug monitoring (TDM), mainly for drugs characterized by a narrow therapeutic index. TDM is generally defined as the clinical laboratory measurement of a chemical parameter that, with appropriate medical interpretation, will directly influence drug prescribing procedures.[2] Most of the time, conventional TDM refers to the individualization of drug dosage by maintaining plasma or blood drug concentrations within a targeted therapeutic range. In this way, an individualized dose is proposed to the clinician according to the drug plasma or blood concentration using an a posteriori approach. More than 20 years ago, clinical laboratory started advocating the use of pharmacogenetics (PGx) as a complementary tool for optimizing drug efficacy.[3] It took years before the National Academy of Clinical Biochemistry (NACB) published Guidelines and Recommendations for "*Laboratory Analysis and Application of Pharmacogenetics to Clinical Practice*."[4] This has set the framework for the laboratory community to begin and bring application of PGx to practice. The consensus is now being used by clinical laboratories as an additional tool to refine dose selection or, more interestingly to select, a priori, the first dose to administer.[5] It is defined by the drug agencies [European Medicines Agency (EMA) and US Food and Drug Administration (FDA)] as "*The study of variations in DNA sequence as related to drug response*."[6] According to this definition, drug response has to be considered broadly and include not only the therapeutic efficacy but also the potential occurrence of side effects [also known as adverse drug reactions (ADRs)]. PGx and, by extension, pharmacogenomics combines pharmacology (as the science of drugs) and genetics/genomics (as the study of genes and their functions) to develop effective and safe medications and propose drug dosing (as its main application related to TDM) that will be tailored to a person's genetic makeup. The final objective of the PGx is to predict the appropriate drug and/or dose to achieve the best clinical response with a high therapeutic efficacy and a low occurrence of side effects or ADRs. In practice, it is based on the identification of constitutive genetic markers located in the genes encoding proteins involved in drug pharmacokinetics (PK) and/or pharmacodynamics (PD). To date, the vast majority of genes explored in the context of PGx are those coding for metabolizing enzymes or membrane drug transporters and therefore mainly influencing drug PK parameters. Indeed, among the 94 PharmGKB PGx-based drug dosing guidelines,[1] currently published by the Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch

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From the *Louvain Centre for Toxicology and Applied Pharmacology, Institut de Recherche Expérimentale et Clinique, Université Catholique de Louvain; †Department of Clinical Chemistry, Cliniques Universitaires Saint-Luc, Brussels, Belgium; and ‡CHU Limoges, Université de Limoges, Inserm, IPPRITT, U1248, F-87000 Limoges, France.
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Pharmacogenetics Working Group, 81 (86%) are associated with the genotype determination of either a metabolizing enzyme or a membrane drug transporter, whereas only 13 (14%) are associated with the genotype determination of a PD-associated gene (mainly *G6PD*, *HLA*, *VKORC1*, and *IFNL3*, see below). The PGx analyses are usually performed on blood samples collected in EDTA-containing tubes but can also be performed using saliva or hair samples. The request must be accompanied by patient consent (as for any genetic analysis), but other preanalytical constraints are very limited. The genetic variations explored are mostly single-nucleotide polymorphisms (SNPs) that require simple and rapid analytical techniques. Their cost is generally comparable with that of a drug concentration determination. These PGx biomarkers have well-defined functionalities enabling rapid interpretation (with the help of validated and published guidelines), perfectly compatible with the clinical decision process. However, their routine use is still quite limited mostly because of the lack of formal proof that clinical outcome really improves after genotype-based dosing using well-designed randomized control trial data.

In this article, we describe selected PGx biomarkers that predict or could predict PD (both in terms of efficacy and toxicity). Only constitutive genetic markers are taken into consideration excluding the case of targeted therapies in medical oncology involving acquired genetic variations. First, the most relevant clinical applications already subject to validated international guidelines, and ready to be implemented in routine, are discussed to illustrate the clinical potential of PD-associated PGx biomarkers. Then, to illustrate not only the research potential of such biomarkers but also the complexity of PGx–PD relationships, the case of immunosuppressive (IS) drugs (for which conventional TDM is widely accepted) is extensively described with the potential to include some of these PGx biomarkers in future PGx dosing guidelines.

G6PD Deficiency as an Historical Example of PGx Biomarker Predictive of ADRs

Glucose-6-phosphate dehydrogenase (G6PD) deficiency was among the first enzyme defect of genetic origin to be related to variable drug response. The enzyme is not involved in drug PK, but it protects cells from oxidative damages. G6PD converts glucose-6-phosphate to 6-phosphogluconolactone.[7] At the same time, it generates nicotinamide adenine dinucleotide phosphate (NADPH), which is necessary to produce reduced glutathione (GSH). G6PD deficiency decreases NADPH production and lowers GSH levels, which in turn impairs cell ability to scavenge reactive oxygen species (ROS; eg, superoxide anion, hydrogen peroxide, and hydroxyl radicals). ROS are produced under physiological conditions and have important roles for normal cellular functions (eg, immunity and cell signaling). They are also related to many pathological conditions (eg, cancer and hypertension). Some drugs can also trigger the production of ROS in high amounts. G6PD is expressed in all cells, but its role is particularly important in red blood cells (RBCs). RBCs do not have mitochondria to generate NADPH and are therefore dependent on G6PD to protect hemoglobin β chain from oxidative damage. Although individuals with G6PD deficiency have intrinsically abnormal RBCs, they are asymptomatic under normal conditions, but their susceptibility to ROS is increased. Hemolysis results from the action of an exogenous factor (including drug intake) are associated with oxidative stress, which can ultimately result in acute hemolytic anemia (AHA).

The Discovery of G6PD Deficiency

G6PD deficiency was first discovered in 1956[8] from experiments conducted with hemolyzates of RBCs obtained from patients developing adverse reactions after administration of the antimalarial drug primaquine and patients with no adverse reactions. Primaquine (and more generally, 8-aminoquinoline compounds) was known to cause AHA in certain individuals, but the mechanism for such sensitivity remained obscure. It was shown later in *in vitro* experiment that the metabolism of primaquine by cytochrome P450 enzymes (CYPs) contributes to the toxicity: primaquine metabolites induce the formation of methemoglobin and ROS.[9] It was also quickly realized that G6PD deficiency was the underlying cause of "favism," a term used for more than 2000 years to describe acute and even lethal toxicity caused by the ingestion of fava beans (also known as broad beans).[10]

The Genetic Basis of G6PD Deficiency

G6PD deficiency is considered the most prevalent enzyme deficiency worldwide, with almost 5% of the population affected.[11] The World Health Organization (WHO) constituted a working group dedicated to G6PD deficiency. The group made very comprehensive recommendations regarding the enzyme characteristics to consider for the identification and the classification of *G6PD* variants (eg, RBC G6PD activity, electrophoretic migration, Michaelis constants, etc.).[12] They proposed to consider 5 classes of variants. Class I variants are very rare. They are associated with severe deficiency and result in chronic nonspherocytic hemolytic anemia. Class II and III variants are associated with severely deficient (less than 10% of residual activity) and moderately deficient activities (10%–60% residual activity), respectively. Class IV variants have normal activities, and class V variants are associated with increased G6PD activity. The detailed classification is available in several review articles and in the WHO working group report.[8,12,13]

G6PD gene is located on the X chromosome. The deficiency is thus inherited in a sex-dependent fashion: it is fully expressed in hemizygous men and homozygous women. The diagnosis of G6PD deficiency in heterozygous women is more difficult. In each cell, only one X chromosome is active, and the pattern of inactivation is random. Heterozygous women have therefore G6PD-deficient RBCs combined with RBCs with normal G6PD activity, with an average of 50% of each.[12]

G6PD Pharmacogenetics

The WHO working group proposed a first list of drugs implicated in AHA in G6PD-deficient individuals in 1967.[14] Over the years, the list has been shortened because some

drugs were withdrawn from the market. Several regulatory agencies have introduced warnings or precautions in drug labels to alert the risk of adverse reactions in individuals with G6PD deficiency. PharmGKB web site provides an updated list of labels containing PGx information related to G6PD for drugs approved by the US FDA, the EMA, the Pharmaceuticals and Medical Devices Agency, Japan (PMDA), and Health Canada (Santé Canada).[1] The scientific curators of the web site have also highlighted 21 clinical annotations (ie, variant–drug pairs) of notable interest (the drugs concerned include antimalarial drugs such as primaquine, mefloquine, chloroquine, and artesunate; antibiotics such as chloramphenicol, nitrofurantoin, and sulfamethoxazole/trimethoprim; the antidiabetic glibenclamide; etc.). Two clinical annotations are ranked with the highest levels of evidence. These annotations concern rasburicase (level 1A) and chlorproguanil/dapsone (level 1B). The CPIC has emitted guidelines regarding rasburicase.[13]

The case of rasburicase is exemplary. The drug is used for prophylaxis and treatment of hyperuricemia during chemotherapy in adults and children with lymphoma, leukemia, and solid tumors. The FDA, the EMA, and the PMDA have contraindicated the use of the drug in patients with G6PD deficiency.[13] However, the G6PD status of a patient is often unknown at treatment initiation, and the time to obtain results may preclude routine screening, as emergent therapy may be needed to prevent organ damage from hyperuricemia.[15] The WHO recommends testing patients from high prevalence ethnicities.[12] Inclusion of G6PD results in patient medical records, in particular for those at-risk patients, is urgently needed to guide treatment selection before TDM. This might be facilitated by the advent of preemptive PGx.

HLA-Related Hypersensitivity Drug Reactions

Many PGx biomarkers have been developed to prevent the occurrence of ADRs, and the human leukocyte antigen B (HLA-B) genes have found an essential place in this domain. Applications are currently limited, but they are related to life-threatening effects.

ADRs have historically been divided into type A (intrinsic) reactions and type B (idiosyncratic) reactions.[16] Type A ADRs are predictable, as they are dose dependent and related to the pharmacological action of the drugs. Conventional TDM is of great value for this type of ADRs. This is usually not the case for type B reactions, which are unpredictable and typically not explained by dose or expected pharmacologic response. Type B ADRs are often immunoallergic reactions. Several of them have been related to the presence of specific HLA alleles over the past decade. In HLA-mediated ADRs, the drug or a reactive metabolite of the drug either covalently binds to and modifies an endogenous peptide (hapten model) or interacts directly with the HLA molecule leading to type IV hypersensitivity reactions (Fig. 1).[17]

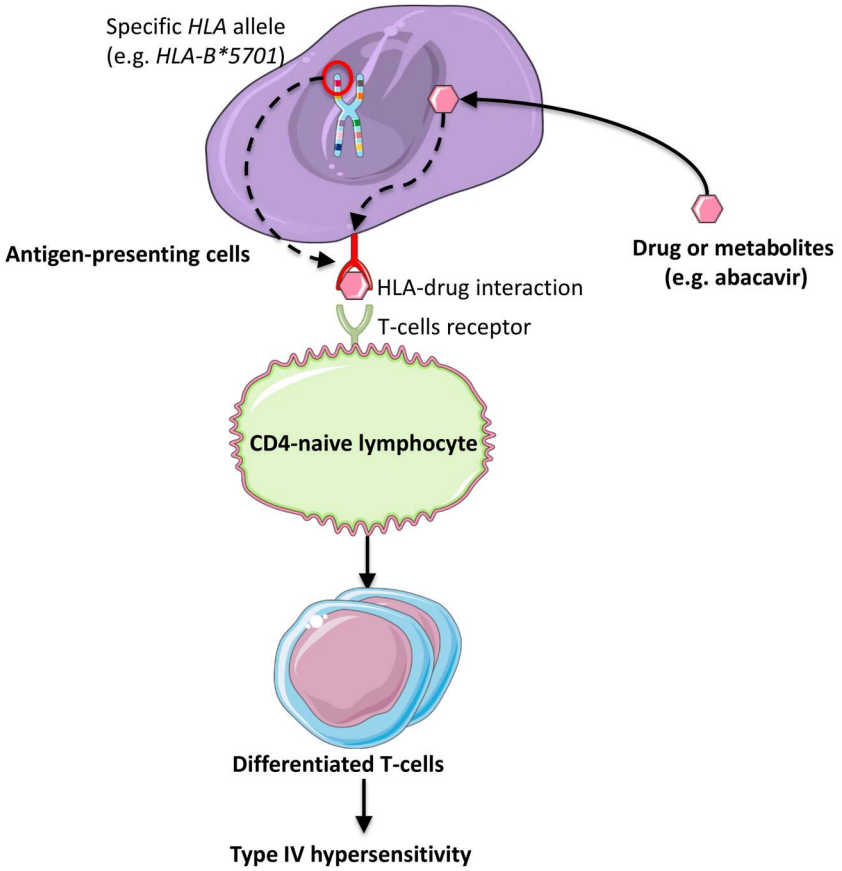

FIGURE 1. HLA-related hypersensitivity drug reactions: example of abacavir and *HLA-B*5701*.

Abacavir and HLA-B*57:01

Abacavir is a nucleoside reverse transcriptase inhibitor used in drug combinations for the management of type 1 HIV. The use of abacavir is associated with a frequent (4.2% based on drug label) and potentially life-threatening type IV hypersensitivity reaction that requires the discontinuation of the treatment. Several studies have found an association between the occurrence of hypersensitivity to abacavir and the presence of the *HLA-B*57:01* allele.[18] In a double-blind, prospective, randomized study, the determination of *HLA-B*57:01* before the prescription of abacavir was found of definite clinical interest.[19] The study involved 1956 HIV type 1 patients from 19 countries. The patients had not previously received abacavir. A lower incidence of clinically diagnosed hypersensitivity was found in the prospective-screening group (3.4%) as compared to the control group (7.8%; $P < 0.001$), and the screening totally eliminated immunologically confirmed hypersensitivity reactions (0% in the prospective-screening group versus 2.7% in the control group, $P < 0.001$). Given the value of the determination of the *HLA-B*57:01* allele before abacavir treatment, PGx testing is now required before treatment initiation. The prevalence of hypersensitivity is, however, subject to interethnic variations that might impact the cost–benefit balance in areas of low allelic prevalence.[20]

Carbamazepine and HLA-B*15:02

Carbamazepine is commonly used in the prevention of seizures and in the treatment of bipolar disorder or neuropathic pain. Among the ADRs reported for this drug, skin manifestations are frequent and can lead to serious complications such as the Stevens–Johnson syndrome or the toxic epidermal necrolysis. It is estimated that the incidence of Stevens–Johnson syndrome and toxic epidermal necrolysis is 1–4 cases per 10,000 exposed persons.[21]

It has been shown that particular HLA alleles may be considered as predisposing factors to these cutaneous reactions to carbamazepine. In populations of Asian origin, the *HLA-B*15:02* allele was associated with significantly increased risk.[22–24] The prevalence of this allele is highly variable depending on ethnicity. *HLA-B*15:02* is very rare in individuals who are not of Asian origin (eg, Whites, African Americans, Hispanics, and Native Americans).[25] It does not seem to be a marker for carbamazepine-induced hypersensitivity in these populations.[24,26,27] By contrast, the prevalence of *HLA-B*15:02* among residents of Southeast Asian countries was reported to be as high as 8%.[25] Since 2008, the US FDA recommends the screening of patients with ancestry in genetically at-risk populations (patients of Asian descent) before carbamazepine treatment.[28]

A systematic review showed that other HLA alleles (*HLA-A*24:02*, *HLA-A*31:01*, *HLA-B*15:11*, and *HLA-B*40:01*) influence the susceptibility to carbamazepine hypersensitivity.[29] *HLA-A*31:01* appears as a risk factor in both Asians and Whites patients.[29] However, systematic screening of this allele is not yet recommended.

These 2 examples illustrate the potential of HLA PGx for the prevention of type B ADRs and its added value to conventional TDM with regards to dose adjustments based on drug levels.

VKORC1 as a Molecular Target of Anti–vitamin K Drugs

Another well-described example of clinical validation including PD-associated gene in PGx dosing guideline involves the anticoagulation therapy and, in particular, the prescription of warfarin as a vitamin K antagonist.

In routine clinical practice, warfarin is usually dosed empirically with an initial dose (the prescription is based on data from the drug information label) typically followed by regular measurements of the international normalized ratio (INR) and subsequent dose adjustments to reach the target INR defined according to the pathological conditions. So, the initial dose of warfarin is mainly based on population averages (eg, 4–5 mg/d for a target INR of 2–3), with additional prescription of loading doses during the first few days of anticoagulation in some specific settings. Given the very large interpatient variability, stable doses to achieve an INR of 2–3 typically range from 1 to 20 mg/d, irrespective of the method used to initiate warfarin therapy (ie, with or without using loading dose). Therefore, the iterative process to define a personalized dose can take weeks to months and, during this sensitive period, patients have an increased risk of overanticoagulation or underanticoagulation, which can result in bleeding or thromboembolism.[30]

Warfarin Pharmacogenetics

It has been clearly established that polymorphisms in genes coding for metabolizing enzymes, and particularly *CYP2C9*, influence the warfarin dose requirement and are to be considered in preemptive genotyping strategies to define a personalized initial warfarin dose.[30] Beside warfarin (half-live, $t_{1/2}$: 32–43 hours), other anti–vitamin K drugs such as acenocoumarol ($t_{1/2}$: 2–8 hours) and phenprocoumon ($t_{1/2}$: 156–178 hours) are also metabolized by CYP2C9.[31] The most frequent *CYP2C9* coding SNPs characterized by a decreased enzyme activity in European ancestry population are *CYP2C9*2* (c.430C>T, p.Arg144Cys, and rs1799853) and *CYP2C9*3* (c.1075A>C, p.Ile359Leu, and rs1057910). In vitro and in vivo studies suggest that *CYP2C9*2* and **3* impair metabolism of *S*-warfarin (the more active stereoisomer in the racemic mixture administered as a drug) by around 30%–40% and 80%–90%, respectively.[32] It is also to be noted that additional *CYP2C9* alleles characterized by decreased enzyme activity (*CYP2C9*5*, **6*, **8*, and **11*) have to be determined in African ancestry population where they are more frequent than *CYP2C9*2* and *3 and contribute more to warfarin dose variability in that population.[33]

Interestingly, and in line with this short review, not only the plasma drug concentration (influenced by *CYP2C9* genetic polymorphisms as briefly described above) is of importance but also the level of expression of the molecular target of warfarin and other anti–vitamin K drugs. Indeed, one can easily understand that the lower the expression level of the target, the lower the dose requirement will be to inhibit it. Also, this molecular target of anti–vitamin K drug is the vitamin K epoxide reductase protein (Fig. 2) encoded by

VKORC1 gene and catalyzing the conversion of oxidized vitamin K (inactive form as a cofactor for vitamin K–dependent carboxylase involved itself in the conversion from inactive to functional clotting factors) to reduced vitamin K, which is the rate-limiting step in vitamin K recycling.[34] In this respect, a common variant upstream of *VKORC1* (c.-1639G>A, rs9923231) has been significantly associated with a low relative expression of *VKORC1* mRNA (and VKORC1 protein) and subsequent higher sensitivity to warfarin (including increased bleeding risk) at a standard dosage in most ethnic groups.[35] The impact of this SNP on the expression of the molecular target of anti–vitamin K and its very large interethnic variation in terms of minor allelic frequency is such that it largely explains by itself the difference in average dose requirements between European, African, and Asian ancestry populations.[36,37] For this reason, this SNP is included (in association with *CYP2C9* SNPs) in validated algorithms allowing to predict a personalized dose of warfarin.[30,38] It must be stressed that clinical laboratories typically report *VKORC1* genotype results using either upstream c.-1639G>A genotype or another *VKORC1* SNP (1173C>T, rs9934438), which is in complete linkage disequilibrium with the previous upstream SNP.

Based on the vitamin K cycle presented in Figure 2, one can easily understand that any factor reducing the level of active (reduced) vitamin K by a mechanism independent of VKORC1 activity will potentiate the activity of warfarin and other related anti–vitamin K drugs. The apparently increased drug activity should therefore be associated with lower warfarin requirement to reach a target INR. CYP4F2 is a primary liver vitamin K oxidase catalyzing the metabolism of reduced vitamin K to hydroxy-vitamin K1 and removing vitamin K from the vitamin K cycle.[39] Its activity counteracts that of VKORC1 and results in limiting excessive accumulation of vitamin K. A coding nonsynonymous SNP in *CYP4F2* gene (*CYP4F2*3*, c.1297G>A, p.Val433Met, and rs2108622) has been associated with a reduced activity of the protein and higher warfarin dose requirements.[40,41] Furthermore, it has been demonstrated that including *CYP4F2*3* genotype in warfarin dosing models (including *CYP2C9* and *VKORC1* genotypes and clinical factors such as age and weight) allowed to further increase the explained variability (up to 60%) in drug dose requirements.[42] However, this gain in term of predictability was only validated thereafter in populations with European and Asian ancestry but not confirmed in African ancestry populations.[43,44] Based on guidelines recently updated by the CPIC group, although it is strongly recommended to take into consideration both *CYP2C9* and *VKORC1* genotypes to predict warfarin dose using validated and published PGx algorithms in non-African ancestry populations, the inclusion of *CYP4F2*3* is still considered as an option.[30] Although less studied than warfarin, PGx algorithms including *CYP2C9*, *VKORC1*, and *CYP4F2* polymorphisms (and clinical factors) also demonstrated superiority compared with clinical algorithms for acenocoumarol[45–48] and phenprocoumon[49] with some contradictory results.[50]

Therefore, the example of warfarin, including *VKORC1* and *CYP4F2*3* in addition to metabolizing enzyme genotype, illustrates the potential added value of integrating PD-associated genes in drug dosing algorithms for routine PGx implementation. Furthermore, given the increasing prescription of new direct oral anticoagulants (DOACs) and the lack of absolute evidence of their therapeutic superiority over anti–vitamin K drugs,[51–54] it would have been interesting to be able to conduct randomized controlled studies comparing the clinical response of DOACs with anti–vitamin K drugs using

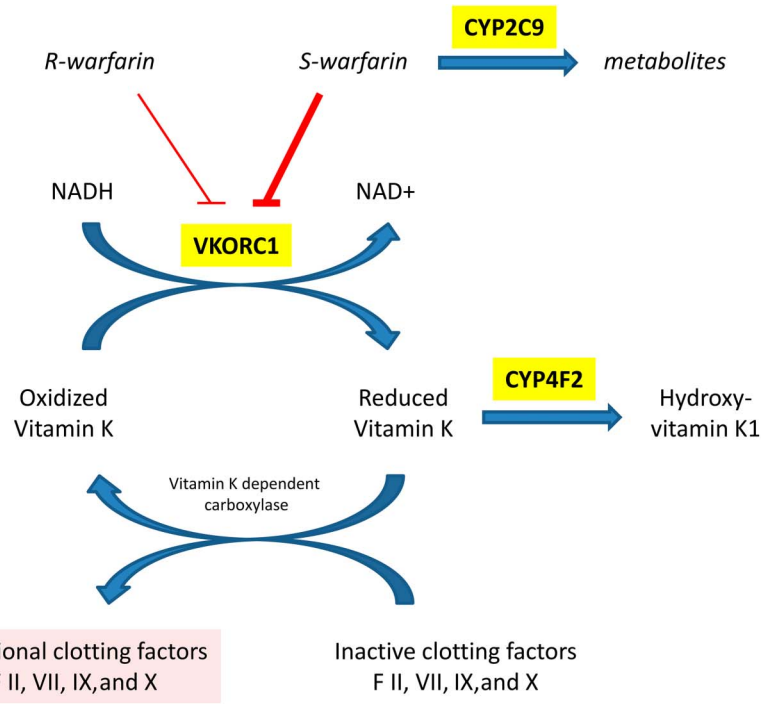

FIGURE 2. Warfarin metabolism and mechanism of action in relation to the vitamin K cycle.

PGx-based dosing algorithms. Such strategy, potentially profitable from an economic point of view, could allow for better use of already existing drugs.

PGx Biomarkers Predictive of Immunosuppression in Solid Organ Transplantation

A long history of work on metabolizing enzymes and membrane transporters involved in the PK of IS drugs has shown that PGx can affect dose–concentration relationships in solid organ transplantation (SOT) and be used as a complement to conventional TDM.[5] However, investigations of genetic variations related to proteins of the PD pathways targeted by these drugs and of their impact on interindividual variability in drug response are only starting to emerge in the literature. Therefore, unlike the cases of *G6PD*, *HLA*, and *VKORC1* described in previous paragraphs, the following candidate PGx biomarkers (categorized according to the 3 main pharmacological classes of IS) are still under study and not yet included in any validated dosing guidelines (CPIC or other). One reason for that is the complexity of IS PD pathways. Contrary to the example of warfarin, numerous genes have to be considered, and it is likely that compensatory mechanisms may occur.

Calcineurin Inhibitors

Cyclosporin A (CsA) and tacrolimus (TAC) are inhibitors of the calcineurin pathway, which is normally upregulated after T-cell activation. The drugs require intracellular immunophilins to inhibit calcineurin [cyclophillin A and FKBP12 (FK506-binding protein 12) for CsA and TAC, respectively]. Physiologically, immunophillins play a role in the regulation of immune and basic cellular processes. In complexes with calcineurin inhibitor (CNI), they block the association of the calcineurin A catalytic subunit (CnA), calcineurin B regulatory subunit (CnB), calmodulin, and calcium, preventing the dephosphorylation of the nuclear factor of activated T cells (NFAT) and thus its migration into the nucleus. The transcription of interleukin 2 (IL2) and CD25, the IL2 receptor α chain, is inhibited, which decreases T-cell activation and results in immunosuppression.[55] Although it is now well established that *CYP3A5*3*, and to a lesser extent *CYP3A4*22*, can be determined to individualize TAC first doses,[5] few studies have investigated the variability of CNI target genes in relation to clinical outcomes in SOT.[56]

Immunophilins Pharmacogenetics

No PGx study was reported on FKBP12 (*FKBP1A* gene). Genetic research on this gene has failed to identify frequent variants in its coding or promoter region.[57–59] Therefore, *FKBP1A* could not be considered yet as a potential candidate in SOT PGx.

There are more data available regarding cyclophilin A (*PPIA* gene) and most notably a promoter SNP (rs8177826, C>G) (Table 1).[60–62] Although in vitro assays suggest that rs8177826 has a functional consequence with higher promoter activity for rs8177826-G variant allele,[60–62] lower *PPIA* mRNA levels were found in peripheral blood mononuclear cells among rs8177826-G carriers.[63]

The SNP was investigated in 290 renal transplant recipients. No association was found for acute renal rejection or delayed graft function. However, the risk of nephrotoxicity was related to the rs8177826 variant. The authors supported their findings with experimental data, suggesting a nephrotoxic effect due to increased cyclophilin expression, which contradicts previous findings.[61] The candidate gene study conducted by Pouché et al in 221 renal transplant patients treated with CsA did not confirm this finding, and no significant relationships between rs8177826 and biopsy-proven acute rejection (BPAR) or infections were found either.[64] Moore et al also explored this SNP and other *PPIA* variants in a renal transplant population (n = 670). No association was found for death-censored allograft survival (Kaplan–Meier analyses, $P > 0.05$).[65]

Calcineurin and Calmodulin Pharmacogenetics

The genetic variability of calcineurin isoforms (encoded by *PPP3CA*, *PPP3CB*, and *PPP3R1*) and calmodulin (encoded by *CALM1*, *2*, and *3*) has not been studied in depth in relation to PGx. Pouché et al[64] investigated 7 variants related to the calcineurin–calmodulin complex in 381 renal transplant recipients receiving CsA (n = 221) or TAC (n = 160), and mycophenolate mofetil (MMF). Three of the 7 candidates had been identified through a very comprehensive literature review[65]: *PPP3CA*-rs45441997, *PPP3R1*-rs1868402, and *CALM1*-rs12885713. All 3 had been tested in at least one well-designed clinical study, and their functional effects were supported by a clear mechanistic rationale. Among these candidates, none showed associations with BPAR and serious infections.

NFAT Pharmacogenetics

Three genes are encoding NFAT in lymphocytes: *NFATC1*, *NFATC2*, and *NFATC3*. No study has been able to associate genetic variation in these genes with the effects of CNI drugs. Coexpression of the 3 isoforms may balance the lack of individual members.[66] It is thus likely that genetic variations impacting expression levels or affinities would ultimately be compensated. NFAT is also involved in insulin secretion, and research reveals that variants in *NFATC4* (not expressed in lymphocytes but present in pancreatic β cells) may modulate the risk of new-onset diabetes after transplantation (Table 1).[67]

IL2 Pharmacogenetics

The most widely tested SNPs in IL2 are a promoter SNP (rs2069762, frequently reported as −330T>G) and a synonymous SNP in the first exon (rs2069763). Both SNPs were found to be associated with a number of outcomes as recently reviewed by Stojanova et al.[68] A meta-analysis of 8 case–control studies (1405 renal transplant recipients) on rs2069762 concluded that the variant is not associated with the risk of acute rejection.[69] The rs2069763 SNP seems to be more promising, as it was associated with increased risk of acute rejection in kidney transplant recipients (Table 1).[70] This deserves further investigation in SOT.

TABLE 1. Most Convincing PGx Associations Reported in SOT Regarding Variants in Genes Related to the PD Pathways of IS Drugs

Gene	Variants	Drugs	Population	Clinically Significant Effect Reported	Reference
PPIA	rs8177826	Cyclosporine	KTR (n = 290)	Increased risk of nephrotoxicity: OR = 3.49; CI 95% (1.47–8.24), $P = 0.006$	61
NFATC4	rs10141896	CNI	Hispanic KTR (n = 319)	Lower incidence of NODAT in the first posttransplant year: variant frequency in control versus NODAT patients: 5.7% versus 1.9%	67
IL2	rs2069763	CNI	Turkish KTR (n = 90) receiving grafts from living related donors	Increased risk of acute rejection: OR = 6.3; CI 95% (1.8–22.15), $P = 0.005$	70
mTOR	Haplotype comprising: rs1770345, rs2300095, rs2076655, rs1883965, and rs12732063	Sirolimus	KTR (n = 179) switched from a CNI to sirolimus (discovery group: n = 113; and validation group: n = 66)	Decreased hemoglobin level in variant carriers: discovery group: $\beta = -0.82$ g/dL, $P = 0.0076$; validation group: $\beta = -1.58$ g/dL, $P = 0.0308$	71
IMPDH2	rs11706052	MPA	de novo KTR (n = 237)	Increased risk of BPAR: OR = 3.39, CI 95% (1.42–8.09), $P = 0.006$	72
			KTR (n = 80)	Increased IMPDH area under the time–effect curve (AUC) over 12 h in variant allele carriers versus noncarriers: AUC = 336, CI 95% (216–521) versus 227, CI 95% (198–260) μmol XMP/s/mol AMP, $P = 0.04$	73
			Healthy volunteers	Reduced antiproliferative effect of MPA on lymphocytes (approx. −50%) in n = 8 heterozygous carriers versus 12 noncarriers	74
IMPDH1	rs2278293	MPA	KTR (n = 191)	Decreased risk of BPAR: OR = 0.34, CI 95% (0.15–0.76), $P = 0.008$	78
	rs2278294	MPA	KTR (n = 191)	Decreased risk of BPAR: OR = 0.40, 95% CI (0.18–0.89), $P = 0.02$	78
			KTR (n = 456)	Decreased risk of BPAR: OR = 0.54, CI 95% (0.34–0.85), $P = 0.0075$	77
			Pediatric cardiac renal transplants (n = 59)	Association with MMF gastrointestinal intolerance (rs2228075 with rs2278294)	76 and 83

AMP, adenosine monophosphate; CI, confidence interval; KTR, kidney transplant recipients; NODAT, new-onset diabetes after transplantation; OR, odds ratio; XMP, xanthosine-monophosphate.

mTOR Inhibitors (mTORi)

mTORi (sirolimus and everolimus) are inhibitors of the mechanistic (formerly named "mammalian") target of rapamycin (mTOR), a phosphatidylinositol kinase also known as the FRAP (FKBP–rapamycin-associated protein). These drugs bind FKBP12, and the complex inhibits the kinase activity of mTOR. mTOR regulates protein synthesis through the phosphorylation and inactivation of the repressor of mRNA translation eukaryotic initiation factor 4E binding protein (4EBP1), and through the phosphorylation and activation of the phosphatidylinositol 3-kinase-p70 ribosomal S6 Kinase (p70S6K, encoded by *RPS6KB1 gene*). The mTOR protein (encoded by *MTOR* gene) is included in 2 complexes, mTORC1 and mTORC2, of which only the former is sensitive to mTORi. In mTORC1, mTOR forms a stoichiometric complex with RAPTOR (the regulatory-associated protein of mTOR, encoded by *RPTOR* gene) and is associated with a G protein beta-subunit–like protein, the target of rapamycin complex subunit LST8 (mLST8). In the setting of transplantation, mTORi decrease the cellular response to IL2, reducing lymphocytes proliferation.

Variants in *MTOR*, *RPS6KB1*, and *RPTOR* have been tested in a renal transplant cohort of individuals switched from CNI to sirolimus at least 3 months posttransplantation (n = 113 discovery group; n = 66 validation group).[71] A tag-SNP approach was used for *MTOR* and *RPS6KB1*, whereas 4 SNPs in *RPTOR* were selected based on the literature (rs2289759, rs7212142, rs7211818, and rs11653499). A

significant association was found between a variant haplotype of *MTOR* and decreased hemoglobin levels in both discovery and validation populations (Table 1). The effect size was minor, and the association was observed before mTOR treatment initiation, suggesting a physiological rather than a PGx mechanism. No variants were found associated with other sirolimus adverse effects including dyslipidemia, edemas, and cutaneous reactions.[71]

Mycophenolic Acid

Mycophenolic acid (MPA) effect is essentially attributed to the inhibition of type II inosine monophosphate dehydrogenase (IMPDH), a rate-limiting enzyme involved in the de novo purine synthesis pathway. This enzyme has 2 isoforms encoded by *IMPDH1* and *IMPDH2*. MPA is a more potent inhibitor of IMPDH2, which is expressed in activated lymphocytes as compared to IMPDH1, which is expressed in most cell types. Both enzymes have been the subject of PGx research in addition to SNPs of genes coding for MPA-specific metabolizing enzymes such as *UGT1A9*.[5]

IMPDH2 Pharmacogenetics

A SNP located in *IMPDH2* intron 7 (rs11706052, usually referred to as 3757T>C) has been extensively studied with as many positive as negative associations reported. Grinyò et al[72] were the first to report a significant association between this SNP and the risk of BPAR (Table 1). Two subsequent clinical studies suggest that this SNP may be associated with a poor clinical response to MPA (Table 1). The first study was conducted in 80 renal transplant patients treated with MMF (the MPA prodrug). The authors found that IMPDH activity was increased in carriers of the variant allele (C) as compared to noncarriers.[73] The second study was conducted in healthy volunteers, and rs11706052 SNP was found to reduce the antiproliferative effect of MPA on lymphocytes by approximately 50%.[74] A study sought to replicate the association between this SNP and the risk of acute kidney rejection in a large cohort of 969 kidney transplant recipients, but the authors only found a modest association, which was not significant with adjustment for multiple testing.[75] In pediatric heart transplant recipients, the rs11706052 variant surprisingly associated with more frequent neutropenia requiring dose withholding ($P = 0.046$).[76] This latter study contradicts the above-mentioned studies, suggesting poorer response to MPA. Furthermore, it must be stressed that the association with the risk of acute rejection was not confirmed in at least 3 studies in relatively large groups of renal transplant recipients (191, 456, and 1040 patients).[77–79]

Among other *IMPDH2* candidates, at least 3 SNPs were predicted to affect IMPDH2 activity using in vitro or in silico experiments. *IMPDH2* rs121434586 (c.787C>T), located in exon 7[80] and 2 promoter SNPs: c.-95C>T[81] and rs4974081.[82] Both rs121434586 (c.787C>T) and c.-95C>T SNPs have extremely low minor allelic frequency (<1%). Any contribution to the interpatient variability in MPA effects observed clinically is thus very unlikely. However, they might be the cause of unexplained effects in a few individuals. On the other hand, rs4974081 is frequently found in Whites, but no association between this SNP and clinical outcomes of MPA (BPAR occurrence, leucopenia, and infections) was found in 456 renal transplant recipients.[77]

IMPDH1 Pharmacogenetics

Two SNPs within *IMPDH1* intron 7 (rs2278293 and rs2278294) were found to be associated with a decreased risk of BPAR following the 1st year after renal transplantation (Table 1).[78] This finding was replicated for rs2278294 (Table 1).[77] Carriers of the rs2278294 variant also had a 1.6-fold increased risk of leucopenia, reinforcing the direction of the functional effect. In addition, rs2278294 was related to MMF gastrointestinal intolerance, defined using relatively robust criteria, in pediatric heart transplant recipients (Table 1).[76,83]

However, at least 3 other studies, including 2 studies in large cohorts of renal transplant recipients (n = 1000 approx.) found no association of rs2278293 or rs2278294 with rejection,[75,79,84] or graft or patient survival at 5 years.[79]

In conclusion, comprehensive data regarding the genetic variability of proteins involved in the PD of IS drugs are quite limited and still controversial. Further well-designed clinical studies are therefore needed to demonstrate potential clinical utility in SOT for some of them, if any.

CONCLUSION

PGx is gradually taking its place in hospital laboratories, but its use for treatment personalization is still restricted to a few clinical areas. Clinical implementation has required important research efforts and long periods of time to obtain evidence to justify clinical use. The research has been predominantly focused on the genetic variability of drug disposition pathways (eg, metabolizing enzymes and membrane transporters) mainly for 2 major reasons. First, it is easier to focus on the genetic factors that can affect dose–concentration relationships as compared to dose–effect relationships: blood concentration can be easily measured in most cases and represents a valuable surrogate. Second, pharmacogenes such as drug-metabolizing enzymes or membrane drug transporters bear functional and relatively frequent genetic variations, which have been characterized in depth. This is not always the case for drug targets that are frequently part of large signaling pathways involving compensatory mechanisms. Already available for some years, next-generation sequencing could be particularly interesting for studying these pathways. Today, its cost still remains too high to be used on studies on large populations in most laboratories, but it will undoubtedly decrease and render this approach more accessible. Next-generation sequencing might therefore be a formidable opportunity for future research in PGx and particularly in this less explored field of PD. As in the case of warfarin for which both PK- and PD-associated PGx biomarkers are already used to define a personalized dose before the start of treatment, it can be expected that such genotype-based dosing guidelines combining PK- and PD-associated PGx biomarkers will become widespread for many other drugs in the future.

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