Pharmacogenetics and pharmacogenomics of immunosuppressive agents: perspective for individualized therapy

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Immunosuppressive therapy has markedly improved over the past years with the advent of highly potent and rationally targeted immunosuppressive agents. Since these drugs are characterized by a narrow therapeutic index, major efforts have been carried out to define therapeutic windows based on the blood levels of each immunosuppressant, and relating those concentrations to clinical events. Although pharmacokinetic-based approaches are currently used as useful tools to guide drug dosing, they present several limitations. Pharmacogenomics – a science that studies the inherited basis of differences between individual responses to drugs in order to identify the best dose and therapy for each patient – might represent a complementary support. Preliminary studies that have focused on polymorphisms of genes encoding enzymes involved in drug metabolism, drug distribution, and pharmacological target, have shown promising results. Indeed, pharmacogenomics holds promise for improvement in the ability to individualize pharmacological therapy based on the patient's genetic profile.

Organ transplant recipients respond in different ways to immunosuppressive agents, so that drug-related toxicity or graft rejection may occur, even with conventional dose regimens. Individual differences in response to pharmacotherapy can be related to several factors, including age, sex, concomitant diseases, and drug–drug interactions, as well as inheritance [1,2]. As an additional confounding factor, most of the immunosuppressants currently used in clinical practice are characterized by a narrow therapeutic index and considerable variability in their absorption and metabolism, eventually resulting in a marked difference between patients' exposure to the drugs [3].

In the past, it was assumed that measurement of drug concentrations in biological fluids could help to limit such differences in drug response. Since then, a large number of studies have shown that therapeutic drug monitoring with pharmacokinetic approaches is a useful tool to guide drug dosing and, eventually, predict clinical outcome [4,5]. Although easily applicable in routine clinical practice, this approach still has some pitfalls. Indeed, some studies failed to document a significant correlation between pharmacokinetic parameters, daily drug exposure, and clinical response to a given treatment [6]. These limitations provide the rationale for searching complementary strategies to manage immunosuppressive agents beyond pharmacokinetics.

Among the different factors claimed to influence drug disposition, genetic variations

seem to play a pivotal role. As a matter of fact, functionally significant variations in DNA sequences of genes encoding proteins can result in disturbances in drug absorption, distribution, metabolism, or pharmacological action. These mutations may explain the individual variations in response to pharmacotherapy. Accordingly, the identification of an individual's genetic make-up may be useful to choose the best treatment for each patient. Pharmacogenetic studies, which focus on how single genes modulate the effect of a drug, have been implemented to solve this task, and these have now been supplemented by pharmacogenomics, a new genetic science focusing on how the genome as a whole can affect the action of a drug, referring to the contribution of individual genes as well as to gene–gene interactions [2]. As compared to pharmacokinetic studies, pharmacogenomic studies can be conducted even before the beginning of treatment, they do not require the assumption of steady-state conditions, and can be performed less invasively using saliva, hair root, or samples of buccal swab. In addition, the results are constant for an individual's lifetime and can provide predictive value for multiple drugs [7]. This promising approach could be used to predict which individual will benefit most from a given drug. This applies to all drugs but is of particular importance for those characterized by a narrow therapeutic index, such as the immunosuppressive agents that

represent the ideal candidates for pharmacogenomic approaches. Thus, this review will focus on the genetics of the drug-metabolizing system that affects the bioavailability of the main immunosuppressive drugs.

Azathioprine

Azathioprine (AZA) is a mercaptopurine analog of adenine and hypoxanthine used for the treatment of childhood leukemia and autoimmune diseases, as well as an adjunctive immunosuppressive agent after organ transplantation [8]. AZA is a prodrug that is converted in the liver to 6-mercaptopurine (6-MP), which is catabolized by three enzymes: xanthine oxidase (XO; EC 1.1.3.22) to the inactive 6-thiouric acid; thiopurine *S*-methyltransferase (TPMT; EC 2.1.1.67) to the inactive methylmercaptopurine; and hypoxanthine phosphoribosyltransferase (HPRT; EC 2.4.2.8) to active 6-thioguanine nucleotides (TGNs). TPMT can also catalyze the methylation of cytotoxic TGNs, which are potent inhibitors of *de novo* purine synthesis. This inhibition, together with the incorporation of fraudulent metabolites into RNA and DNA, has a primary role in the mechanism of action of AZA [8]. Thus, TPMT is a principal enzyme in the neutralization of cytotoxic TGNs in hematopoietic tissues. Low activity of these enzymes leads to the accumulation of TGN metabolites, which induce many serious side effects, such as hematopoietic cell toxicity (neutropenia, anemia and thrombocytopenia), hepatotoxicity, pancreatitis, and gastrointestinal disturbances [9,10]. To this, it can be reasonably speculated that adverse events observed after AZA treatment mainly depend on the expression and activity of TPMT.

Early observations have documented that the population frequency distribution of TPMT activity displays a trimodal pattern: low/low, low/high, and high/high [11]. After cloning of the *TPMT* gene (a 27-kb gene on chromosome 6p22.3 composed by 10 exons) several alterations in the amino acid sequence, as a result of SNPs, have been discovered. The wild-type allele, associated with high TPMT activity, has been designated as *TPMT**1. Subsequently, approximately 11 variant alleles have been identified and associated with low TPMT activity [11]. Three alleles, *TPMT**2, *TPMT**3A and *TPMT**3C, account for 80–95% of intermediate or low enzyme activity whereas *TPMT**3B, *3D, *5, *6, *7 and *8 are less frequent. Patients with *TPMT**3A have complete loss of TPMT

catalytic activity; those with *TPMT**3B present a 9-fold reduction; and patients with *TPMT**3C have a 1.4-fold decrease in catalytic activity [11]. Nishida *et al.* [12] measured TPMT enzymatic activity in the red blood cells of 44 healthy unrelated Japanese subjects. In this study, the TPMT activity of the subject with *TPMT**1/*3C was 40% lower than the mean value of individuals with *TPMT**1/*1. The mutant allele *TPMT**2 contains a 238G>C transversion in the open reading frame, leading to an amino acid substitution at codon 80 (Ala>Pro). It is worthy to note that the frequency and pattern of mutant *TPMT* alleles show differences among various ethnic populations. For example, the *TPMT**3A polymorphism occurs most frequently in the French Caucasian population, the *TPMT**3C variant is characteristic of the Ghanaian population, and *TPMT**2 is prevalent with the same frequency in both British and French Caucasians [11]. At variance, Asians have a lower frequency of mutant *TPMT* alleles and all mutant alleles identified, to date, are *TPMT**3A [11].

Approximately 90% of individuals inherit high enzyme activity (wild type), 10% have intermediate activity (those who are heterozygous for mutation), and 0.3–1% have low or no detectable enzyme activity because of two non-functional *TPMT* alleles (individuals homozygous for the mutation) [13]. A clinically important consequence of this finding is that patients with inherited TPMT deficiency accumulate a higher concentration of cytotoxic TGNs and are at a greater risk of myelosuppression, despite using standard doses of AZA, as compared with wild-type subjects. Conversely, patients with high TPMT activity might be at risk of poor clinical response [8]. As a consequence, patients who are homozygous for the *TPMT* defective alleles should be treated with 6–10% of the standard dose of AZA while heterozygous patients should start with 65% of the standard doses and will require frequent monitoring. At variance, patients with high TPMT activity should receive doses at the upper limit of those generally recommended [8,11].

As a consequence of decreased TPMT activity due to inherited differences, altered response to AZA has been observed in organ transplant recipients (Table 1). Indeed, *TPMT* mutations have been associated with a greater likelihood of myelosuppression in heart and kidney transplant patient who receive standard doses of AZA [14,15]. Recently, an AZA-induced fatal

Table 1. Pharmacogenetic studies of azathioprine in organ transplant recipients.

	Patients	Results	Ref.
Thiopurine S-methyltransferase	39 heart and kidney Tx	Patients with mutant <i>TPMT</i> alleles are at a high risk of accumulating cytotoxic nucleotides	[14]
	30 heart Tx	Patients with mutant <i>TPMT</i> alleles developed agranulocytosis and neutropenia	[15]
	1 adult kidney Tx	<i>TPMT</i> mutation was associated with fatal immunosuppression	[16]
	22 pediatric kidney Tx	High <i>TPMT</i> activity was shown to be associated with an increased risk of rejection	[17]
	68 adult kidney Tx	High <i>TPMT</i> activity was associated with worse clinical outcome	[18]
Hypoxanthine phosphoribosyltransferase	27 heart Tx	Increased frequency of <i>HPRT</i> mutants correlated with rejection	[21]

HPRT: Hypoxanthine phosphoribosyltransferase; *TPMT*: Thiopurine S-methyltransferase; *Tx*: Transplant recipient.

myelosuppression in a renal transplant recipient with a *TPMT**1/*3C genotype has been reported [16]. Despite AZA discontinuation at day 16 post-transplantation, the patient developed pneumonia (from *Xanthomonas maltophilia* and *Pseudomonas aeruginosa*) and peritonitis (from *Xanthomonas maltophilia* and *Candida albicans*). He died at day 24 from cardiopulmonary arrest caused by severe hypoxia secondary from pulmonary hemorrhage, underlying the importance to screen patients for *TPMT* mutations. On the other hand, those patients with high *TPMT* activity could no longer benefit from AZA therapy. As a matter of fact, studies in pediatric [17] and adult [18] renal transplant recipients have shown that high *TPMT* activity was associated with a poor clinical outcome possibly related to a high rate of AZA catabolism.

The conversion from AZA to 6-MP is mediated by *HPRT*, an enzyme required by mammalian cells to synthesize DNA via the salvage pathway [8]. During extensive T-cell proliferation, as during the active immune response to a graft, the *HPRT* gene (located on the X chromosome at position Xq26) undergoes spontaneous mutations at multiple regions of the sequence [19,20]. In these conditions, the administration of AZA could lead to the death of T cells carrying wild-type *HPRT*, allowing for the selective enrichment and survival of T cells carrying *HPRT* mutants. These mutations can alter the cellular phenotype by rendering mutants resistant to the cytotoxicity of AZA. Indeed, it has been shown that the frequency of T cells with *HPRT* mutations in the peripheral blood is positively correlated with the incidence of rejection in heart transplant recipients [21].

Mutations in *TPMT* and *HPRT* may significantly affect the clinical outcome of patients

treated with AZA (Table 1), thus monitoring for these mutations should allow more effective use of this immunosuppressant.

Mycophenolic acid-releasing formulations

Mycophenolic acid (MPA), the active derivative of the prodrugs mycophenolate mofetil (MMF) and an enteric-coated formulation of mycophenolate sodium (EC-MPS), is routinely used as an immunosuppressant in solid organ transplantation [22]. This drug is usually given at a fixed daily dose and, up to now, no pharmacogenetic or pharmacogenomic studies on MPA have been carried out. However, indirect evidence is available that inheritance difference might affect MPA exposure.

A recent retrospective analysis of MPA levels has identified different patterns of drug exposure [23], suggesting the involvement of mutations in the enzymes responsible for MPA metabolism in the gastrointestinal tract. This drug is primarily metabolized to phenolic and acyl glucuronides by phase II reactions catalyzed by the uridine diphosphate glucuronosyltransferase (UDP-GT) enzyme family [24]. Several genetic polymorphisms have been found for this family of enzymes, which are associated with altered drug bioavailability and drug-related toxicity [25,26]. The reported allele frequency of these mutations ranged from < 1 to 55%, suggesting that UDP-GT activity in the tissues can greatly differ between individuals. As a consequence of inter-individual polymorphic *UGT* regulation, differences in MPA metabolism, therapeutic efficacy and toxicity may be postulated.

In spite of the well-documented efficacy of MPA pharmacokinetic monitoring in predicting clinical outcome after organ transplantation [4,22], several patients experienced acute rejection

Table 2. Influence of *MDR1* genotypes on the pharmacokinetics of cyclosporine A.

SNP position	Results	Ref.
Exon 26 (<i>C3435T</i>)	No influence on cyclosporine A trough levels or rejection episodes	[33]
	22% increase of cyclosporine A AUC in the <i>C/T</i> and <i>T/T</i> genotype compared with <i>C/C</i> [†]	[34]
	Lower cyclosporine A AUC ₀₋₄ values in the <i>C/C</i> genotype compared with the <i>T/T</i> genotype [†]	[35]
	Higher oral cyclosporine A clearance in heterozygous <i>C/T</i> as compared with <i>C/C</i>	[36]
	No correlation with cyclosporine A dose-adjusted predose concentrations	[49]
Exon 12 (<i>T1236C</i>) Exon 21 (<i>G2677A/T</i>) Exon 26 (<i>C3435T</i>)	Higher cyclosporine A exposure in patients with the <i>T-T-T</i> haplotype compared to the <i>C-G-C</i> haplotype	[37,38]
Exon 21 (<i>G2677A/T</i>) Exon 26 (<i>C3435T</i>)	No influence of <i>MDR1</i> haplotypes on cyclosporine A pharmacokinetics	[39]
Exon 12 (<i>T1236C</i>) Exon 21 (<i>G2677A/T</i>) Exon 26 (<i>C3435T</i>)	No association between cyclosporine A blood levels and <i>MDR1</i> SNPs	[61]

[†]Not statistically significant.

AUC: Area under the concentration–time curve; MDR: Multi-drug resistance.

episodes despite MPA levels falling within the expected therapeutic ranges. As a potential explanation, it can be speculated that inherited differences in the pharmacological target may affect MPA efficacy. The immunosuppressive action of MPA on the proliferation of both T and B lymphocytes is exerted by selective inhibition of inosine monophosphate dehydrogenase (IMPDH; EC 1.1.1.205), a key enzyme in the *de novo* biosynthesis of guanine nucleotides [27]. IMPDH is expressed in two isoforms, types I and II, which share 85% identity at the amino acid level and show almost identical kinetic properties. IMPDH type I is expressed in all tissues, whereas the type II isoform is usually silent and can be induced by T-cell activation or after long-term treatment with MMF [28]. *In vitro* experiments [29], as well as genetic analysis in humans [30], have documented mutations in both IMPDH isoforms, suggesting a potential mechanism to explain the lack of efficacy observed in some patients given MPA. Nevertheless, prospective studies are required to assess whether the aforementioned mutations may be of relevance in the management of transplanted patients treated with MPA-releasing formulations.

Calcineurin inhibitors

Calcineurin is a calcium/calmodulin-dependent phosphatase whose activity is required for the induction of T-cell lymphokine production and proliferation [31]. Both cyclosporine A (CsA) and tacrolimus (TRL) are calcineurin inhibitors currently used to prevent allograft rejection after organ or tissue transplantation. Although CsA and TRL

are structurally different, both of them show common pathways for distribution, metabolism, and excretion, as well as for pharmacological targets.

Transport of these drugs through the intestine and the blood–brain barrier is restricted by P-glycoprotein (P-gp), a protein encoded by the multi-drug resistance-1 (*MDR1*) gene, which is located on 7q21.1 chromosome and consists of a core promoter region and 28 exons [32]. P-gp is a glycosylated 170-kDa membrane protein with six putative transmembrane segments and an intracellular binding site for ATP. This protein is expressed in the liver, kidneys, small and large intestine, brain, testis, muscle, placenta, and adrenals [32]. P-gp acts as an efflux pump to remove lipophilic molecules from the intracellular space, protecting cells from toxic substances. Its absence results in accumulation of several drugs in the brain and many other tissues, an effect of great relevance for narrow therapeutic index agents, such as calcineurin inhibitors. Recently, 29 SNPs have been reported for the *MDR1* gene [32]. Of these, variants in exons 12, 21 and 26 correlate with expression of the *MDR1* gene and function of P-gp [23] and are, therefore, the ideal candidates for pharmacogenomic studies.

Actually, only a few studies have investigated the impact of these polymorphisms on the bioavailability of calcineurin inhibitors, most of them showing contradictory results. A detailed description of the available literature on the potential influence of *MDR1* genotype on the pharmacokinetics of CsA is given in Table 2. Early pharmacogenetic studies failed to document an association between *MDR1* exon 26 SNP and

Table 3. Influence of *MDR1* genotypes on the pharmacokinetics of tacrolimus.

SNP position	Results	Ref.
Exon 21 (G2677A/T)	Positive predictor of tacrolimus exposure and drug neurotoxicity	[40]
Exon 26 (C3435T)	No correlation with tacrolimus dose-adjusted predose concentrations	[49]
Exon 26 (C3435T)	Limited reduction of tacrolimus levels in patients with the C/C genotype	[52]
Exon 12 (T1236C) Exon 21 (G2677A/T) Exon 26 (C3435T)	No association between tacrolimus blood levels and <i>MDR1</i> SNPs	[62,63]
Exon 21 (G2677A/T) Exon 26 (C3435T)	Both SNPs significantly associated with dose-normalized tacrolimus levels	[41,42]
Exon 12 (T1236C) Exon 21 (G2677A/T) Exon 26 (C3435T)	Tacrolimus concentration/dose ratio was 36% lower in wild-type patients for exon 21; the T-T-T haplotype was associated with higher tacrolimus levels as compared to C-G-C	[43]
Exon 12 (T1236C) Exon 21 (G2677A/T) Exon 26 (C3435T)	No association between tacrolimus blood levels and <i>MDR1</i> SNPs or haplotypes	[61]

MDR: Multi-drug resistance.

CsA pharmacokinetic parameters in stable renal transplant patients [33], as well as in small cohorts of healthy subjects [34] and heart transplant recipients [35]. However, in the last two studies a trend of higher area under the curve (AUC) values in subjects carrying C/T and T/T in exon 26 as compared with the C/C group was reported, although the finding was not statistically significant. Therefore, it can not be excluded that the low number of patients considered may have caused bias in the results. In contrast to previous findings, it has been reported that stable transplant recipients carrying the C/C genotype in exon 26 had significantly higher CsA values as compared with T/T genotypes [36]. More interestingly, recent studies in heart [37] and renal [38] transplant recipients have shown that CsA exposure was higher in patients with the *MDR1* T-T-T haplotype as compared to those with C-G-C, suggesting that haplotypes, rather than single SNP genotypes could influence CsA disposition. At variance, Mai *et al.* [39] argued against this conclusion, showing that *MDR1* haplotypes did not affect CsA pharmacokinetics.

Data on the influence of genetic polymorphisms of the *MDR1* gene on TRL bioavailability are more consistent (Table 3). Indeed, Yamauchi *et al.* [40] found that a mutation at position 2677 in exon 21 is a positive predictor of TRL exposure and drug-induced neurotoxicity. Consistent with this finding, others have shown that not only exon 21, but also a SNP on exon 26 is associated with altered TRL levels [41,42]. This pattern has been confirmed in a cohort of 81 renal transplant recipients treated with TRL [43]. The concentration/dose ratio was

36% lower in the wild-type patients for exon 21, suggesting that, for a given dose, their TRL blood concentrations are lower. As observed with CsA, this study has shown that patients with haplotype T-T-T have higher TRL levels compared with C-G-C. These results underline the influence of polymorphisms in exons 12, 21 and 26 of the *MDR1* gene on calcineurin inhibitor bioavailability, and the importance of studying haplotypes rather than a single SNP of this gene.

Beside *MDR1*, other factors affecting drug disposition include cytochromes (CYPs), mainly CYP3A4 and CYP3A5, two subfamilies of enzymes with complementary activity [44]. The former is expressed in the liver, jejunum, colon, and pancreas, whereas the latter is mainly found in the small intestine and stomach. It is noteworthy that the genes encoding CYP3A4/5 are located on the same chromosome as *MDR1*; 7q22.1 [32].

With regards to the CYP3A4 gene, more than 20 SNPs have been described [45]. Among these, an A to G mutation in the 5'-flanking region, which is referred to as CYP3A4*1B or CYP3A4-V and has a frequency of 4% in Caucasians and 70% in Blacks, has been associated with impaired enzyme activity [46]. This mutation has been studied in relation to the pharmacokinetics of CsA (Table 4). Early studies in renal transplant patients [33,47] or in healthy volunteers [48] found no significant difference in the apparent clearance of CsA between different genotypes of CYP3A4. However, it should be pointed out that CsA trough levels were used as surrogate markers of CsA clearance. In contrast, a separate study [48] showed that, in a small group of normal individuals, CsA exposure was affected by the presence

Table 4. Influence of genotypes related to the expression of the *CYP3A4* gene on the pharmacokinetics of calcineurin inhibitors.

Variant	SNP	Results	Ref.
<i>CYP3A4</i> *1B	5'-regulatory region (A290G)	No association with cyclosporine A clearance	[47]
		No influence on cyclosporine A levels or rejection episodes	[33]
		Cyclosporine A AUC 83% higher in G homozygotes	[48]
		Higher tacrolimus doses required in mutants as compared with wild-type carriers	[49]

AUC: Area under the concentration–time curve; CYP: Cytochrome P450.

of the *A* allele of the *CYP3A4**1B polymorphism. Indeed, the dose-normalized CsA AUC was 83% higher in homozygous individuals as compared with wild-type healthy volunteers. Moreover, genotyping of *CYP3A4* has been conducted in a large cohort of kidney transplant recipients receiving CsA or TRL [49], and this study documented that patients carrying the *CYP3A4**1B variant had lower TRL, but not CsA, dose-adjusted trough levels compared with those with the wild-type genotype.

The polymorphisms associated with the expression of *CYP3A5* seem to have a greater impact on calcineurin inhibitor bioavailability than those of *CYP3A4* (Table 5). It has been reported that ~30% of Caucasians and 50% of Africans have a genetic inability to express functional *CYP3A5* [45]. One of these alleles is associated with a premature stop codon and determines the complete absence of *CYP3A5* activity [50]. The above-mentioned study by Hesselink *et al.* [49] has indeed shown that patients with the *CYP3A5**3 genotype require a lower TRL dose to reach the target predose concentration compared with *CYP3A5**1 allele carriers. Similarly, in a cohort of adult renal transplant recipients, a significant difference in TRL dose-adjusted blood level was found between *CYP3A5* expressor versus non-expressor genotypes [51], with the former requiring higher TRL doses to maintain the same blood level. At variance with that observed for TRL, Anglicheau *et al.* [38] studied 106 stable renal transplant patients and did not observe any association between the *CYP3A**1/*3 genotype, which was present in 8.5% of the patients, and CsA pharmacokinetic parameters. To date, the reason for the absence of an association between CsA requirements and *CYP3A4**1B or *CYP3A5**3 alleles is still unknown, and represents an intriguing challenge for the future.

Recently, MacPhee *et al.* [52], studying a SNP in the *CYP3A1* pseudogene, found that this allele is associated with the dose requirement for TRL. However, the real impact of this

polymorphism is doubtful because these SNPs are in the promoter region of the pseudogene *CYP3A1* and, thus, cannot be the basis for polymorphic *CYP3A5* expression [50]. It can be speculated that the observed results of this study can be explained by the linkage disequilibrium of the pseudogene with *CYP3A5**1, which can result in either the presence or absence of enzyme activity [50,53].

Inherited differences may also be present in the drug targets. CsA and TRL exert their immunosuppressive effects by forming complexes with immunophilins (cyclophilin for CsA, and FK-binding protein [FKBP] for TRL) and inhibiting calcineurin activity [54]. Thus, it can be postulated that potential variants in the calcineurin gene might affect drug effects. Nonetheless, although mutations in the calcineurin gene have been documented in yeast cells [55], no mutations have been found in human studies, suggesting that the calcineurin gene is highly conserved [56] and is not accountable for any individual variation in response of patients to CsA or TRL.

Rapamycins

Rapamycins are a new class of anticancer and immunosuppressive drugs, including sirolimus and its derivatives, CCI-779 and everolimus. After binding to the immunophilin FKBP, these drugs inhibit the mammalian target of rapamycin (mTOR) kinase whose downstream targets include p70 S6 kinase and the negative regulator of translation initiation 4E-BP, ultimately arresting the cell cycle in the G1 phase [57].

Although clinical data are lacking, it has been suggested that genetic variants of the mTOR and FKBP genes may influence sensitivity to rapamycins, leading to drug resistance [58]. Moreover, rapamycins are transported by P-gp and metabolized by *CYP3A4*, -3A5 and -2C8 [59]. Thus, the possibility exists that, as observed with calcineurin inhibitors, genetic variants of the *MDR1*, *CYP3A4* and *CYP3A5* genes may affect rapamycin bioavailability, providing a strong rationale

Table 5. Influence of genotypes related to the expression of the *CYP3A5* gene on the pharmacokinetics of calcineurin inhibitors.

Variant	SNP	Results	Ref.
<i>CYP3A5</i> *3	A6986G	The mutant genotype requires a lower TRL dose as compared with <i>CYP3A5</i> *1 allele carriers <i>CYP3A5</i> *1 carriers require larger TRL doses to maintain the same blood levels compared with <i>CYP3A5</i> *3 patients <i>CYP3A5</i> *3/*3 carriers have higher dose-normalized TRL levels No association between CsA pharmacokinetic parameters and <i>CYP3A5</i> genetic polymorphisms CsA oral clearance was significantly higher in patients carrying the <i>CYP3A5</i> *3/*3 genotype CsA and TRL levels were significantly higher in <i>CYP3A5</i> *3*3 patients than in <i>CYP3A5</i> *1*3 patients The TRL concentration-to-dose ratio was decreased in patients carrying the <i>CYP3A5</i> *1/*1 genotype	[49] [42,64] [51] [38] [36] [61] [63]
<i>CYP3AP1</i>	Pseudogene (A/G-44)	Patients with <i>CYP3AP1</i> *1 have lower TRL concentrations with significant delay in achieving target levels	[52,65]

CsA: Cyclosporine A; CYP: Cytochrome P450; TRL: Tacrolimus.

to plan and perform *ad hoc* pharmacogenomic studies for this new class of immunosuppressants.

Conclusion

In the last few years, novel immunosuppressive agents (sirolimus, everolimus, MPA, and TRL) as well as new formulations of traditional drugs (CsA, Neoral®) have been introduced into clinical practice for the management of transplant recipients. Since these agents are characterized by a narrow therapeutic index, pharmacokinetic approaches have been successfully used in the past to tailor the best drug dosage for each patient.

The advent of the genomic era has produced new insights into the molecular basis of human genetic disorders, and pharmacogenomics is undoubtedly a potential source of additional information. Genomic tools could help in identifying patients who are likely to respond to or are at a high risk of adverse events from immunosuppressive drugs prior to surgery (i.e., while patients are on the waiting list or during the pretransplant evaluation). This approach would allow physicians to choose the most appropriate drug regimen based on the genetic profile of a patient. Indeed, early studies have shown that pharmacogenetics may provide information on how individual genes may affect a patient's response to medications. However, since the inherited component of the response to drugs is often polygenic, it should be expected that the study of multiple genes, as well as gene–gene interactions, might be eventually more informative. Therefore, it can be speculated that pharmacogenomics undoubtedly holds promise for an interesting and, hopefully, better future by providing an improved ability to individualize

immunosuppressive therapy based on the genetic profile of the patient. However, the clinical applicability, quality and cost effectiveness of this approach is still to be proven.

Outlook

As a complementary field to pharmacokinetics, it should be expected that in the near future a patient's genetic profile will need to be taken into account when prescribing a drug. However, several critical issues remain to be addressed before pharmacogenomics can be applied to clinical practice.

In the near future, pharmacogenomic studies will need to be focused on identifying candidate genes whose polymorphisms can predict not only drug disposition but, even more importantly, pharmacological effects and clinical outcomes. In addition, because of marked population heterogeneity, pharmacogenomic relations must also be validated in different ethnic groups to avoid confounding factors. The development of a predictive dosage algorithm incorporating genetic testing represents an additional challenge for the future.

To address some of these issues, new techniques, such as DNA microarray technology, by performing genome-wide searches can lead to the detection of small variations in the DNA or gene structures, allowing the simultaneous assessment of the expression profile of thousands of genes at the entire genome level in a single run, which will identify candidate genes and, ultimately, predict response to several drugs [60].

Therefore, to prove the usefulness of pharmacogenomics in transplantation medicine, prospective clinical studies are needed to show whether genotype determination before

Highlights

- Immunosuppressants are narrow therapeutic index agents that require close monitoring.
- Pharmacogenetic studies have been shown to provide additional information on drug exposure when compared with pharmacokinetic approaches.
- The study of multiple genes, as well as gene–gene interactions, has the potential to tailor the best immunosuppressive regimen for each patient before transplantation.
- The clinical applicability of pharmacogenomic approaches remains to be proven.
- Genome-wide studies must be performed for the identification of candidate genes that are capable of predicting drug response and clinical outcome.
- Pharmacogenomic studies must be validated in special populations and different ethnic groups.
- Predictive dosage algorithms based on genetic testing must be developed.

transplantation would allow better use of a given drug when compared with a pharmacokinetic approach, and whether it is capable of improving safety and clinical efficacy.

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