Pediatr Nephrol (2012) 27:1489–1498
DOI 10.1007/s00467-012-2105-0

EDUCATIONAL REVIEW

The role of genetics in drug dosing

Nicholas Ware

Received: 12 March 2011 / Revised: 23 December 2011 / Accepted: 23 December 2011 / Published online: 23 February 2012

Abstract Renal transplantation is the optimal form of renal replacement therapy (RRT) for the majority of patients. Both short- and long-term graft rejection are well recognized complications following transplantation, and optimal immunosuppression is often difficult to achieve. Pharmacodynamics (PD) and pharmacokinetics (PK) are hard to predict in all patients, and best practice involves the use of standard dosing based on weight and therapeutic drug monitoring (TDM). Pharmacogenetics (PG) is the use of genetic screening to predict metabolic responses to different immunosuppressive drugs and enables more accurate predictions of PD and PK to be made. This has the potential to improve graft outcome by reducing both short- and long-term graft rejection.

Keywords ABCB1 · CYP3A5 · Immunosuppression · Pharmacogenetics · Therapeutic drug monitoring · Transplantation

Introduction

Organ transplantation is the optimal treatment for many patients with end-stage organ failure. It is well documented that transplantation is a more optimal form of renal replacement therapy than dialysis, with the additional benefits of better quality of life both in terms of physical health and social situation. This not only benefits the child but also the parents, carers, and extended family. Following transplantation, recipients require immunosuppressive therapy to prevent graft rejection. A gradual improvement in short-term graft survival has occurred over the last 10 years as immunosuppressive drugs and regimens have improved. However, there has been minimal improvement in long-term graft survival over the same period, partly due to drug side effects, such as nephrotoxicity [1, 2]. The personalisation of immunosuppressive regimens is one way that future graft outcome may be improved. Pharmacogenetics (PG) is the study of genetic variation that gives rise to different drug responses, and theoretically, if patients can be genetically screened prior to transplantation, then their immunosuppressive therapy can be tailored to optimise their short- and long-term graft outcome.

N. Ware (✉)
Renal Department, Great Ormond Street Hospital for Children,
Great Ormond Street,
London WC1N 3JH, UK
e-mail: nicholasware@doctors.org.uk

Immunosuppression posttransplantation

There is no standard immunosuppressive regimen in the UK for paediatric patients undergoing renal transplantation, but drug regimens usually include the combination of a calcineurin inhibitor (CNI; such as tacrolimus or ciclosporin) with an antiproliferative agent (azathioprine or mycophenolate) and corticosteroids. Regimens often use an induction therapy that includes antibodies to lymphocytes, such as basiliximab or daclizumab, anti-CD25 antibodies that bind to interleukin (IL)-2 receptors on activated T-lymphocytes. Mammalian target of rapamycin (mTOR) inhibitors, such as sirolimus and everolimus, are sometimes used in combination with or instead of a CNI when proven intolerance to CNIs necessitates their withdrawal [3]. Immunosuppressive drugs have narrow therapeutic ranges: underdosing can lead to organ rejection, whereas overdosing can result in infection, malignancy and direct organ toxicity [3]. Episodes of acute rejection often occur within the first few weeks of transplantation but can occur any time if there are inadequate levels of

immunosuppression. The response may be cell or antibody mediated and lead to injury or destruction of the cellular structures of the graft. The response can be more aggressive and include a vascular reaction. Clinically, acute rejection tends to occur as an acute episode causing a reduction in graft function (with associated reduced urine output and urine biochemistry changes) and by clinical features such as pyrexia, graft tenderness and oedema. Chronic allograft dysfunction is the most common cause of delayed graft loss and is a gradual process occurring months or years posttransplantation. The course is generally unremitting and inevitably leads to loss of graft function and either a return to dialysis or a further attempt at transplantation.

There is wide interindividual variation in the dose of immunosuppressive drugs required to achieve target blood concentrations. The best approach to achieve whole-blood or plasma concentrations within a defined therapeutic range is the use of therapeutic drug monitoring (TDM). The most critical period for organ rejection is the first 72 h posttransplantation, when inadequate drug exposure increases the risk of acute rejection in patients treated with ciclosporin [4], tacrolimus [5] or mycophenolate [6]. TDM cannot optimise drug exposure during this early timeframe; such optimisation can only be achieved by the use of an ideal initial dose. Therefore, the use of a PG approach (through genetic screening) to predict the most appropriate initial dose may offer a complementary strategy to TDM. It is possible that the use of potent induction therapy may render less critical the need to attain an optimal drug concentration in the regimen [7]. A PG strategy may be particularly useful in guiding the initial dose for drugs with a long half-life, such as sirolimus, as TDM will inevitably take longer to respond these cases [8].

Several genetic polymorphisms have been identified in drug targets, drug-metabolising enzymes and drug transporters. Theoretically, individuals could be screened for specific polymorphisms, effectively acting as biomarkers, facilitating more specific and individualised choice of drug and dose. This strategy may enable therapeutic concentrations to be attained more quickly. The PG of immunosuppression for solid organ transplantation have been reviewed extensively for adult populations [9–11] but far less work has concentrated on paediatric populations. Despite this, there have been a number of advances in the understanding of genetic associations with immunosuppressive pharmacokinetics (PK) and pharmacodynamics (PD) in the context of their use in a PG strategy to guide drug dosing in both adults and children. Several strategies have emerged with potential for use in clinical practice, the most promising of which is the use of the cytochrome P450 (CYP)3A5 (CYP3A5) genotype to predict the optimal initial dose for tacrolimus [12].

Much of the content of this article relates to adult studies, although paediatric studies have been undertaken in small numbers. Hopefully, as further research is done, our knowledge of paediatric PG will improve and contribute to improved outcomes for solid organ transplants in children.

Pharmacogenetic strategies based on pharmacokinetics

The CNIs ciclosporin and tacrolimus, as well as sirolimus, have an oral bioavailability of 20–30%. Two key components of the barrier that prevents drug absorption are subject to expression-level genetic variability. The oxidative enzymes CYP3A4 and CYP3A5 are responsible for first-pass metabolism in the enterocyte and the liver, and the drug transporter P-glycoprotein (P-gp), the product of the *ABCB1* (previously known as *MDR-1*) gene, expels tacrolimus from the enterocyte, thereby preventing absorption. The expression of CYP3A decreases, whereas the expression of P-gp increases along the length of the gastrointestinal tract (moving from the proximal to the distal end) [13].

The *CYP3A5* genotype and pharmacokinetics

Tacrolimus, ciclosporin, sirolimus, everolimus and corticosteroids are all metabolised by CYP3A4 and CYP3A5 [14–16]. There is significant variability in the expression of CYP3A4 in the intestine and the liver, but no clear genetic basis has been identified for this heterogeneity or for the differences in immunosuppressive drug PK and PD presumed to be associated with CYP3A4 (reviewed in reference [9]); therefore, the *CYP3A4* genotype is unlikely to be of value in the prediction of optimal drug dosing. CYP3A5 has a similar protein sequence to CYP3A4 and similar substrate specificity. Individuals with at least one wild-type *CYP3A5*1* allele are able to synthesise a functional protein that constitutes up to 50% of their total hepatic CYP3A. These individuals are referred to as functional CYP3A5 expressers. *CYP3A5*3* homozygotes, who express low levels of CYP3A5, are referred to as functional non-expressers [17]. The prevalence of these genotypes differs between ethnic groups, with CYP3A5 expression more common in populations whose genetic origin is close to the equator [18]. Approximately 85% of individuals with a sub-Saharan African genetic origin, 50% of south Asians and 15% of caucasians are CYP3A5 expressers [12], revealing a reliable genotype–phenotype association across a wide range of ethnic and geographically different populations.

In addition to the ethnic and geographical variation in CYP3A5 expression, there is also age-related variation in the expression of CYP3A enzymes. In foetal life, CYP3A7 plays a major role, with CYP3A7 expression being highest in the first trimester. It is then gradually replaced by CYP3A4 throughout the developmental period [19]. At the same time, it

is thought that the expression of CYP3A5 remains relatively constant independent of age. Although there is no definitive study on the induction capability of CYP3A enzymes, a study by Yukawa et al. suggests that enzyme induction is stronger in infants than in adults [20]. As a result, when reviewing studies, it is important that age and developmental stage are considered in addition to CYP3A5 genotype, although their influence is not well established.

CYP3A5 and tacrolimus

Research across a range of adult populations has confirmed that expression of CYP3A5 is associated with reduced tacrolimus exposure following oral administration [21]. The genetic component appears to affect drug absorption rather than the rate of drug elimination [22]. CYP3A5 expressers exhibit a significant delay in achieving target blood concentrations when using conventional initial doses and subsequent TDM-based adjustment [23, 24]. This delay has been associated with an earlier [25] and increased incidence [26–28] of acute rejection in the early period following transplantation when protocols that do not use an induction antibody are used. Other studies have failed to confirm this observation, perhaps because potent regimens with widespread use of induction antibodies were used [7, 24]. An increased incidence of nephrotoxicity has been noted in CYP3A5 nonexpressers following renal transplantation [24, 27]. The authors hypothesised that this was because of increased metabolic activity in the kidney resulting in protection against toxicity in CYP3A5 expressers.

A recent study by Zhao et al. investigated the effect of age and PG on tacrolimus drug disposition in 50 de novo paediatric renal transplant recipients [29]. Looking at a number of variables they showed that CYP3A5 expression (in addition to body weight and haematocrit level) had significant effects on PK variability, whilst the other variables (including demographic, clinical and other PG variables including *CYP3A4*, *ABCB1* and *ABCB2*) had no effect. They also showed that the standard starting tacrolimus dose of 0.15 mg/kg twice a day was associated with underdosing in children who are expressers, and that higher dosages (0.2–0.3 mg/kg twice a day) should be recommended, principally in children with low haematocrit levels and weighing less than 20 kg. In contrast, for children who are non-expressers, the lower dose of 0.1 mg/kg twice a day should be recommended, primarily in children weighing more than 40 kg. They concluded by recommending a body weight-based dosing regimen based on *CYP3A5* polymorphism and haematocrit levels for better individualisation of tacrolimus dosage.

A similar study by Ferraresso et al. looking retrospectively at 30 adolescent renal transplant recipients showed a two-fold increase of tacrolimus daily doses in functional expressers compared with non-expressers was needed in order to reach the desired therapeutic range [30]. They also showed a high incidence of acute rejection episodes among expressers, which is consistent with the need for higher tacrolimus doses in this group. Another study on paediatric heart transplant patients found that at 3, 6 and 12 months post-transplantation, a significant difference in tacrolimus blood level was found between the functional expressers and non-expressers, with the expressers requiring a larger tacrolimus dose to maintain the same blood concentration [31]. They concluded that specific genotypes of *MDR1* and *CYP3A5* in paediatric heart transplant patients require larger tacrolimus doses to maintain their tacrolimus blood concentration, and that this information could be used prospectively to manage immunosuppressive therapy.

An important multi-centre randomised controlled trial by Thervet et al. assigned adult renal transplant recipients to receive tacrolimus either according to *CYP3A5* genotype or according to the standard daily regimen [32]. The primary end point was the proportion of patients achieving a therapeutic trough concentration. In the group receiving the adapted dose, they required fewer dose modifications, and the targeted trough concentration was achieved by 75% of these patients more rapidly. This is the first study to show that prospective adaptation of tacrolimus daily dose according to *CYP3A5* genotype increased the proportion of patients reaching the therapeutic target range.

CYP3A5 and ciclosporin

Despite extensive research in adults, no clear genetic predictors of ciclosporin absorption have been identified. There have been a few studies demonstrating a correlation between genetic predictors and ciclosporin absorption but there is not sufficiently strong evidence for the development of a PG strategy. The majority of studies displayed no correlation between genotype and dose-normalised blood concentrations. The largest of these studies concluded that ciclosporin dose requirements based on blood concentration measurements at 0 h following drug dosing (C_0), blood pressure and long-term graft survival were not influenced by the *CYP3A5*1* genotype in Caucasian patients [33]. A large study in patients of differing ethnicities has also failed to demonstrate any association when measuring both C_0 and C_2 (measurements taken at 2 h following dosing) [34]. To date there have been no studies looking at *CYP3A5* and ciclosporin PG in paediatric patients.

CYP3A5 and sirolimus

Sirolimus has a long half-life (approximately 60 h) and, accordingly, a slow response time for TDM [35], thereby

rendering the use of a PG approach for dosing with this drug extremely desirable. Again only adult studies have looked at CYP3A5 and sirolimus. In the absence of treatment with a CNI, oral bioavailability of sirolimus was reduced in individuals expressing CYP3A5 [36]. This observation was confirmed in two further studies, with dose-normalised blood concentrations for CYP3A5 expressers reaching only 50 to 80% of the concentrations observed in non-expressers [37]. However, two further similar studies failed to demonstrate any association between oral bioavailability of sirolimus and CYP3A5 expression [38, 39]. It is conceivable that ciclosporin may inhibit or saturate the barrier to drug absorption, nullifying the effect of CYP3A5 expression. Following these observations, a study using the *CYP3A5* genotype to predict the initial sirolimus dose in patients not previously treated with a CNI would be the next logical step, although in children they tend only to be used when proven intolerance to CNIs necessitates their withdrawal. One concern with this approach is the risk of acute rejection in the early period after transplantation. The use of a PG strategy to optimise the sirolimus dose may offer a solution to this problem.

There are no published data on the influence of the *CYP3A5* genotype on everolimus, the other mTOR inhibitor that is metabolised by CYP3A5, in either adult or paediatric populations [40].

ABCB1 genotype and pharmacokinetics

Tacrolimus, ciclosporin, sirolimus, everolimus and corticosteroids are all substrates for P-gp [41, 42]. Sequencing of the *ABCB1* gene (which encodes P-gp) has revealed more than 50 single nucleotide polymorphisms (SNPs), which vary in frequency according to ethnicity (reviewed in reference [43]). The three most common, and most widely researched, are the synonymous SNPs *1236C>T* in exon 12 and *3435C>T* in exon 26 that do not alter the protein sequence, and the non-synonymous SNP *2677G>(T,A)* in exon 21 which does result in an amino acid substitution. In general, wild-type *ABCB1* alleles have been associated with increased tissue expression of P-gp, although this has not been reported consistently [44]. It has been suggested that the haplotype of the three SNPs is more predictive of phenotype than the individual SNP genotype [45]. A *3435C>T* mutation linked to one of the other mutations results in altered protein folding, which can modify substrate specificity [46] or alter mRNA stability [47]. There are a number of conflicting reports regarding the *ABCB1* genotype-phenotype association, and the robustness of the association has been questioned [48]. For any PG test to be effective it is imperative that there is a strong relationship between genotype and phenotype. As a result, the heterogeneous genotype-phenotype relationship with *ABCB1* is a major limiting factor for its potential use in PG.

ABCB1 and tacrolimus

In intestinal biopsies of adult patients undergoing living-donor liver transplantation, *ABCB1* mRNA levels were negatively correlated with dose-adjusted tacrolimus blood concentrations [49]. High levels of *ABCB1* mRNA were associated with increased acute cellular rejection and poorer survival rates in the first year post-transplantation [49]. Research into the association between the *ABCB1* genotype and tacrolimus exposure has yielded mixed results, with some studies showing a small but significant increase in dose-adjusted tacrolimus blood concentrations in patients with the mutated genotype, while other studies have reported no correlation.

A recent study on 51 paediatric patients post liver transplantation demonstrated a significant association between *ABCB1* genetic polymorphisms and tacrolimus-associated nephrotoxicity [50]. This suggests that *ABCB1* polymorphisms in the gastrointestinal tract do have a significant influence on tacrolimus dose requirements in the stable phase post-transplantation. The study also showed that there was no correlation between different *ABCB1* polymorphisms and tacrolimus PK at 6 months post-transplantation, although they were significantly correlated to the incidence of renal dysfunction at the same point.

ABCB1 and ciclosporin

As with tacrolimus, there is no consistently observed relationship between *ABCB1* polymorphisms and the PK of ciclosporin although ciclosporin is recognised as an inhibitor of P-gp. Cattaneo et al. evaluated the associations between *ABCB1* genotypes and ciclosporin-related outcomes in 147 adult renal transplant recipients [51]. Carriers of T allelic variants in exons 21 or 26 exhibited a 3-fold increase in the risk of delayed graft function, a trend toward slower recovery of renal function and lower glomerular filtration rate (GFR) at study end, as well as a significantly higher incidence of new-onset diabetes and cytomegalovirus (CMV) reactivation when compared with carriers of the wild-type genotype. It must be assumed that the renal effects in this study occurred as a result of PK factors, as only the genotype of the recipients was determined [51].

A study by Fanta et al. looking at pretransplant children with end-stage kidney disease showed that genetic variations of *ABCB1* has an age-dependent effect on the oral bioavailability of ciclosporin and on the oral dose requirements in the oldest patients [52]. Carriers of the *ABCB1 1236C>T* or *2677G>T* variant allele had oral bioavailability approximately 1.3–1.6 times higher than noncarriers amongst children

>8 years. They also had a lower prehepatic extraction ratio of ciclosporin than noncarriers in the same age group. The association did not exist in children <8 years old. In summary, if the *ABCB1* genotype has a real influence on the PK of immunosuppressive drugs, it is likely to be only minor and therefore of little value in planning drug dosing, although there is a need for more research in this area.

Drug formulations and pharmacogenetics

The observations described in the *Pharmacogenetic Strategies Based on Pharmacokinetics: An Active Barrier to Drug Absorption* section were made with branded products, specifically Prograf (tacrolimus) and Neoral (ciclosporin). It is possible that genetic influences will be less pronounced with the prolonged-release formulation of tacrolimus, Advagraf. Moving from the proximal to the distal end of the gastrointestinal tract, epithelial cells tend to express less CYP4503A and more P-gp [13], suggesting that the *ABCB1* genotype may have a more pronounced influence on drug absorption with Advagraf. Generic formulations of tacrolimus and ciclosporin are now available in several countries, and economic pressures are likely to result in their increased use worldwide. Excipients contained within these preparations may influence the barrier to enteric absorption, and it cannot be assumed that genetic influences applicable to one formulation will apply to all. For example, vitamin E, a known inhibitor of P-gp [53], is used in the Neoral preparation of ciclosporin and may enhance absorption. The granular formulation Modigraf, which is often used in paediatric patients as it is manufactured in liquid form, has an 18% increased bioavailability when compared with Prograf and is therefore not bioequivalent to Prograf or Advagraf and hence may have a different PG profile. Intravenous routes for immunosuppressive drugs in the period immediately posttransplantation may also affect their PG profiles.

Thiopurine-*S*-methyltransferase and azathioprine

Elimination of azathioprine occurs via metabolism by the enzyme thiopurine-S-methyltransferase (TPMT). Approximately 1 in 300 individuals are deficient in TPMT because of variant alleles and only require 6–10% of the standard dose of azathioprine (reviewed in reference [54]). Although the number of individuals at risk is small, administration of azathioprine to TPMT-deficient individuals may result in catastrophic myelotoxicity. Several SNPs predict the TPMT phenotype, and in many medical specialties, including both adult and paediatric rheumatology and gastroenterology, genotyping for the *TPMT* gene or direct measurement of TPMT activity prior to the administration of azathioprine has become standard practice. This method is the best-established PG strategy for therapeutic immunosuppression. In transplantation, however, the approach has not been adopted widely, and TPMT deficiency is not typically perceived as a major concern, perhaps as a result of the intensive monitoring that occurs immediately after transplantation, including the measurement of a number of haematological parameters. Given the existence of a practical PD assay in transplantation with a clinically relevant response time, PG is unlikely to contribute significantly to clinical practice.

Corticosteroids

Genetic influences on corticosteroids have not been extensively studied, possibly because researchers did not always measure corticosteroid blood concentrations. Miura et al. demonstrated that neither the *CYP3A5* nor the *ABCB1* genotype influenced plasma concentrations of prednisolone measured 28 days after renal transplantation in adults [55]. The steroid and xenobiotic receptor (SXR) plays an important role in the regulation of *CYP3A4* and *ABCB*1 expression by both endogenous and xenobiotic substrates [56]. One finding of potential interest was that individuals with the *NR1I2 7635G* allele of SXR exhibited reduced maximum concentrations of prednisolone in plasma. Miki et al. showed that the amounts of SXR messenger RNA (mRNA) in the liver and intestine reached maximal levels in young adults (15–38 years old) and then subsequently decreased to less than half of the maximal levels with ageing. The authors proposed that age-related differences in the body's capacity to metabolise steroids and xenobiotic compounds result in an important role for SXR and its target genes [56]. An interesting study by Zheng et al. on paediatric heart transplant patients used regression analysis to try to identify predictors of steroid dependency posttransplantation [57]. The study confirmed *ABCB1 3435C>T* and cytokine IL-10 polymorphisms as independent risk factors for steroid dependency at 1 year posttransplantation. [The anti-inflammatory effects of IL-10 are known to promote protection in certain allografts (heart, lung and liver) and are also associated with fewer complications following bone marrow transplantation] [58]. Further studies looking at the genetic influences on corticosteroids are required before useful evidence can be translated into practice, but the potential role in paediatric transplant patients is clear. In addition to their role in steroid metabolism, SXR SNPs also influence the metabolism of CYP3A4 and ABCB1 substrates, including tacrolimus and ciclosporin, by altering the expression of CYP3A4 and ABCB1. Some studies have shown that SXR SNPs increase the oral clearance of tacrolimus, although further investigation is required to clarify the extent of this

effect [59, 60]. Similarly, the effect of SXR SNPs on ciclosporin needs further investigation before definitive conclusions can be drawn regarding their influence.

Mycophenolate

The active agent mycophenolic acid (MPA) can be delivered either as the prodrug mycophenolate mofetil (MMF) or as enteric-coated mycophenolate sodium. MPA is an antiproliferative agent that inhibits the activity of the target enzyme inosine monophosphate dehydrogenase (IMPDH), which exists as two isoforms. Variations in IMPDH activity are thought to play a role in the heterogeneous response to MPA. All studies on mycophenolate have been done in adult populations, but the findings are likely to be similar in paediatric populations. Sombogaard et al. demonstrated that the *IMPDH type II .3757T* polymorphism was associated with increased IMPDH activity in mycophenolate-treated renal transplant recipients (n=101) and that this polymorphism accounted for 8% of the interindividual variability in IMPDH activity [61]. In another multicentre study, this polymorphism was predictive of acute rejection, as patients with at least one C allele exhibited a threefold increased risk of acute rejection [62]. SNPs in the *IMPDH1* gene have also been associated with acute rejection [63]. MPA is conjugated by uridine glucuronyl transferases (UGTs), and the glucuronide is either eliminated in urine or excreted in bile, where it is subject to enterohepatic recirculation. Biliary secretion is mediated by the drug transporter ABCC2 (MRP2) and, as a result, SNPs in the *ABCC2* gene can have an impact on MPA exposure [64]. *UGT1A9* gene products are localised in the liver and kidney and are likely to play a role in MPA glucuronidation. One study identified UGT1A9 as the key UGT responsible for glucuronidation of MPA to MPAG in the liver. Individuals with the g.275T and g.2152C alleles in the *UGT1A9* promoter region exhibited reduced exposure to the active drug MPA of a magnitude that is likely to be of clinical significance and result in increased incidence of acute rejection [65]. The influence of SNPs in the *UGT1A9* gene may be dose dependent. MPA exposure was significantly reduced in patients treated daily with a 2-g MMF dose compared with patients treated with 1 g [66]. Whereas these studies identified potential targets for a PG strategy for mycophenolate, the targets have not been tested. One potential limitation is the small proportion of interpatient variability in MPA PK that is predicted by genetic factors. Among the different UGT enzymes, there is wide variation in levels of expression depending on age. UGT1A1 and UGT2B7 reach adult levels by 3 months of age, whereas UGT1A6, UGT1A9 and UGT2B7 can take up to 10 years to reach adult levels [67]. This adds further difficulty to the PG profiling of paediatric patients with respect to UGT expression.

Pharmacogenetic strategies to predict pharmacodynamics

Genetic influence on intracellular drug concentrations

A number of adult studies have addressed the influence of genetic factors on the intracellular concentration of drugs both in the lymphocytes of the target organs for efficacy and in the renal tubular epithelial cells for toxicity. The correlation between whole-blood and intralymphocyte concentrations of ciclosporin is poor [68, 69]. Methods have been established to measure intracellular concentrations of both ciclosporin [68–71] and tacrolimus [72]. Although these measurements have not been adopted in clinical practice, intracellular drug concentrations correlated better with acute rejection than did whole-blood concentrations [70, 73]. Individuals with the *ABCB1 C3435CT CC* genotype exhibited significantly lower intracellular ciclosporin concentrations than a pooled CT/TT group [68]. In another study, an association between lymphocyte P-gp activity and intracellular ciclosporin concentrations was observed, although there was no correlation with the *ABCB1* genotype [66], supporting studies in which inhibition of lymphocyte activation by ciclosporin correlated inversely with the level of P-gp expression [74]. Whereas the hypothesis that high levels of P-gp expression on lymphocytes predicted by the *ABCB1* genotype will lead to an increased incidence of graft rejection suggested by these in vitro data is attractive, there are few supportive clinical studies. In a study of adults post renal transplantation, there was a statistically significant association between the *ABCB1* haplotype and acute rejection, but this association accounted for only a small proportion of the risk [75]. Data supporting the hypothesis that variation in renal tubular P-gp expression levels accounts for the differential susceptibility to CNI nephrotoxicity, as a result of the influence of P-gp on intracellular drug concentrations, have been obtained from patients transplanted with nonrenal organs. However, recipients of liver transplants with the *ABCB1 2677TT* homozygote genotype appeared to be less susceptible to nephrotoxicity in one study [76] than another [50]. Moreover, several studies failed to identify any association between the *ABCB1* or *CYP3A5* genotypes and nephrotoxicity in bone marrow [77] or cardiac transplant recipients [78]. In renal transplant recipients, the incidence of ciclosporin nephrotoxicity was significantly higher when the donor, but not the recipient, had the *ABCB1 3435TT* genotype [79]. Lower levels of P-gp expression were noted in renal biopsies of patients with CNI nephrotoxicity [80]. A limitation of these studies is the difficulty in making or excluding with confidence the diagnosis of CNI nephrotoxicity. If based on histological criteria, all patients would need to be biopsied to robustly define the presence or absence of CNI toxicity, rather than only those being investigated for renal dysfunction. A drop in serum creatinine concentration with reduced CNI dose may be used as an alternative

measure, but again, is not routinely tested in all patients. Sirolimus is known to enhance the nephrotoxicity of ciclosporin. In vitro, the susceptibility of cultured human proximal tubular epithelial cells to CNI toxicity was increased by P-gp inhibition, including that caused by sirolimus, and the inhibition was associated with increased intracellular ciclosporin concentrations [81]. Similar data regarding genetically determined differences in P-gp expression are awaited.

Genomics of immunosuppressive drug targets

Genetic predictors for particularly high or low risk of rejection could potentially be used to create individualised immunosuppressive regimens. A number of SNPs in candidate genes for cytokines and cell-surface molecules involved in the immune response have been associated with the incidence of acute rejection following transplantation, but none of the reported associations have been replicated consistently. As a result, no clear candidate has arisen to help guide immunosuppressive therapy (for a recent review, see reference [82]).

Role of donor polymorphisms

This review has focussed on the genotype of transplant recipients. There have been relatively few studies looking at the role of polymorphisms in graft donors, and their importance remains unclear. Woillard et al. concluded from a study on renal transplant patients that the presence of ABCB1 polymorphisms in donors influences long-term graft outcome [83]. In adult renal transplant recipients, the incidence of ciclosporin nephrotoxicity was significantly higher when the donor, but not the recipient, had the *ABCB1 3435TT* genotype [79]. Further studies are required to evaluate the role of donor polymorphisms, although it is likely that in paediatric and adult renal transplantation, the role of recipient polymorphisms will be more important.

Weight-based dosing of immunosuppressive drugs in paediatric patients

In addition to the issues of PG discussed, there are also other considerations when dealing with immunosuppressive drug dosing in paediatric transplant patients. For example, tacrolimus dosing in children follows a similar weight-based protocol as for adults. Kausman et al. have shown that there is a strong relationship between age, body size and the development of supratherapeutic tacrolimus levels when following a strict dose initiation based on weight [84]. It is therefore likely that individual dosing needs to be adjusted based on weight, age and body surface area, although further evidence is required to develop accurate protocols. This adds further complexity to the PG considerations already discussed.

Conclusion

There is a large evidence base relating to genetic influences on the pharmacology of immunosuppressive drugs. Much of this evidence comes from studies in adult populations, but more work is being undertaken on paediatric populations. For a PG test to be useful, the genotype must have a major influence on the PK or PD of a drug with a narrow therapeutic index, for which the rapid achievement of target blood concentrations is important. The only strategy to have fulfilled these requirements to date is the *CYP3A5* genotype, which can predict the optimal initial dose of tacrolimus. Whether a PG dosing strategy for tacrolimus can improve clinical outcomes by improving efficacy or reducing toxicity remains to be proven and is a difficult outcome to measure. A randomised controlled trial in needed that uses genetically predicted doses from the time of transplantation in a sample including patients at high risk of rejection and toxicity. There are no known reliable genetic markers for the heterogeneity in the PK of ciclosporin. The pharmacology of mycophenolate is complex, but several genetic predictors of plasma MPA concentrations and efficacy failure have emerged that are worthy of further research; however, there is concern that the genetic influences will be lost in the complexity of MPA PK, which seem to be influenced to a greater degree by environmental rather than genetic factors.

Measurement of intracellular drug concentrations is an exciting new development in TDM and will potentially be an improved indicator of whole-blood concentrations than using efficacy and toxicity. Genetic polymorphisms in drug transporters such as P-gp may predict the partitioning characteristics of a drug between cells and plasma. This may aid either in determining the therapeutic range or avoiding specific drugs with a particularly high risk of efficacy failure or toxicity. Due to the relatively low number of paediatric organ transplants that take place, it is difficult to perform trials and obtain data on paediatric patients. However, there is plenty of further work to be done in this area, and at some point, genetic screening of all transplant patients may become routine. The use of PG strategies in transplantation is close to reaching the clinic, but further research is required.

Multiple choice questions

Answers appear following the reference list

1. Which ethnic subgroup has the highest proportion of CYP3A5 expressers?

   a) Caucasian
   b) Sub-Saharan African
   c) South Asian

2. P-gp is encoded by which gene?
   a) *ABCB1*
   b) *CYP3A*
   c) *CYP4A*
   d) *TPMT*
3. Mycophenolic acid inhibits the activity of which target enzyme?
   a) P-gp (P-glycoprotein)
   b) TPMT (thiopurine-S-methyltransferase)
   c) IMPDH (inosine monophosphate dehydrogenase)
   d) UGT (uridine glucuronyl transferases)
4. Which of these drugs has the longest half-life?
   a) Tacrolimus
   b) MMF
   c) Ciclosporin
   d) Sirolimus
5. The CNIs ciclosporin and tacrolimus, as well as sirolimus, have an oral bioavailability of what percentage?
   a) 10–20%
   b) 20–30%
   c) 30–40%
   d) 40–50%

References

1. Golshayan D, Buhler L, Lechler RI, Pascual M (2007) From current immunosuppressive strategies to clinical tolerance of allografts. Transpl Int 20:12–24
2. Kaneku HK, Terasaki PI (2006) Thirty year trend in kidney transplants: UCLA and UNOS Renal Transplant Registry. Clin Transpl 1–27
3. Halloran PF (2004) Immunosuppressive drugs for kidney transplantation. N Engl J Med 351:2715–2729
4. Clase CM, Mahalati K, Kiberd BA, Lawen JG, West KA, Fraser AD, Belitsky P (2002) Adequate early cyclosporin exposure is critical to prevent renal allograft rejection: Patients monitored by absorption profiling. Am J Transplant 2:789–795
5. Undre NA, van Hoof J, Christiaans M, Vanrenterghem Y, Donck J, Heeman U, Kohnle M, Zanker B, Land W, Morales JM, Andres A, Schafer A, Stevenson P (1999) Low systemic exposure to tacrolimus correlates with acute rejection. Transplant Proc 31:296–298
6. van Gelder T, Silva HT, de Fijter JW, Budde K, Kuypers D, Tyden G, Lohmus A, Sommerer C, Hartmann A, Le Meur Y, Oellerich M, Holt D, Tonschoff B, Keown B, Campbell S, Mamelok R (2008) Comparing mycophenolate mofetil regimens for de novo renal transplant recipients: The fixed-dose concentration-controlled trial. Transplantation 86:1043–1051
7. Hesselink DA, van Schaik RH, van Agteren M, de Fijter JW, Hartmann A, Zeier M, Budde K, Kuypers DR, Pisarski P, Le Meur Y, Mamelok RD, Van Gelder T (2008) CYP3A5 genotype is not associated with a higher risk of acute rejection in tacrolimus-treated renal transplant recipients. Pharmacogenet Genomics 18:339–348
8. Le Meur Y, Djebli N, Szelag JC, Hoizey G, Toupance O, Rérolle JP, Marquet P (2006) CYP3A5*3 influences sirolimus oral clearance in de novo and stable renal transplant recipients. Clin Pharmacol Ther 80:51–60
9. Ng FL, Holt DW, MacPhee IA (2007) Pharmacogenetics as a tool for optimising drug therapy in solid-organ transplantation. Expert Opin Pharmacother 8:2045–2058
10. Ekbal NJ, Holt DW, MacPhee IA (2008) Pharmacogenetics of immunosuppressive drugs: Prospect of individual therapy for transplant patients. Pharmacogenomics 9:585–596
11. Cattaneo D, Baldelli S, Perico N (2008) Pharmacogenetics of immunosuppressants: Progress, pitfalls and promises. Am J Transplant 8:1374–1383
12. Macphee IA, Holt DW (2008) A pharmacogenetic strategy for immunosuppression based on the CYP3A5 genotype. Transplantation 85:163–165
13. Zhang Y, Benet LZ (2001) The gut as a barrier to drug absorption. Combined role of cytochrome P4503A and P-glycoprotein. Clin Pharmacokinet 40:159–168
14. Dai Y, Iwanaga K, Lin YS, Hebert MF, Davis CL, Huang W, Kharasch ED, Thummel KE (2004) In vitro metabolism of cyclosporine A by human kidney CYP3A5. Biochem Pharmacol 68:1889–1902
15. Dai Y, Hebert MF, Isoherranen N, Davis CL, Marsh C, Shen DD, Thummel KE (2006) Effect of CYP3A5 polymorphism on tacrolimus metabolic clearance in vitro. Drug Metab Dispos 34:836–847
16. Kamdem LK, Streit F, Zanger UM, Brockmoller J, Oellerich M, Armstrong VW, Wojnowski L (2005) Contribution of CYP3A5 to the in vitro hepatic clearance of tacrolimus. Clin Chem 51:1374–1381
17. Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, Watkins PB, Daly A, Wrighton SA, Hall SD, Maurel P, Relling M, Brimer C, Yasuda K, Venkataramanan R, Strom S, Thummel K, Boguski MS, Schuetz E (2001) Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. Nat Genet 27:383–391
18. Thompson EE, Kuttab-Boulos H, Witonsky D, Yang L, Roe BA, Di Rienzo A (2004) CYP3A variation and the evolution of salt-sensitivity variants. Am J Hum Genet 75:1059–1069
19. Stevens JC, Hines RN, Gu C, Koukouritaki SB, Manro JR, Tandler PJ, Zaya MJ (2003) Developmental expression of the major human hepatic CYP3A enzymes. J Pharmacol Exp Ther 307:573–582
20. Yukawa E (2007) Approach to individualized pharmacotherapy: analysis of population pharmacokinetic parameters. TDM Research 24:8–16
21. Macphee IA, Fredericks S, Tai T, Syrris P, Carter ND, Johnston A, Goldberg L, Holt DW (2002) Tacrolimus pharmacogenetics: Polymorphisms associated with expression of cytochrome P4503A5 and P-glycoprotein correlate with dose requirement. Transplantation 74:1486–1489
22. Haufroid V, Wallemacq P, Vankerckhove V, Elens L, De Meyer M, Eddour DC, Malaise J, Lison D, Mourad M (2006) CYP3A5 and ABCB1 polymorphisms and tacrolimus pharmacokinetics in renal transplant candidates: Guidelines from an experimental study. Am J Transplant 6:2706–2713
23. MacPhee IA, Fredericks S, Tai T, Syrris P, Carter ND, Johnston A, Goldberg L, Holt DW (2004) The influence of pharmacogenetics on the time to achieve target tacrolimus concentrations after kidney transplantation. Am J Transplant 4:914–919
24. Kuypers DR, de Jonge H, Naesens M, Lerut E, Verbeke K, Vanrenterghem Y (2007) CYP3A5 and CYP3A4 but not MDR1 single-nucleotide polymorphisms determine long-term tacrolimus disposition and drug-related nephrotoxicity in renal recipients. Clin Pharmacol Ther 82:711–725
25. MacPhee IA, Fredericks S, Mohamed M, Moreton M, Carter ND, Johnston A, Goldberg L, Holt DW (2005) Tacrolimus pharmacogenetics: The CYP3A5*1 allele predicts low dose-normalised

tacrolimus blood concentrations in whites and South Asians. Transplantation 79:499–502
26. Quteineh L, Verstuyft C, Furlan V, Durrbach A, Letierce A, Ferlicot S, Taburet AM, Charpentier B, Becquemont L (2008) Influence of CYP3A5 genetic polymorphism on tacrolimus daily dose requirements and acute rejection in renal graft recipients. Basic Clin Pharmacol Toxicol 103:546–552
27. Chen JS, Li LS, Cheng DR, Ji SM, Sun QQ, Cheng Z, Wen JQ, Sha GZ, Liu ZH (2009) Effect of CYP3A5 genotype on renal allograft recipients treated with tacrolimus. Transplant Proc 41:1557–1561
28. Singh R, Srivastava A, Kapoor R, Sharma K, Mittal D (2009) Impact of CYP3A5 and CYP3A4 gene polymorphisms on dose requirement of calcineurin inhibitors, cyclosporine and tacrolimus, in renal allograft recipients of North India. Naunyn Schmiedebergs Arch Pharmacol 380:169–177
29. Zhao W, Elie V, Roussey G, Brochard K, Niaudet P, Leroy V, Loirat C, Cochat P, Cloarec S, André J, Garaix F, Bensman A, Fakhoury M, Jacqz-Aigrain E (2009) Population pharmacokinetics and pharmacogenetics of tacrolimus in de novo pediatric kidney transplant recipients. Clin Pharmacol Ther 86:609–618
30. Ferraresso M, Tirelli A, Ghio L, Grillo P, Martina V, Torresani E, Edefonti A (2007) Influence of the CYP3A5 genotype on tacrolimus pharmacokinetics and pharmacodynamics in young kidney transplant recipients. Pediatric Transplant 11:296–300
31. Zheng HX, Webber S, Zeevi A, Schuetz E, Zhang J, Bowman P, Boyle G, Law Y, Miller S, Lamba J, Burckart GJ (2003) Tacrolimus dosing in pediatric heart transplant patients is related to CYP3A5 and MDR1 gene polymorphisms. Am J Transplantation 3:477–483
32. Thervet E, Loriot MA, Barbier S, Buchler M, Ficheux M, Choukroun G, Toupance O, Touchard G, Alberti C, Le Pogamp P, Moulin B, Le Meur Y, Heng AE, Subra JF, Beaune P, Legendre C (2010) Optimization of initial tacrolimus dose using pharmacogenetic testing. Clin Pharmacol Ther 87:721–726
33. Kreutz R, Zurcher H, Kain S, Martus P, Offermann G, Beige J (2004) The effect of variable CYP3A5 expression on cyclosporine dosing, blood pressure and long-term graft survival in renal transplant patients. Pharmacogenetics 14:665–671
34. Fredericks S, Jorga A, MacPhee IA, Reboux S, Shiferaw E, Moreton M, Carter ND, Holt DW, Johnston A (2007) Multi-drug resistance gene-1 (MDR-1) haplotypes and the CYP3A5*1 genotype have no influence on ciclosporin dose requirements as assessed by C0 or C2 measurements. Clin Transplant 21:252–257
35. MacDonald A, Scarola J, Burke JT, Zimmerman JJ (2000) Clinical pharmacokinetics and therapeutic drug monitoring of sirolimus. Clin Ther 22(Suppl B):B101–B121
36. Anglicheau D, Le Corre D, Lechaton S, Laurent-Puig P, Kreis H, Beaune P, Legendre C, Thervet E (2005) Consequences of genetic polymorphisms for sirolimus requirements after renal transplant in patients on primary sirolimus therapy. Am J Transplant 5:595–603
37. Miao LY, Huang CR, Hou JQ, Qian MY (2007) Association study of ABCB1 and CYP3A5 gene polymorphisms with sirolimus trough concentration and dose requirements in Chinese renal transplant recipients. Biopharm Drug Dispos 29:1–5
38. Mourad M, Mourad G, Wallemacq P, Garrigue V, Van Bellingen C, Van KV, De Meyer M, Malaise J, Eddour DC, Lison D, Squifflet JP, Haufroid V (2005) Sirolimus and tacrolimus trough concentrations and dose requirements after kidney transplantation in relation to CYP3A5 and MDR1 polymorphisms and steroids. Transplantation 80:977–984
39. Renders L, Frisman M, Ufer M, Mosyagin I, Haenisch S, Ott U, Caliebe A, Dechant M, Braun F, Kunzendorf U, Cascorbi L (2007) CYP3A5 genotype markedly influences the pharmacokinetics of tacrolimus and sirolimus in kidney transplant recipients. Clin Pharmacol Ther 81:228–234
40. Webster AC, Lee VW, Chapman JR, Craig JC (2006) Target of rapamycin inhibitors (sirolimus and everolimus) for primary immunosuppression of kidney transplant recipients: A systematic review and meta-analysis of randomized trials. Transplantation 81:1234–1248
41. Saeki T, Ueda K, Tanagawara Y, Hori R, Komano T (1993) Human P-glycoprotein transports cyclosporin A and FK505. J Biol Chem 268:6077–6080
42. Miller DS, Fricker G, Drewe J (1997) p-Glycoprotein-mediated transport of a fluorescent rapamycin derivative in renal proximal tubule. J Pharmacol Exp Ther 282:440–444
43. Marzolini C, Paus E, Buclin T, Kim RB (2004) Polymorphisms in human MDR1 (P-glycoprotein): Recent advances and clinical relevance. Clin Pharmacol Ther 75:13–33
44. Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmoller J, Johne A, Cascorbi I, Gerloff T, Roots I, Eichelbaum M, Brinkmann U (2000) Functional polymorphisms of the human multidrug-resistance gene: Multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. Proc Natl Acad Sci USA 97:3473–3478
45. Anglicheau D, Verstuyft C, Laurent-Puig P, Becquemont L, Schlageter M-H, Cassinat B, Beaune P, Legendre C, Thervet E (2003) Association of the multidrug resistance-1 gene single-nucleotide polymorphisms with the tacrolimus dose requirements in renal transplant recipients. J Am Soc Nephrol 14:1889–1896
46. Kimchi-Sarfaty C, Oh JM, Kim IW, Sauna ZE, Calcagno AM, Ambudkar SV, Gottesman MM (2007) A "silent" polymorphism in the MDR1 gene changes substrate specificity. Science 315:525–528
47. Wang D, Johnson AD, Papp AC, Kroetz DL, Sadee W (2005) Multidrug resistance polypeptide 1 (MDR1, ABCB1) variant 3435C>T affects mRNA stability. Pharmacogenet Genomics 15:693–704
48. Chowbay B, Li H, David M, Cheung YB, Lee EJ (2005) Meta-analysis of the influence of MDR1 C3435T polymorphism on digoxin pharmacokinetics and MDR1 gene expression. Br J Clin Pharmacol 60:159–171
49. Masuda S, Goto M, Fukatsu S, Uesugi M, Ogura Y, Oike F, Kiuchi T, Takada Y, Tanaka K, Inui K (2006) Intestinal MDR1/ABCB1 level at surgery as a risk factor of acute cellular rejection in living-donor liver transplant patients. Clin Pharmacol Ther 79:90–102
50. Hawwa AF, McKiernan PJ, Shields M, Millership JS, Collier PS, McElnay JC (2009) Influence of ABCB1 polymorphisms and haplotypes on tacrolimus nephrotoxicity and dosage requirements in children with liver transplant. Br J Clin Pharmacol 68:413–421
51. Cattaneo D, Ruggenenti P, Baldelli S, Motterlini N, Gotti E, Sandrini S, Salvadori M, Segoloni G, Rigotti P, Donati D, Perico N, Remuzzi G (2009) ABCB1 genotypes predict cyclosporine-related adverse events and kidney allograft outcome. J Am Soc Nephrol 20:1404–1415
52. Fanta S, Niemi M, Jönsson S, Karlsson MO, Holmberg C, Neuvonen PJ, Hoppu K, Backman JT (2008) Pharmacogenetics of cyclosporine in children suggests an age-dependent influence of ABCB1 polymorphisms. Pharmacogenet Genomics 18:77–90
53. Dintaman JM, Silverman JA (1999) Inhibition of P-glycoprotein by D-alpha-tocopheryl polyethylene glycol 1000 succinate (TPGS). Pharm Res 16:1550–1556
54. Evans WE (2004) Pharmacogenetics of thiopurine S-methyltransferase and thiopurine therapy. Ther Drug Monit 26:186–191
55. Miura M, Satoh S, Inoue K, Kagaya H, Saito M, Inoue T, Habuchi T, Suzuki T (2008) Influence of CYP3A5, ABCB1 and NR1I2 polymorphisms on prednisolone pharmacokinetics in renal transplant recipients. Steroids 73:1052–1059
56. Miki Y, Suzuki T, Tazawa C, Blumberg B, Sasano H (2005) Steroid and xenobiotic receptor (SXR), cytochrome P450 3A4 and multidrug resistance gene 1 in human adult and fetal tissues. Mol Cell Endocrinol 231:75–85
57. Zheng HX, Webber SA, Zeevi A, Schuetz E, Zhang J, Lamba J, Boyle GJ, Wilson JW, Burckart GJ (2004) The impact of

pharmacogenomic factors on steroid dependency in pediatric heart transplant patients using logistic regression analysis. Pediatr Transplant 8:551–557
58. Middleton PG, Taylor PR, Jackson G, Proctor SJ, Dickinson AM (1998) Cytokine gene polymorphisms associating with severe acute graft-versus-host disease in HLA-identical sibling transplants. Blood 92:3943–3948
59. Benkali K, Prémaud A, Picard N, Rérolle JP, Toupance O, Hoizey G, Turcant A, Villemain F, Le Meur Y, Marquet P, Rousseau A (2009) Tacrolimus population pharmacokinetic-pharmacogenetic analysis and Bayesian estimation in renal transplant recipients. Clin Pharmacokinet 48:805–816
60. Li L, Li CJ, Zheng L, Zhang YJ, Jiang HX, Si-Tu B, Li Z (2011) Tacrolimus dosing in Chinese renal transplant recipients: a population-based pharmacogenetics study. Eur J Clin Pharmacol 67:787–795
61. Sombogaard F, van Schaik RH, Mathot RA, Budde K, van der WM, Vulto AG, Weimar W, Glander P, Essioux L, van Gelder T (2009) Interpatient variability in IMPDH activity in MMF-treated renal transplant patients is correlated with IMPDH type II 3757T>C polymorphism. Pharmacogenet Genomics 19:626–634
62. Grinyo J, Vanrenterghem Y, Nashan B, Vincenti F, Ekberg H, Lindpaintner K, Rashford M, Nasmyth-Miller C, Voulgari A, Spleiss O, Truman M, Essioux L (2008) Association of four DNA polymorphisms with acute rejection after kidney transplantation. Transpl Int 21:879–891
63. Wang J, Yang JW, Zeevi A, Webber SA, Girnita DM, Selby R, Fu J, Shah T, Pravica V, Hutchinson IV, Burckart GJ (2008) IMPDH1 gene polymorphisms and association with acute rejection in renal transplant patients. Clin Pharmacol Ther 83:711–717
64. Naesens M, Kuypers DR, Verbeke K, Vanrenterghem Y (2006) Multidrug resistance protein 2 genetic polymorphisms influence mycophenolic acid exposure in renal allograft recipients. Transplantation 82:1074–1084
65. van Schaik RH, van Agteren M, de Fijter JW, Hartmann A, Schmidt J, Budde K, Kuypers D, Le Meur Y, van der WM, Mamelok R, van Gelder T (2009) UGT1A9–275T>A/-2152C>T polymorphisms correlate with low MPA exposure and acute rejection in MMF/tacrolimus-treated kidney transplant patients. Clin Pharmacol Ther 86:319–327
66. Kuypers DR, Naesens M, Vermeire S, Vanrenterghem Y (2005) The impact of uridine diphosphate-glucuronosyltransferase 1A9 (UGT1A9) gene promoter region single-nucleotide polymorphisms T-275A and C-2152T on early mycophenolic acid dose-interval exposure in de novo renal allograft recipients. Clin Pharmacol Ther 78:351–361
67. Mirochnick M, Capparelli E, Connor J (1999) Pharmacokinetics of zidovudine in infants: a population analysis across studies. Clin Pharmacol Ther 66:16–24
68. Crettol S, Venetz JP, Fontana M, Aubert JD, Ansermot N, Fathi M, Pascual M, Eap CB (2008) Influence of ABCB1 genetic polymorphisms on cyclosporine intracellular concentration in transplant recipients. Pharmacogenet Genomics 18:307–315
69. Ansermot N, Rebsamen M, Chabert J, Fathi M, Gex-Fabry M, Daali Y, Besson M, Rossier M, Rudaz S, Hochstrasser D, Dayer P, Desmeules J (2008) Influence of ABCB1 gene polymorphisms and P-glycoprotein activity on cyclosporine pharmacokinetics in peripheral blood mononuclear cells in healthy volunteers. Drug Metab Lett 2:76–82
70. Barbari AG, Masri MA, Stephan AG, El Ghoul B, Rizk S, Mourad N, Kamel GS, Kilani HE, Karam AS (2006) Cyclosporine lymphocyte maximum level monitoring in de novo kidney transplant patients: A prospective study. Exp Clin Transplant 4:400–405
71. Falck P, Guldseth H, Asberg A, Midtvedt K, Reubsaet JL (2007) Determination of ciclosporin A and its six main metabolites in isolated T-lymphocytes and whole blood using liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 852:345–352
72. Capron A, Musuamba F, Latinne D, Mourad M, Lerut J, Haufroid V, Wallemacq PE (2009) Validation of a liquid chromatography-mass spectrometric assay for tacrolimus in peripheral blood mononuclear cells. Ther Drug Monit 31:178–186
73. Falck P, Asberg A, Guldseth H, Bremer S, Akhlaghi F, Reubsaet JL, Pfeffer P, Hartmann A, Midtvedt K (2008) Declining intracellular T-lymphocyte concentration of cyclosporine a precedes acute rejection in kidney transplant recipients. Transplantation 85:179–184
74. Singh D, Alexander J, Owen A, Rustom R, Bone M, Hammad A, Roberts N, Park K, Pirmohamed M (2004) Whole-blood cultures from renal-transplant patients stimulated ex vivo show that the effects of cyclosporine on lymphocyte proliferation are related to P-glycoprotein expression. Transplantation 77:557–561
75. Bandur S, Petrasek J, Hribova P, Novotna E, Brabcova I, Viklicky O (2008) Haplotypic structure of ABCB1/MDR1 gene modifies the risk of the acute allograft rejection in renal transplant recipients. Transplantation 86:1206–1213
76. Hebert MF, Dowling AL, Gierwatowski C, Lin YS, Edwards KL, Davis CL, Marsh CL, Schuetz EG, Thummel KE (2003) Association between ABCB1 (multidrug resistance transporter) genotype and post-liver transplantation renal dysfunction in patients receiving calcineurin inhibitors. Pharmacogenetics 13:661–674
77. Woodahl EL, Hingorani SR, Wang J, Guthrie KA, McDonald GB, Batchelder A, Li M, Schoch HG, McCune JS (2008) Pharmacogenomic associations in ABCB1 and CYP3A5 with acute kidney injury and chronic kidney disease after myeloablative hematopoietic cell transplantation. Pharmacogenomics J 8:248–255
78. Klauke B, Wirth A, Zittermann A, Bohms B, Tenderich G, Korfer R, Milting H (2008) No association between single nucleotide polymorphisms and the development of nephrotoxicity after orthotopic heart transplantation. J Heart Lung Transplant 27:741–745
79. Hauser IA, Schaeffeler E, Gauer S, Scheuermann EH, Wegner B, Gossmann J, Ackermann H, Seidl C, Hocher B, Zanger UM, Geiger H, Eichelbaum M, Schwab M (2005) ABCB1 genotype of the donor but not of the recipient is a major risk factor for cyclosporine-related nephrotoxicity after renal transplantation. J Am Soc Nephrol 16:1501–1511
80. Joy MS, Nickeleit V, Hogan SL, Thompson BD, Finn WF (2005) Calcineurin inhibitor-induced nephrotoxicity and renal expression of P-glycoprotein. Pharmacotherapy 25:779–789
81. Anglicheau D, Pallet N, Rabant M, Marquet P, Cassinat B, Meria P, Beaune P, Legendre C, Thervet E (2006) Role of P-glycoprotein in cyclosporine cytotoxicity in the cyclosporine-sirolimus interaction. Kidney Int 70:1019–1025
82. Goldfarb-Rumyantzev AS, Naiman N (2010) Genetic predictors of acute renal transplant rejection. Nephrol Dial Transplant 25:1039–1047
83. Woillard JB, Rerolle JP, Picard N, Rousseau A, Guillaudeau A, Munteanu E, Essig M, Drouet M, Le Meur Y, Marquet P (2010) Donor P-gp polymorphisms strongly influence renal function and graft loss in a cohort of renal transplant recipients on cyclosporine therapy in a long-term follow-up. Clin Pharmacol Ther 88:95–100
84. Kausman JY, Patel B, Marks SD (2008) Standard dosing of tacrolimus leads to overexposure in pediatric renal transplantation recipients. Pediatr Transplant 12:329–335

Answers:

1. b
2. a
3. c
4. d
5. b