Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines for *CYP3A5* Genotype and Tacrolimus Dosing

KA Birdwell[1,2], B Decker[3], JM Barbarino[4], JF Peterson[2,5], CM Stein[2,6], W Sadee[7], D Wang[7], AA Vinks[8,9], Y He[10], JJ Swen[11], JS Leeder[12], RHN van Schaik[13], KE Thummel[14], TE Klein[4], KE Caudle[15] and IAM MacPhee[16]

Tacrolimus is the mainstay immunosuppressant drug used after solid organ and hematopoietic stem cell transplantation. Individuals who express CYP3A5 (extensive and intermediate metabolizers) generally have decreased dose-adjusted trough concentrations of tacrolimus as compared with those who are CYP3A5 nonexpressers (poor metabolizers), possibly delaying achievement of target blood concentrations. We summarize evidence from the published literature supporting this association and provide dosing recommendations for tacrolimus based on *CYP3A5* genotype when known (updates at www.pharmgkb.org).

INTRODUCTION

Tacrolimus is a widely used immunosuppressive medication with a narrow therapeutic index and large between-patient pharmacokinetic variability, which is partly because of genetic variations in *CYP3A5*. The purpose of this guideline was to provide information relevant to the interpretation of *CYP3A5* genotype results to guide dosing of tacrolimus. Detailed guidelines for use of tacrolimus as well as analyses of cost effectiveness are not discussed. Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines are periodically updated at http://www.pharmgkb.org.

FOCUSED LITERATURE REVIEW

A systematic literature review focused on *CYP3A5* genotype and tacrolimus use (**Supplementary Material** online) was conducted.

GENE: *CYP3A5*

Enzymes in the cytochrome P450 (CYP) 3A family are responsible for the oxidative metabolism of tacrolimus. Four genes in this family have been described, but only *CYP3A4* and *CYP3A5* are thought to be relevant in adults. *CYP3A7* is only expressed in fetal liver and *CYP3A43* is of uncertain significance. There is a high degree of sequence homology between *CYP3A4* and *CYP3A5* and, thus, substrate overlap.[1] Both first pass metabolism and systemic clearance of drugs metabolized by *CYP3A5* are susceptible to genetically determined differences in enzyme expression. Variant alleles for *CYP3A5* (**3*, **6*, or **7*) may result in truncated mRNA with loss of expression of the functional protein in homozygotes or compound heterozygotes, or encode nonfunctional protein.[2] The physiological function of *CYP3A5* is unclear. Whereas *CYP3A4* poor metabolizers are rare, absence of functional *CYP3A5* is the norm in many populations. This is most notable for white people with 80–85% of the population being homozygous for the variant *CYP3A5*3* allele.[3] It seems that retention of *CYP3A5* expression has been under some evolutionary selection pressure in populations originating close to the equator, and loss of this positive selection pressure with migration away from the equator, possibly related to the benefits derived from a sodium retaining phenotype in hot climates.[4]

Genetic test interpretation

Each named * allele is defined by the genotype at one or more specific single-nucleotide polymorphisms (**Supplementary**

[1]Division of Nephrology Department of Medicine, Vanderbilt University, Nashville, Tennessee, USA; [2]Department of Medicine, Vanderbilt University, Nashville, Tennessee, USA; [3]Division of Nephrology and Division of Clinical Pharmacology, Indiana University School of Medicine, Indianapolis, Indiana, USA; [4]Department of Genetics, Stanford University, Stanford, California, USA; [5]Department of Biomedical Informatics, Vanderbilt University, Nashville, Tennessee, USA; [6]Department of Pharmacology, Vanderbilt University, Nashville, Tennessee, USA; [7]Center for Pharmacogenomics, School of Medicine, The Ohio State University, Columbus, Ohio, USA; [8]Division of Clinical Pharmacology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA; [9]Department of Pediatrics, College of Medicine, University of Cincinnati, Cincinnati, Ohio, USA; [10]Institute of Clinical Pharmacology, Central South University, Changsha, Hunan, Peoples Republic of China; [11]Department of Clinical Pharmacy and Toxicology, Leiden University Medical Center, Leiden, The Netherlands; [12]Division of Clinical Pharmacology and Therapeutic Innovation, Department of Pediatrics, Children's Mercy Hospitals and Clinics, Kansas City, Missouri, USA; [13]Department of Clinical Chemistry, Erasmus MC Rotterdam, The Netherlands; [14]Department of Pharmaceutics, University of Washington, Seattle, Washington, USA; [15]Department of Pharmaceutical Sciences, St. Jude Children's Research Hospital, Memphis, Tennessee, USA; [16]Institute of Medical and Biomedical Education, Renal Medicine, St. George's, University of London, London, UK. Correspondence: K Birdwell (kelly.birdwell@vanderbilt.edu; cpic@pharmgkb.org)

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Table 1 Assignment of likely metabolism phenotypes based on *CYP3A5* diplotypes

Likely phenotype	Genotypes	Examples of diplotypes[a]
Extensive metabolizer (CYP3A5 expresser)	An individual carrying two functional alleles	*1/*1
Intermediate metabolizer (CYP3A5 expresser)	An individual carrying one functional allele and one nonfunctional allele	*1/*3, *1/*6, *1/*7
Poor metabolizer (CYP3A5 nonexpresser)	An individual carrying two nonfunctional alleles	*3/*3, *6/*6, *7/*7, *3/*6, *3/*7, *6/*7

[a]Additional rare variants, such as *CYP3A5*2*, *8*, and *9 may be found, which are of unknown functional significance. However, if a copy of *1 is present, expected phenotype would be intermediate metabolizer.

Table S1 online). The function associated with these allelic variants is summarized in **Supplementary Table S2** online. The assignment of the likely *CYP3A5* phenotype, based on * allele diplotypes, is summarized in **Table 1**. *CYP3A5* alleles have been extensively studied in groups with diverse geographic ancestries (**Supplementary Table S3** online). One of the limitations inherent in a genotype-only test is that rare or *de novo* variants may not be included in commercially available genotyping tests.

Available genetic test options

See Supplementary material online and www.PharmGKB.org for more information on commercially available clinical testing options.

Incidental findings

No clear diseases or conditions have been linked to variation in *CYP3A5* unrelated to medication use.

Other considerations

Most genetic tests examine the presence of the *CYP3A5*3* allele. Less commonly observed and possibly not included, depending on the assay, are *CYP3A5*6* and *CYP3A5*7* alleles, which are associated with decreased *CYP3A5* activity, similar to that observed for *CYP3A5*3*. Additional rare variants, such as *2, *8, and *9 may be found, which are of unknown functional significance.

In this guideline, we use phenotype nomenclature consistent with other CYP enzymes (i.e., extensive metabolizer, intermediate metabolizer, and poor metabolizer). Typically with other CYP enzymes, an extensive metabolizer would be classified as a "normal" metabolizer, and, therefore, the drug dose would not change based on the patient's genotype. However, in the case of *CYP3A5* and tacrolimus, a *CYP3A5* expresser (i.e., *CYP3A5* extensive metabolizer or intermediate metabolizer) would require a higher recommended starting dose and the *CYP3A5* nonexpresser (i.e., poor metabolizer) would require the standard recommended starting dose. *CYP3A5* expressers constitute the minority in European populations. Additional clinician education might be necessary to avoid confusion.

DRUG: TACROLIMUS

Tacrolimus was approved in 1994 by the US Food and Drug Administration as an antirejection medication for liver transplantation. Tacrolimus is a macrolide that binds to its cytoplasmic protein receptor, FK binding protein 12, in T lymphocytes. This complex binds calcineurin, preventing dephosphorylation and nuclear translocation of nuclear factor of activated T cells, ultimately inhibiting interleukin-2 production and T lymphocyte activation.[5] Today, tacrolimus is one of the most widely prescribed immunosuppressive medications in solid organ transplantation and is also increasingly being used in the treatment of glomerulonephritis and graft-vs.-host disease. Tacrolimus in clinical use is complicated by its high between-patient variability in pharmacokinetics as well as its narrow therapeutic index. This may lead to underexposure, potentially increasing the risk of rejection, or overexposure, with risk of toxicity, including nephrotoxicity, hypertension, neurotoxicity, and hyperglycemia.[6] As a result, current management of tacrolimus usually includes therapeutic drug monitoring (TDM).[7] Clinically, TDM is done using tacrolimus whole blood trough concentrations, which have been shown to correlate with area under the curve measurements.[8,9] Previous studies have shown a relationship between lower tacrolimus exposure and acute rejection and higher exposure and toxicities (see **Supplementary Table S4** online). The addition of induction therapies and mycophenolate has allowed lower target trough concentrations, as exemplified by the Symphony study in kidney transplant, in which patients randomized to low dose tacrolimus with target concentrations of 3–7 ng/mL had less rejection and better kidney function.[10] Although TDM is helpful for adjusting subsequent doses based on blood concentrations, it provides no information for the initial dose. Individual differences in first pass metabolism (see below) may delay reaching target blood concentrations with the initial selected dose. Furthermore, although achieving target blood concentrations does not always ensure efficacy or diminish adverse events, target blood concentrations specific for organ type and time posttransplant are available in the package insert and established by consensus guidelines.[7,11]

Demethylation and hydroxylation of tacrolimus occurs by hepatic and intestinal CYP3A isoforms (CYP3A4 and CYP3A5). Tacrolimus is also a substrate for the multidrug efflux transporter P-glycoprotein (encoded by the *ABCB1* gene, previously called *MDR1*), which is expressed on various epithelial and endothelial cells and lymphocytes. First pass metabolism by CYP3A4 and CYP3A5 in the intestine and liver contributes to the poor oral bioavailability of tacrolimus, which is only around 20%. Tacrolimus is cleared through hepatic metabolism by CYP3A4 and CYP3A5 with biliary excretion of metabolites.

Linking genetic variability to variability in drug-related phenotypes

Blood concentrations of tacrolimus are strongly influenced by *CYP3A5* genotype, with substantial evidence linking *CYP3A5*

Table 2 Dosing recommendations for tacrolimus based on *CYP3A5* phenotype

CYP3A5 phenotype[a]	Implications for tacrolimus pharmacologic measures	Therapeutic recommendations[b]	Classification of recommendations[c]
Extensive metabolizer (CYP3A5 expresser)	Lower dose-adjusted trough concentrations of tacrolimus and decreased chance of achieving target tacrolimus concentrations.	Increase starting dose 1.5–2 times recommended starting dose.[d] Total starting dose should not exceed 0.3 mg/kg/day. Use therapeutic drug monitoring to guide dose adjustments.	Strong
Intermediate metabolizer (CYP3A5 expresser)	Lower dose-adjusted trough concentrations of tacrolimus and decreased chance of achieving target tacrolimus concentrations.	Increase starting dose 1.5–2 times recommended starting dose.[a] Total starting dose should not exceed 0.3 mg/kg/day. Use therapeutic drug monitoring to guide dose adjustments.	Strong
Poor metabolizer (CYP3A5 nonexpresser)	Higher ("normal") dose-adjusted trough concentrations of tacrolimus and increased chance of achieving target tacrolimus concentrations.	Initiate therapy with standard recommended dose. Use therapeutic drug monitoring to guide dose adjustments.	Strong

[a]Typically, with other CYP enzymes, an extensive metabolizer would be classified as a "normal" metabolizer, and, therefore, the drug dose would not change based on the patient's genotype. However, in the case of CYP3A5 and tacrolimus, a CYP3A5 expresser (i.e., CYP3A5 extensive metabolizer or intermediate metabolizer) would require a higher recommended starting dose and the CYP3A5 nonexpresser (i.e., poor metabolizer) would require the standard recommended starting dose. [b]This recommendation includes the use of tacrolimus in kidney, heart, lung, and hematopoietic stem cell transplant patients, and liver transplant patients in which the donor and recipient genotypes are identical. [c]Rating scheme is described in **Supplementary Data** online. [d]Further dose adjustments or selection of alternative therapy may be necessary because of other clinical factors (e.g., medication interactions, or hepatic function).

genotype with phenotypic variability (see **Supplementary Table S4** online). The application of a grading system to the evidence linking genotypic to phenotypic variability indicates a high quality of evidence in the majority of cases (see **Supplementary Table S4** online). The evidence described below and in **Supplementary Table S4** online provides the basis for the dosing recommendations in **Table 2**.

In kidney, heart, and lung transplant patients, over 50 studies have found that individuals with the *CYP3A5*1/*1* or *CYP3A5*1/*3* genotype have significantly lower dose-adjusted trough concentrations of tacrolimus as compared to those with the *CYP3A5*3/*3* genotype, with **1* carriers requiring 1.5–2 times the dose to achieve similar blood concentrations (see **Supplementary Table S4** online). *CYP3A5*1/*3* is believed to explain up to 45% of the variability in tacrolimus dose.[12] Because of the rarity of the *CYP3A5*6* and *CYP3A5*7* alleles in most populations (see **Supplementary Table S3** online), their impact on tacrolimus dose-adjusted trough concentrations has only been examined in combined analyses with *CYP3A5*3*.[13,14] However, because both alleles result in a nonfunctional protein,[13] their impact on tacrolimus clearance and dose-adjusted trough concentrations is presumed to be identical to *CYP3A5*3*.

Therapeutic recommendations

This guideline is not intended to recommend for or against *CYP3A5* genotype testing in transplants. The current evidence for utility of *CYP3A5* genotyping to guide tacrolimus dosing is limited to *CYP3A5*'s effect on tacrolimus pharmacokinetic parameters, with no direct evidence for improved clinical immunosuppressant outcome. As a result, we are not recommending whether or not to test for the *CYP3A5* genotype in transplants, but we are providing recommendations on how to use *CYP3A5* genotype information if it is known. Because it is typical clinical practice to achieve target blood concentrations as quickly as possible, we do recommend if *CYP3A5* genotype is known, to individualize initial tacrolimus treatment using *CYP3A5* genotype to guide tacrolimus dosing, as outlined in **Table 2**. Transplant recipients with the poor metabolizer phenotype (**Table 1**) should receive the standard dosing of medication based on the tacrolimus package insert. Those recipients with an extensive or intermediate metabolizer phenotype will generally require an increased dose of tacrolimus to achieve therapeutic drug concentrations. We recommend a dose 1.5–2 times higher than standard dosing, but not to exceed 0.3 mg/kg/day, followed by TDM, given the risk of arterial vasoconstriction, hypertension, and nephrotoxicity that can occur with supratherapeutic tacrolimus concentrations.[15,16] In addition, concomitant medications, abnormal liver function, or presence of clinical conditions, such as diarrhea, must be taken into consideration when dosing tacrolimus (see Other Considerations below).

Given the availability of TDM, genetic testing is most helpful before initiation of the drug in order to more rapidly achieve therapeutic drug concentrations. This was illustrated in a randomized controlled trial by Thervet *et al.*,[17] in which target tacrolimus blood concentrations were achieved earlier in new kidney transplant recipients whose tacrolimus dose was chosen based on *CYP3A5* genotype vs. a control group that started tacrolimus based on standard weight-based dosing. In this study, patients received induction therapy with either basiliximab or antithymocyte globulin. Extensive metabolizers in the genotyped-dosed group had an increase in tacrolimus dose to 0.3 mg/kg/day, whereas the poor metabolizers had a decrease to 0.15 mg/kg/day, and the control group received 0.2 mg/kg/day. Therapeutic drug monitoring was used in both groups. At three days after starting

treatment with tacrolimus, significantly more of the transplant recipients in the genotyping group compared with control recipients had achieved target range (43.2% vs. 29.1%, respectively).[17] However, it should be noted that tacrolimus was not started until day seven while awaiting genotyping test results, which may differ from standard treatments with a start of tacrolimus at the time of transplantation. No differences were seen in patient survival, nephrotoxicity, or acute rejection between the groups over the three-month follow-up. With this study as the only published randomized control trial, more data are needed to understand if dosing tacrolimus by genotype will affect clinical outcomes. However, a recent meta-analysis including 21 studies evaluating the effect of *CYP3A5* polymorphism on kidney transplant recipients concluded that there is a significantly increased risk for transplant rejection for those with the *CYP3A5*1/*1* or *CYP3A5*1/*3* genotype ($P = 0.04$; odds ratio = 1.32). Furthermore, patients with the *CYP3A5*3/*3* (nonexpresser) genotype exhibited dose-adjusted trough concentrations 1.8–2.5 times higher than *CYP3A5* expressers during the first year after transplantation.[18]

Thus, at present, there is no definitive evidence to indicate that genotype-guided dosing for tacrolimus affects long-term clinical outcomes. However, there is strong evidence to support its effect on achieving target trough whole blood concentrations, which is routine clinical practice for most centers (see **Supplementary Table S4** online). Besides initial dose, genotype-guided dosing may also be useful in patients in whom achieving therapeutic blood concentrations has been difficult, where the genotype may provide some additional information to discern the reason.

In liver transplant recipients, the *CYP3A5* genotype of the donor liver may not be the same as the *CYP3A5* genotype of the recipient intestine. In these cases, it may be necessary to account for both the donor and recipient genotypes when determining the dose. However, studies to date have been inconclusive as to the relative influence of the donor and recipient genotypes, and whether donor liver and recipient intestinal genotypes come into play at different points posttransplant. Although some studies show that the donor genotype affects dose-adjusted trough concentrations from the first week posttransplant,[19,20] others show that it does not begin to play a role until the second week or even the sixth month posttransplant.[21–23] Evidence is also conflicting for recipient intestinal genotype: a few studies show that it never significantly affects tacrolimus concentrations,[20,22,23] whereas others show its influence on concentrations is only significant up to the point at which the donor genotype becomes significant.[21,24] Because of the small number of studies analyzing these cases, as well as inconsistent results, this guideline recommendation only includes kidney, heart, lung, and hematopoietic stem cell transplant patients, and liver transplant patients in which the donor and recipient genotypes are identical.

Pediatrics

The effect of *CYP3A5* genotype on dose-corrected tacrolimus concentration in pediatric populations has been studied in several clinical settings, including heart[25,26] and liver transplantation,[27] but most extensively after kidney transplantation.[28–33] Unfortunately, available data vary in terms of study duration after transplant and inclusion of additional factors that impact the dose-exposure relationship. In general, although the dose-exposure relationship changes over time regardless of genotype, dose-corrected tacrolimus trough concentrations are 1.5 to 2-fold higher in kidney transplant patients with *CYP3A5*3/*3* genotypes compared with patients with *CYP3A5*1/*1* or **1/*3* genotypes over the first two[31] to four[29] weeks posttransplant, at six months,[32] and throughout the first year posttransplant.[28,30,33] However, patient age and concurrent drug therapy also contribute to variability in the tacrolimus dose-exposure relationship in children. For example, postpubertal renal transplant patients (age >12 years) have higher dose-corrected tacrolimus concentrations compared with younger children in the first two to three week posttransplantation period[31,34] or over the first year posttransplant,[30,33,34] indicative of a lower dose requirement to achieve a comparable target concentration.[33] Thus, for children and adolescents with at least one *CYP3A5*1* allele, a 1.5 to 2-fold increase in dose followed by TDM as recommended for adults seems appropriate.

The **Supplementary Material** online contains example clinical decision support tools that can be used within electronic health records, which assist clinicians to use genetic information to optimize drug therapy. Clinical implementation resources include cross-references for drug and gene names to widely used terminologies and standardized nomenclature systems (**Supplementary Tables S5 and S6** online), workflow diagrams (**Supplementary Figures S1 and S2** online), and example text for documentation in the electronic health record and point-of care alerts (**Supplementary Tables S7 and S8** online).

Recommendations for incidental findings

Not applicable.

Other considerations

Several drugs (drug-drug interactions) are important to consider, especially nondihydropyridine calcium channel blockers and azole antifungals that are commonly coadministered in the transplant population. The drug interaction with the azole antifungals has been reported to be less profound in CYP3A5 expressers.[35,36] For additional information on tacrolimus drug interactions, see the review by van Gelder.[37] Specific patient factors, such as fasting or diarrhea, may cause altered absorption that can affect tacrolimus concentrations. This has been extensively reviewed in an article by Staatz and Tett.[38]

Additional genetic variants described in the literature but with unclear effects on tacrolimus metabolism because of either limited or conflicting studies include *CYP3A4*22*, *POR*28*, *PPAR alpha*, and *ABCB1*. A critical issue in predicting tacrolimus clearance *in vivo* is the relative contribution of CYP3A4 compared to CYP3A5 to its metabolism. Because of the complete loss of metabolic activity with the *CYP3A5*3* allele, the impact of variation in *CYP3A4* may be high in those with no CYP3A5 expression. Of note, donor *CYP3A5* genotype may play a role in pharmacodynamics. In kidney transplant recipients, the *CYP3A5* genotype together with the donor *ABCB1* genotype may affect the susceptibility of the kidney for tacrolimus nephrotoxicity.[39] Although

the current guideline refers to using the recipient *CYP3A5* genotype to guide selection of the optimal initial dose for tacrolimus, we can expect the potential for greater predictive value in polygenic algorithms.

A further confounding factor is the influence of ethnicity. Although it was initially hypothesized that individuals of African origin require high doses of tacrolimus because of expression of CYP3A5, these individuals have a high dose requirement for tacrolimus, irrespective of *CYP3A5* genotype.[40] This finding suggests other factors besides *CYP3A5* genotype are important in individuals of sub-Saharan African descent.

Potential benefits and risks for the patient

Tacrolimus dosing is routinely directed by TDM. Yet, for patients who have an existing *CYP3A5* genotyping result, *CYP3A5* genotype-guided dosing can achieve initial target tacrolimus concentrations more quickly after transplantation even when TDM-based titration is used.[17] Faster achievement of target concentrations could potentially reduce the risk of graft rejection because of underexposure and toxicity because of overexposure. However, prospective clinical trials are needed to assess if *CYP3A5* genotype-guided dosing improves these outcomes.

CYP3A5 genotyping cannot replace therapeutic drug monitoring, as other factors (i.e., demographic factors, drug–drug interactions, and genetic variation affecting tacrolimus pharmacodynamics) also influence tacrolimus dose requirements. As with any genetic test, a possible risk is the misreporting or misinterpretation of genotype test results. An error in genotyping could result in an increase in tacrolimus dose and subsequently overexposure. However, anticipated effects are limited because of stringent TDM.

Caveats: appropriate use and/or potential misuse of genetic tests

Dose alterations based on *CYP3A5* genotype may result in faster achievement of target tacrolimus concentrations with fewer dose adjustments.[17] In addition, several clinical caveats apply: (1) clinical factors (e.g., age, concomitant drugs) affect tacrolimus concentrations; (2) variants in genes other than *CYP3A5* may affect tacrolimus pharmacokinetics and therefore overall exposure; (3) the relationship between tacrolimus concentration and efficacy and toxicity varies among individuals (pharmacodynamic variability); (4) the genetic determinants of tacrolimus efficacy and toxicity (pharmacodynamics) are not defined; (5) altering initial tacrolimus dosing based on *CYP3A5* genotype has not been shown to improve efficacy or reduce toxicity; and (6) monitoring of tacrolimus blood concentration remains indicated during treatment. With the expansion of our knowledge base, further refinement of the genotype-based dosing recommendations may be required.

DISCLAIMER

Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines reflect expert consensus based on clinical evidence and peer-reviewed literature available at the time they are written, and are intended only to assist clinicians in decision-making, as well as to identify questions for further research. New evidence may have emerged since the time a guideline was submitted for publication. Guidelines are limited in scope and are not applicable to interventions or diseases not specifically identified. Guidelines do not account for all individual variations among patients and cannot be considered inclusive of all proper methods of care or exclusive of other treatments. It remains the responsibility of the health care provider to determine the best course of treatment for the patient. Adherence to any guideline is voluntary, with the ultimate determination regarding its application to be solely made by the clinician and the patient. CPIC assumes no responsibility for any injury to persons or damage to property related to any use of CPIC's guidelines, or for any errors or omissions.

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CONFLICT OF INTEREST

W.S. and D.W. have a patent pending for a combined *CYP3A4/5* genotype panel. All other authors declare no conflicts.

Additional Supporting Information may be found in the online version of this article.

1. Finta, C. & Zaphiropoulos, P.G. The human cytochrome P450 3A locus. Gene evolution by capture of downstream exons. *Gene* **260**, 13–23 (2000).
2. Kuehl, P. *et al*. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat. Genet.* **27**, 383–391 (2001).
3. van Schaik, R.H., van der Heiden, I.P., van den Anker, J.N. & Lindemans, J. CYP3A5 variant allele frequencies in Dutch caucasians. *Clin. Chem.* **48**, 1668–1671 (2002).
4. Thompson, E.E., Kuttab-Boulos, H., Witonsky, D., Yang, L., Roe, B.A. & Di Rienzo, A. CYP3A variation and the evolution of salt-sensitivity variants. *Am. J. Hum. Genet.* **75**, 1059–1069 (2004).
5. Bowman, L.J. & Brennan, D.C. The role of tacrolimus in renal transplantation. *Expert Opin. Pharmacother.* **9**, 635–643 (2008).
6. Kershner, R.P. & Fitzsimmons, W.E. Relationship of FK506 whole blood concentrations and efficacy and toxicity after liver and kidney transplantation. *Transplantation* **62**, 920–926 (1996).
7. Wallemacq, P. *et al*. Opportunities to optimize tacrolimus therapy in solid organ transplantation: report of the European consensus conference. *Ther. Drug Monit.* **31**, 139–152 (2009).
8. Undre, N.A. & Stevenson, P.J. European Tacrolimus Heart Study Group. Pharmacokinetics of tacrolimus in heart transplantation. *Transplant. Proc.* **34**, 1836–1838 (2002).
9. Braun, F. *et al*. Therapeutic drug monitoring of tacrolimus early after liver transplantation. *Transplant. Proc.* **34**, 1538–1539 (2002).

10. Ekberg, H. *et al*. Reduced exposure to calcineurin inhibitors in renal transplantation. *N. Engl. J. Med.* **357**, 2562–2575 (2007).
11. Bouamar, R. *et al*. Tacrolimus predose concentrations do not predict the risk of acute rejection after renal transplantation: a pooled analysis from three randomized-controlled clinical trials(†). *Am. J. Transplant.* **13**, 1253–1261 (2013).
12. Haufroid, V. *et al*. The effect of CYP3A5 and MDR1 (ABCB1) polymorphisms on cyclosporine and tacrolimus dose requirements and trough blood levels in stable renal transplant patients. *Pharmacogenetics* **14**, 147–154 (2004).
13. Santoro, A. *et al*. Pharmacogenetics of calcineurin inhibitors in Brazilian renal transplant patients. *Pharmacogenomics* **12**, 1293–1303 (2011).
14. Zheng, S. *et al*. Measurement and compartmental modeling of the effect of CYP3A5 gene variation on systemic and intrarenal tacrolimus disposition. *Clin. Pharmacol. Ther.* **92**, 737–745 (2012).
15. Morales, J.M., Andres, A., Rengel, M. & Rodicio, J.L. Influence of cyclosporin, tacrolimus and rapamycin on renal function and arterial hypertension after renal transplantation. *Nephrol. Dial. Transplant.* **16** (suppl. 1), 121–124 (2001).
16. Nankivell, B.J., Borrows, R.J., Fung, C.L., O'Connell, P.J., Allen, R.D. & Chapman, J.R. The natural history of chronic allograft nephropathy. *N. Engl. J. Med.* **349**, 2326–2333 (2003).
17. Thervet, E. *et al*. Optimization of initial tacrolimus dose using pharmacogenetic testing. *Clin. Pharmacol. Ther.* **87**, 721–726 (2010).
18. Rojas, L. *et al*. Effect of CYP3A5*3 on kidney transplant recipients treated with tacrolimus: a systematic review and meta-analysis of observational studies. *Pharmacogenomics J.* **15**, 38–48 (2015).
19. Fukudo, M. *et al*. Impact of MDR1 and CYP3A5 on the oral clearance of tacrolimus and tacrolimus-related renal dysfunction in adult living-donor liver transplant patients. *Pharmacogenet. Genomics* **18**, 413–423 (2008).
20. Li, D., Zhu, J.Y., Gao, J., Wang, X., Lou, Y.Q. & Zhang, G.L. Polymorphisms of tumor necrosis factor-alpha, interleukin-10, cytochrome P450 3A5 and ABCB1 in Chinese liver transplant patients treated with immunosuppressant tacrolimus. *Clin. Chim. Acta* **383**, 133–139 (2007).
21. Muraki, Y. *et al*. Impact of CYP3A5 genotype of recipients as well as donors on the tacrolimus pharmacokinetics and infectious complications after living-donor liver transplantation for Japanese adult recipients. *Ann. Transplant.* **16**, 55–62 (2011).
22. Wei-lin, W. *et al*. Tacrolimus dose requirement in relation to donor and recipient ABCB1 and CYP3A5 gene polymorphisms in Chinese liver transplant patients. *Liver Transpl.* **12**, 775–780 (2006).
23. Yu, S. *et al*. Influence of CYP3A5 gene polymorphisms of donor rather than recipient to tacrolimus individual dose requirement in liver transplantation. *Transplantation* **81**, 46–51 (2006).
24. Ji, E., Choi, L., Suh, K.S., Cho, J.Y., Han, N. & Oh, J.M. Combinational effect of intestinal and hepatic CYP3A5 genotypes on tacrolimus pharmacokinetics in recipients of living donor liver transplantation. *Transplantation* **94**, 866–872 (2012).
25. Zheng, H. *et al*. Tacrolimus dosing in pediatric heart transplant patients is related to CYP3A5 and MDR1 gene polymorphisms. *Am. J. Transplant.* **3**, 477–483 (2003).
26. Gijsen, V. *et al*. Age and CYP3A5 genotype affect tacrolimus dosing requirements after transplant in pediatric heart recipients. *J. Heart Lung Transplant.* **30**, 1352–1359 (2011).
27. Guy-Viterbo, V. *et al*. Influence of donor-recipient CYP3A4/5 genotypes, age and fluconazole on tacrolimus pharmacokinetics in pediatric liver transplantation: a population approach. *Pharmacogenomics* **15**, 1207–1221 (2014).
28. Ferraresso, M. *et al*. Influence of the CYP3A5 genotype on tacrolimus pharmacokinetics and pharmacodynamics in young kidney transplant recipients. *Pediatr. Transplant.* **11**, 296–300 (2007).
29. Zhao, W. *et al*. Population pharmacokinetics and pharmacogenetics of tacrolimus in de novo pediatric kidney transplant recipients. *Clin. Pharmacol. Ther.* **86**, 609–618 (2009).
30. Ferraris, J.R. *et al*. Influence of CYP3A5 polymorphism on tacrolimus maintenance doses and serum levels after renal transplantation: age dependency and pharmacological interaction with steroids. *Pediatr. Transplant.* **15**, 525–532 (2011).
31. de Wildt, S.N. *et al*. The interactions of age, genetics, and disease severity on tacrolimus dosing requirements after pediatric kidney and liver transplantation. *Eur. J. Clin. Pharmacol.* **67**, 1231–1241 (2011).
32. García-Roca, P. *et al*. CYP3A5 polymorphism in Mexican renal transplant recipients and its association with tacrolimus dosing. *Arch. Med. Res.* **43**, 283–287 (2012).
33. Lalan, S. *et al*. Effect of CYP3A5 genotype, steroids, and azoles on tacrolimus in a pediatric renal transplant population. *Pediatr. Nephrol.* **29**, 2039–2049 (2014).
34. Kausman, J.Y., Patel, B. & Marks, S.D. Standard dosing of tacrolimus leads to overexposure in pediatric renal transplantation recipients. *Pediatr. Transplant.* **12**, 329–335 (2008).
35. Kuypers, D.R., de Jonge, H., Naesens, M. & Vanrenterghem, Y. Effects of CYP3A5 and MDR1 single nucleotide polymorphisms on drug interactions between tacrolimus and fluconazole in renal allograft recipients. *Pharmacogenet. Genomics* **18**, 861–868 (2008).
36. Chandel, N., Aggarwal, P.K., Minz, M., Sakhuja, V., Kohli, K.K. & Jha, V. CYP3A5*1/*3 genotype influences the blood concentration of tacrolimus in response to metabolic inhibition by ketoconazole. *Pharmacogenet. Genomics* **19**, 458–463 (2009).
37. van Gelder, T. Drug interactions with tacrolimus. *Drug Saf.* **25**, 707–712 (2002).
38. Staatz, C.E. & Tett, S.E. Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation. *Clin. Pharmacokinet.* **43**, 623–653 (2004).
39. Moore, J. *et al*. Donor ABCB1 variant associates with increased risk for kidney allograft failure. *J. Am. Soc. Nephrol.* **23**, 1891–1899 (2012).
40. Macphee, I.A. *et al*. Tacrolimus pharmacogenetics: the CYP3A5*1 allele predicts low dose-normalized tacrolimus blood concentrations in whites and South Asians. *Transplantation* **79**, 499–502 (2005).