Challenges and Opportunities in Pediatric Heart Failure and Transplantation

Pharmacogenomics
Personalizing Pediatric Heart Transplantation

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In recent years, outcomes for pediatric cardiac transplantation (PCTx) have steadily improved, with current 5-year overall survival rates estimated at 83%. This progress may be attributed to improvements in pretransplantation management, selection of donor hearts, surgical technique, prevention and treatment of rejection, and minimization of treatment-related adverse events. Despite these advances, children who receive cardiac transplants experience significant morbidity and mortality, and there is considerable variability in outcomes, much of which cannot be explained by known clinical risk factors.

One source of interindividual variability is genetic variation in the host. There is growing evidence that genomic variation leads to differences in immune response, response to therapies, and susceptibility to adverse outcomes such as malignancy and infection. This review will focus on the effect of variation in genes that encode enzymes, transporters, and drug target molecules that influence drug response. After review of the foundational principles of pharmacogenomics, evidence for the pharmacogenomic effects on cyclosporine, tacrolimus, azathioprine, mycophenolate mofetil (MMF), and the mammalian target of rapamycin (mTOR) inhibitors sirolimus will be reviewed and clinical implications of these findings discussed. Given the limited nature of evidence from studies of PCTx patients, support will also be drawn from non-PCTx research and pediatric renal transplantation. Genetic studies of nonpharmacogenes such as those in immunomodulatory pathways and variation in the donor genotype may contribute to risk prediction but are beyond the scope of this review.

Principles of Pharmacogenomics

Genetic Variation
DNA variation can alter biological function through several mechanisms (Figure 1). Variation within the coding sequence or in intron/exon borders can change the protein product through changes in start codons, stop codons, splice sites, or within the coding sequence, leading to nonsense-mediated decay or altered protein function. Other variants associated with pharmacogenomic outcomes are not predicted to change the encoded protein. These synonymous and intergenic variants may have direct functional effects via altered regulation of expression, or they may serve as a marker for the presence of another functional variant. Thus, important pharmacogenomic variation can occur anywhere in the genome. Individual variants are denoted by their reference single-nucleotide polymorphism cluster ID (rsID, eg, rs1045642). The pattern of variants in an allele defines the haplotype, indicated by the “star allele” designation (eg, CYP3A5*3). The rates of occurrence for specific genotypes and haplotypes vary across populations; some variants are ancestry specific, whereas others have been identified with varying frequency in all ancestries studied to date.

Pharmacogenomic Pathways
Pharmacogenomic variation may influence drug effect through pharmacokinetic mechanisms, affecting absorption, distribution, metabolism, and excretion, or via pharmacodynamic mechanisms, altering drug effects (Figure 1). Variations in drug metabolism genes are among the most well-characterized sources of pharmacogenomic variability. For example, cytochrome P450 (CYP) 2D6 enzyme activity is required for O-demethylation of the prodrug codeine to the active metabolite, morphine. Because of genetic variation, a subset of the population has no CYP2D6 activity (“poor metabolizers”), which results in greatly reduced morphine formation and insufficient pain relief. Conversely, individuals with multiple functional copies of CYP2D6 (“ultrarapid metabolizers”) rapidly convert codeine to morphine, which causes symptoms similar to those of overdose. Variations in drug metabolism genes may also affect conversion of active drugs or metabolites to inactive compounds. Warfarin is inactivated by CYP2C9; individuals with loss-of-function CYP2C9 variants require lower doses of warfarin to reach target levels of anticoagulation.

Pharmacogenomic variants are not restricted to drug metabolism genes but can also be found in genes that encode drug transporters and targets. The OATP1B1 transporter (encoded by SLCO1B1) facilitates hepatocellular uptake of statin medications. Individuals with inactive OATP1B1 are at increased risk for muscle toxicity, specifically with high doses of simvastatin. Vitamin K epoxide reductase (VKORC1) is the target of warfarin. Variation in the promoter region of the VKORC1 gene affects gene transcription and thus modulates the warfarin dose required to achieve therapeutic effect.

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The online-only Data Supplement is available with this article at http://circ.ahajournals.org/lookup/suppl/doi:10.1161/CIRCULATIONAHA.114.001382/-/DC1.

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Circulation is available at http://circ.ahajournals.org DOI: 10.1161/CIRCULATIONAHA.114.001382
knowledge of the pathways that regulate the expression and function of enzymes, transporters, and drug targets, variation in these important components of pharmacological pathways are being discovered.

Pharmacogenomic Outcomes

Many pharmacogenomic studies have focused on pharmacokinetic outcomes such as drug concentration or dose to achieve therapeutic levels. These outcomes are clinically and biologically relevant, particularly for drugs with a narrow therapeutic index for which maintenance of appropriate concentration is critical to achieve benefit without toxicity. However, pharmacodynamic studies of drug efficacy that take serum drug levels into account are required to determine genetic risk factors for ultimate clinical outcomes. Adverse drug events such as drug intolerance caused by side effects or drug toxicity are also crucial events with individual differences in susceptibility, sometimes mediated by genetic variation (Figure 1). A complete personalized therapeutic plan must consider the full spectrum of drug effects, from therapeutic benefit to adverse event, to accurately determine the safest, most effective combination of agents.

Special Considerations for PCTx

The vast majority of pharmacogenomic data are from adult studies. Although genomes are stable throughout life, gene expression and function may vary with age. The developmental ontogeny of drug metabolism and response genes is a topic of active research, because pathways unique to children may contribute to individual differences in drug response. In addition, developmental changes in the pediatric age range can lead to specific drug effects and toxicities in children. For these reasons, it is important to validate pharmacogenomic associations in children rather than extrapolating data from adults.

The specific case of cardiac transplantation also demands consideration of factors unique to organ transplantation. After transplantation, the patient has 2 genomes: Their host genome, present in the majority of cells relevant to drug response, including the liver, kidneys, immune cells, and vasculature; and the donor genome, present in the heart and passenger cells (e.g., leukocytes). Specific variants that affect drug action or toxicity via action in heart cells will be associated with donor, not host, genotype. The interaction of variants in the host and donor genomes is an important topic of current research, but information is very limited at this time in this patient population. Finally, given the need to balance risks for rejection versus drug toxicity, a broad spectrum of clinical outcomes must be studied, including rejection, cardiac function, graft loss and mortality, as well as infection, malignancy, and tissue/organ toxicities, to fully evaluate the effects of variants that later cellular response to therapy.

Maintenance Immunosuppressive Agents in PCTx

Although immune suppression protocols vary among centers, broad principles apply. Therapies include induction therapy (generally intravenous), maintenance therapies (usually multidrug oral medications), and rescue therapy for acute rejection events. Induction therapy use is increasing; currently 71% of PCTx patients receive induction therapy with either interleukin-2 receptor antagonists or T-cell–depleting polyclonal antithymocyte antibodies. The goals of induction therapy include protection against early rejection and delayed introduction of nephrotoxic medications such as calcineurin inhibitors, because renal function is typically impaired in patients immediately after transplantation. The influence of pharmacogenomic variation on the efficacy of induction therapies has not been reported for PCTx patients; however, use of these agents can influence the interpretation and application of pharmacogenomic associations for maintenance therapy as described below. Many studies reported herein were conducted in patients who did not receive induction therapy. Whether the influence of genotype on outcomes such as acute rejection events, long-term steroid dependency, and drug toxicity is attenuated or amplified by induction regimens is the focus of ongoing research.

Medications used for maintenance of immunosuppression in PCTx patients include long-term use of a calcineurin inhibitor (tacrolimus or cyclosporine) in almost all cases. Tacrolimus is currently used in ≈78% of patients at the time of initial hospital discharge (Table 1; Figure 2). Most centers also use an antimetabolite agent (MMF or azathioprine) as adjunctive therapy, with MMF being the most commonly prescribed adjunctive agent at the present time and the use of azathioprine being on the decline. In some cases, a proliferation signal inhibitor (sirolimus or everolimus) is used as the adjunctive agent in lieu of MMF or azathioprine. These proliferation signal inhibitors are only very rarely used as a primary immunosuppressant, because there is insufficient evidence that they offer adequate protection against early rejection in the PCTx population. There is increasing use of steroid avoidance or early steroid withdrawal after pediatric transplantation because of the significant adverse effects of long-term use of corticosteroids, including growth failure. Steroid avoidance may be facilitated by the use of induction therapy. For each of these agents, the clinical use profile, mechanism of action, and potential adverse events will be discussed, followed by a summary of pharmacogenomic associations to...
date based on review of published findings from cohorts of PCTx patients, pediatric renal transplant patients, or adult cardiac transplant patients (Table 2 and Table I in the online-only Data Supplement).

**Tacrolimus**

Tacrolimus, the most commonly used calcineurin inhibitor in PCTx, is a macrolide antibiotic compound derived from the fungus *Streptomyces tsukubaensis.* Tacrolimus binds FK binding protein; this complex binds to and inhibits the phosphatase activity of the calcineurin-calmodulin complex, which leads to decreased translocation of NFAT (nuclear factor of activated T cells) transcription factors to the nucleus. This inhibits the transcription of cytokines including interleukin-2, interleukin-3, interleukin-4, tumor necrosis factor-α, granulocyte-macrophage colony-stimulating factor, and interferon-γ, which causes blunted T-cell activation and proliferation. Adverse events associated with tacrolimus include nephrotoxicity such as acute reversible azotemia, chronic irreversible renal disease, tubular disease, and hemolytic uremic syndrome; hypertension; dyslipidemia; neurotoxicity such as tremor; metabolic derangements such as glucose intolerance, diabetes mellitus, hyperkalemia, and hypomagnesemia; and gastrointestinal side effects such as anorexia, nausea, vomiting, diarrhea, and abdominal discomfort. Like all immunosuppressive agents, tacrolimus is also associated with increased risk for infection and malignancy.

Tacrolimus is inactivated in the liver by cytochrome P450 enzymes in the 3A family (CYP3A). Functional CYP3A alleles have been associated with more rapid tacrolimus inactivation and higher dose requirements in pediatric renal transplant patients and adult cardiac transplant patients. The most well-characterized CYP3A variant is CYP3A5*3, which includes a splicing variant that prevents translation of active enzyme. One or more copies of the functional enzyme (encoded by CYP3A5*3) are present in the majority of black people, but homozygosity for the nonfunctional genotype, denoted by CYP3A5*3/*3, is more common among individuals of European and Asian descent and Hispanic Americans. Several studies have investigated the influence of CYP3A5*3 on tacrolimus disposition in PCTx, consistently finding

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**Table 1. Maintenance Immunotherapy for Pediatric Cardiac Transplantation**

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Clinical Use</th>
<th>Mechanism of Action</th>
<th>Side Effects*</th>
<th>Pharmacokinetic Pathways</th>
<th>Genes Associated With Drug Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tacrolimus</td>
<td>Most commonly used calcineurin inhibitor</td>
<td>Binds FK binding protein, causing inhibition of calcineurin, T-lymphocyte signal transduction, and IL-2 signal transduction</td>
<td>Nephrotoxicity, hypertension, dyslipidemia, neurotoxicity, glucose intolerance, diabetes mellitus, hyperkalemia, hypomagnesemia, gastrointestinal side effects</td>
<td>Substrate for ABCB1 transporter; Metabolized by CYP3A enzymes</td>
<td>ABCB1, CYP3A4, CYP3A5</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>Calcineurin inhibitor with decreasing use</td>
<td>Binds cyclophilin, inhibits calcineurin, decreasing T-lymphocyte signal transduction and IL-2 transcription</td>
<td>Nephrotoxicity, hypertension, dyslipidemia, hypokalemia, hypomagnesemia, gingival hyperplasia, hirsutism, gastrointestinal side effects</td>
<td>Substrate for ABCB1 transporter; Metabolized by CYP3A enzymes</td>
<td>ABCB1, CYP3A5, NR1I2</td>
</tr>
<tr>
<td>MMF/MPA</td>
<td>Increasing use, very common</td>
<td>Binds IMPDH, causing decreased B- and T-cell proliferation and antibody production</td>
<td>Gastrointestinal symptoms, bone marrow suppression</td>
<td>Metabolized by CES-1, CES-2, UGT enzymes</td>
<td>ABCC2, IMPDH1, IMPDH2, UGT2B7, TNF-α</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>Infrequent, decreasing use</td>
<td>Inhibits purine synthesis, decreasing proliferation of leukocytes and lymphocytes</td>
<td>Gastrointestinal side effects, bone marrow suppression</td>
<td>Metabolized by TPMT</td>
<td>TPMT</td>
</tr>
<tr>
<td>Sirolimus and everolimus</td>
<td>Rarely used</td>
<td>Bind to the FK binding protein, blocking T-and B- cell activation via modulation of mammalian target of rapamycin</td>
<td>Bone marrow suppression, hyperlipidemia, gastrointestinal symptoms, progressive interstitial pneumonitis, nephrotoxicity; hemolytic uremic syndrome with cyclosporine</td>
<td>Substrate for ABCB1 transporter; Metabolized by CYP3A4</td>
<td>None reported in pediatric patients</td>
</tr>
</tbody>
</table>

CES indicates carboxylesterase; IL-2, interleukin-2; IMPDH, inosine-5'-monophosphate dehydrogenase; MMF, mycophenolate mofetil; MPA, mycophenolic acid; TPMT, thiopurine methyltransferase; and UGT, UDP glucuronosyltransferase.

*All agents associated with risk of infection and malignancy.

![Image](https://example.com/image.png)

**Figure 2.** Maintenance immunosuppressant use in pediatric heart transplantation. Maintenance immunosuppression at 1-year (solid bars) and 5-year (open bars) follow-up in pediatric heart transplant recipients with visits from 2007 onward. Percentage of all patients receiving each drug is plotted on the y-axis. Sum across medications is >100% because of concomitant use of ≥2 medications. MMF indicates mycophenolate mofetil; MPA, mycophenolic acid; and mTOR, mammalian target of rapamycin. Based on published data.²⁸
significant associations of \textit{CYP3A5}*3 with lower required tacrolimus doses and higher tacrolimus dose-adjusted trough levels.\textsuperscript{11,14,15} Gijsen et al\textsuperscript{14} investigated the effect of \textit{CYP3A4}*22 (defined by a variation in intron 6) and \textit{CYP3A5}*3 and found that \textit{CYP3A} poor metabolizers required 17% less tacrolimus than intermediate metabolizers and 48% less than extensive metabolizers.

Tacrolimus is also a substrate for the drug transporter p-glycoprotein, encoded by the gene \textit{ABCB1}. Homozygosity for the \textit{C} allele for the synonymous rs1045642 variant has been associated with lower dose-adjusted serum tacrolimus levels and increased steroid dependency at 1 year after transplantation in cohorts of >60 patients.\textsuperscript{10–12} The mechanism whereby this synonymous variant influences outcomes is uncertain. Patients homozygous for the missense G allele at rs2032582 had lower dose-adjusted tacrolimus levels measured at 6 and 12 months after transplantation.\textsuperscript{11} A smaller cohort did not find a statistically significant difference in tacrolimus dose-adjusted concentrations or per-kilogram dose to achieve therapeutic goal by rs1045642, rs2032582, or rs1128503 genotype.\textsuperscript{15} In 1 study of 38 pediatric renal transplant patients, \textit{ABCB1} variants were associated with early posttransplant dose-adjusted tacrolimus levels, but other studies of pediatric renal and adult cardiac transplant patients found no effect.\textsuperscript{16,22,24} The inconsistent impact of \textit{ABCB1} variation on tacrolimus may be attributable to small sample size or unique genetic structure in specific populations. Alternatively, the observation of increased steroid dependency with \textit{ABCB1} variation without differences in serum tacrolimus concentration led to the hypothesis that functional p-glycoprotein pumps tacrolimus out of the target cells, which leads to decreased effect despite therapeutic blood levels.\textsuperscript{16}

**Cyclosporine**

Cyclosporine, the older of the 2 calcineurin inhibitors, is an 11 amino acid cyclic peptide derived from the fungus \textit{Toiyopodium inflatum}. Use is decreasing because of the side effect profile and data indicating a higher incidence of rejection compared with tacrolimus.\textsuperscript{1,25,26} Cyclosporine inhibits calcineurin through binding to cyclophilin, with downstream mechanisms as described for tacrolimus. Oral bioavailability is low because of poor absorption, metabolism by enzymes in the bowel mucosa, and first-pass hepatic metabolism, although it is improved with microemulsion formulations compared with the oil-based form. Toxicities are similar to tacrolimus, including infection, malignancy, nephrotoxicity, hypertension, dyslipidemia, hypokalemia, hypomagnesemia, tremor, and gastrointestinal side effects. The incidence of hypertension and hyperlipidemia may be higher than with tacrolimus, with lower risk of new-onset diabetes mellitus, tremor, and gastrointestinal effects in some series. Cyclosporine is unlike tacrolimus in causing gingival hyperplasia and hirsutism.

Pharmacogenomic studies of cyclosporine in PCTx patients have not been reported, perhaps because of the predominant use of tacrolimus in recent years. Cyclosporine is metabolized

<table>
<thead>
<tr>
<th>Gene Name(s)</th>
<th>Protein Names</th>
<th>Cellular Functions</th>
<th>Pharmacovariants</th>
<th>Alternative Variant Name(s)</th>
<th>Associations</th>
<th>MAF in White Patients*</th>
<th>MAF in Black Patients†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ABCB1</strong></td>
<td>ATP-binding cassette, subfamily B, member 1</td>
<td>Membrane efflux pump and drug transporter</td>
<td>rs1045642</td>
<td>C3435T Ile1145Ile</td>
<td>CC homozygotes on tacrolimus with increased steroid dependency, lower tacrolimus concentration\textsuperscript{15–12}</td>
<td>0.571</td>
<td>0.205</td>
</tr>
<tr>
<td><strong>ABCC2</strong></td>
<td>ATP-binding cassette, subfamily C, member 2</td>
<td>Membrane efflux pump and drug transporter</td>
<td>rs717620</td>
<td>-24C&gt;T</td>
<td>CC homozygotes on MMF/MPA with lower discontinuation, less GI intolerance\textsuperscript{13}</td>
<td>0.181</td>
<td>0.035</td>
</tr>
<tr>
<td><strong>CYP3A4</strong></td>
<td>Cytochrome P450, family 3, subfamily A, polypeptide 4</td>
<td>Steroid hormone and drug metabolism</td>
<td>rs35599367</td>
<td>CYP3A4*22</td>
<td>*22 carriers with lower tacrolimus doses to reach target concentration\textsuperscript{14}</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td><strong>CYP3A5</strong></td>
<td>Cytochrome P450, family 3, subfamily A, polypeptide 5</td>
<td>Steroid hormone and drug metabolism</td>
<td>rs776746</td>
<td>CYP3A5*3</td>
<td>*3 homozygotes with higher tacrolimus concentration, lower tacrolimus dose requirement, higher tacrolimus dose-adjusted concentration\textsuperscript{15,17}</td>
<td>0.036</td>
<td>0.64</td>
</tr>
<tr>
<td><strong>IMPDH1</strong></td>
<td>Inosine 5′-monophosphate dehydrogenase 1</td>
<td>Guanine nucleotide synthesis</td>
<td>rs2278294</td>
<td>250-106G&gt;A</td>
<td>GG homozygotes on MMF/MPA with reduced dose holding or discontinuation, less GI intolerance\textsuperscript{15}</td>
<td>0.323</td>
<td>0.377</td>
</tr>
<tr>
<td><strong>IMPDH2</strong></td>
<td>Inosine 5′-monophosphate dehydrogenase 2</td>
<td>Guanine nucleotide synthesis</td>
<td>rs11706052</td>
<td>819+10T&gt;C</td>
<td>C carriers on MMF/MPA with possible increased bone marrow toxicity\textsuperscript{12}</td>
<td>0.115</td>
<td>0.018</td>
</tr>
</tbody>
</table>

GI indicates gastrointestinal; MAF, minor allele frequency; MMF, mycophenolate mofetil; MPA, mycophenolic acid; and NR, not reported in HapMap Data.

*Based on HapMap Utah residents with ancestry from northern and western Europe from the CEPH collection (CEU) population.

†Based on HapMap African ancestry in Southwest USA (ASW) population.
to inactive compounds by the CYP3A family of enzymes, and 1 study of 25 adult cardiac transplant patients reported an increase in dose- and weight-corrected cyclosporine concentrations in CYP3A5 poor metabolizers. However, in larger cohorts, which included 87 teenagers and 104 pediatric patients who underwent renal transplantation, CYP3A5*3 was not associated with variation in cyclosporine pharmacokinetics. Cyclosporine is also a substrate for p-glycoprotein; the influence of ABCC1 variants on cyclosporine pharmacokinetics have been studied in pediatric renal and adult cardiac transplant patients. In all 3 pediatric studies, ABCC1 genotype influenced cyclosporine concentrations, although in the adult cardiac transplant study, the effect was inconsistent and dependent on the time point studied.

An additional candidate gene, NR1I2, encodes the steroid and xenobiotic receptor (SXR), which regulates CYP3A4 expression. Three studies in pediatric renal transplant patients have demonstrated that carriers of rs3842689, a 6-base-pair deletion in the NR1I2 promoter, require lower cyclosporine doses.

**MMF and Mycophenolate Sodium**

MMF is a prodrug that is rapidly metabolized to the active form, mycophenolic acid (MPA). Enteric-coated mycophenolate sodium delivers MPA in the small intestine. MPA reversibly inhibits inosine monophosphate dehydrogenase (IMPDH), which catalyzes purine synthesis. Because activated lymphocytes are dependent on the de novo synthesis of purine nucleotides, IMPDH inhibition causes decreased B- and T-cell proliferation and decreased antibody production. MPA preferentially binds to IMPDH isofrom type II, expressed in active lymphocytes. In addition to malignancy and infection (specifically cytomegalovirus and herpes zoster infections), MMF toxicities include nausea, diarrhea, abdominal cramping, and bone marrow suppression. MMF is not nephrotoxic.

Published pharmacogenomic studies of MMF have focused on drug-related toxicities. Adverse events are common, especially in the infant population. In a cohort of 59 PCTx patients, those homozygous for A at rs11706052 in IMPDH2 experienced less bone marrow toxicity leading to MMF dose holding, although the effect was no longer statistically significant after adjustment for age, race, and sex. In the same patient population, homozygous G genotype at IMPDH1 rs2278294 or rs2228075 was associated with decreased rates of gastrointestinal toxicity. Use of the IMPDH1 haplotype did not lead to greater statistical confidence. These observations have not yet been validated in larger cohorts.

The organic anion transporter multidrug resistance protein 2, encoded by ABCC2, is involved in the enterohepatic circulation of MPA. In the same 59 PCTx patients, ABCC2 rs717620 GG genotype was protective against MMF discontinuation secondary to gastrointestinal side effects, attributed to decreased enterohepatic circulation and lower intestinal MPA concentrations. In 290 patients enrolled in the Pediatric Heart Transplant Study, rs717620 GG genotype conferred increased risk of rejection with hemodynamic compromise and late rejection with hemodynamic compromise, consistent with lower drug exposure in this subset of patients. An evaluation of 4 ABCC2 variants (rs717620, rs2273697, rs8187694, and rs3740066) in 89 PCTx patients found no associations to MMF pharmacokinetics. A study of 32 adult cardiac transplant and 36 lung transplant recipients had mixed results with respect to ABCC2, finding no association for rs717620, although rs3740066 and rs17222723 in ABCC2 were associated with anemia and leukopenia.

MPA is metabolized through phase 2 glucuronidation by UDP glucuronosyltransferases (UGTs). In pediatric renal transplant patients and adult cardiac transplant patients, polymorphisms in UGT2B7 and UGT1A8 have influenced the metabolism, clearance, and side effect profile. The rs1800629 variant in tumor necrosis factor-α has also been studied in pediatric renal transplant patients treated with MMF and was associated with increased rates of myelotoxicity.

**Azathioprine**

Azathioprine is an alternate antimetabolite agent used less frequently than MMF. A prodrug that is metabolized by glutathione in red blood cells to 6-mercaptopurine, this agent inhibits adenine and guanine ribonucleotide production, which results in decreased numbers of circulating B and T lymphocytes, reduced immunoglobulin synthesis, and diminished interleukin-2 secretion. Thiopurine methyltransferase (TPMT) is important for 6-mercaptopurine metabolism. Side effects of azathioprine include gastrointestinal effects (anorexia, nausea, vomiting), infection, malignancy, and bone marrow suppression.

No pharmacogenomic studies of azathioprine in PCTx have been reported. In multiple other patient populations, TPMT variants conferring decreased enzyme function have been shown to increase formation of 6-thioguanine in patients treated with azathioprine, an outcome associated with higher risk for side effects such as bone marrow suppression. However, a recent study of 93 adult cardiac transplant patients paradoxically found that patients with decreased TPMT activity experienced earlier, more severe rejection without an increase in incidence of leukopenia; these data indicate that azathioprine may be less efficacious in patients with decreased TPMT activity, perhaps because of decreased production of active metabolites. Guidelines for the use of TPMT genotype data in clinical care have been published, and the US Food and Drug Administration label recommends TPMT genotype or enzyme function testing in patients treated with azathioprine.

**Sirolimus and Everolimus**

Sirolimus and everolimus are macrolide triene antibiotics that block the response of T- and B-cell activation by cytokines through the binding of FK binding protein and modulation of mTOR. These drugs are infrequent and decreasing use in PCTx. Toxicities include anemia, thrombocytopenia, leukopenia, hyperlipidemia, nephrotoxicity, progressive interstitial pneumonitis, and gastrointestinal effects (constipation, diarrhea, dyspepsia, nausea, and vomiting). Delayed wound healing is sometimes observed, which raises concerns about early use after transplantation surgery. When sirolimus is used in conjunction with cyclosporine, hemolytic uremic syndrome has been reported. These are substrates for CYP3A and p-glycoprotein. CYP3A5 and ABCC1 variants have been studied in adult cardiac transplant patients treated with everolimus (n=30...
and 59 patients), but no significant associations of genotypes to pharmacokinetics were found.43,44

**Clinical Implications**

In the current literature, the evidence for the influence of pharmacogenetic variation on response to maintenance immunotherapy used in PCTxs is sparse and in some cases contradictory. Consistent findings from multiple studies in distinct populations are required for clinical implementation of genotype-guided therapy. Consistent evidence for pharmacokinetic outcomes has been established for azathioprine and *TPMT*, as well as tacrolimus and *CYP3A5*. Additional data, pending validation, suggest that variations in drug transporters and drug targets affect drug disposition and effect, including the influence of *ABCB1* on tacrolimus and the effects of *IMPDH* on MMF intolerance. All of these data are limited by small sample size, evaluation of a limited number of variants, and lack of statistical correction for multiple comparisons. There are several unanswered questions regarding the clinical relevance of these associations. The impact of pharmacovariables on outcomes beyond drug trough concentrations has not been firmly established. Prior knowledge of drug metabolism genotype may facilitate individualized dosing to achieve early therapeutic concentrations and avoid toxic supratherapeutic levels; however, this effect has not been proven. The impact of pharmacovariables on clinical outcomes must be assessed. Given that both morbidity and mortality are increasingly caused by medication toxicities rather than rejection, accurate prediction models for efficacy and toxicity have the potential to individualize therapy and further improve PCTx outcomes.

**Potential Clinical Implementation: Tacrolimus and Azathioprine**

The Pharmacogenomic Resource for Enhanced Decisions in Care & Treatment (PREDICT) program at Vanderbilt University Medical Center was launched to evaluate evidence for clinical pharmacogenomic testing, develop genotype-guided clinical decision support (CDS), and implement pharmacogenomic testing.45,46 Given the well-established associations of drug metabolism variants to tacrolimus and azathioprine disposition and the potential to reduce drug toxicity, the PREDICT program has implemented testing and CDS for *CYP3A5* and *TPMT* variants. Beyond the burden of scientific evidence, obstacles to implementation include technical challenges (eg, laboratory testing protocols and reporting, genotype data storage, development and maintenance of clinical decision support), practical challenges (eg, test turnaround time, physician workflow, communication of results to patients and providers, reimbursement), and educational challenges (for both providers and patients). PREDICT provides proof-of-principle that preemptive genotyping and real-time CDS can be operationalized in clinical practice and offers the opportunity to evaluate the impact of pharmacogenomic testing.

For azathioprine, the US Food and Drug Administration label recommends testing for *TPMT* genotype or phenotype and use of alternative agents or reduced dosage in patients with low or absent *TPMT* activity. Clinical guidelines for use of *TPMT* genotype or phenotype information for dosage adjustment of these drugs in adults and children have been published and are also available at the Pharmacogenomics Knowledgebase (PharmGKB).43,47,48 Given the low prevalence of nonfunctional *TPMT* alleles, patients rarely have 2 such alleles (1 in 178 to 1 in 3736 patients); however, intermediate *TPMT* activity caused by heterozygosity for functional and nonfunctional alleles is much more common (3%-14% of patients) and also merits dosage adjustment. Pre-prescription testing for *TPMT* status before initiation of azathioprine therapy is advocated for many clinical contexts.49-53 Through PREDICT, clinical testing for *TPMT* genotype is available at this institution, with CDS activated for patients with genotypes that indicate low or intermediate *TPMT* activity (Figure 3A). A website has also been developed to provide genotype-specific information and a review of evidence for patients and providers outside of Vanderbilt.54

For tacrolimus, the presence of *CYP3A5*/*1* genotype has been established to increase the tacrolimus dose required to achieve target drug concentrations in multiple populations, including children.11,14-18,20-23,55 In pediatric renal transplant patients, *CYP3A5*/*1* was associated with lower tacrolimus serum concentration despite therapeutic drug monitoring, perhaps because of clinician hesitancy to prescribe the high doses of tacrolimus required in these patients.17,18 Through PREDICT, clinical *CYP3A5* genotyping is available for both pediatric and adult patients receiving tacrolimus. CDS alerts providers of the potential for drug resistance in *CYP3A5*/*1* carriers and homozygotes; further information is available at the MyDrugGenome.org website (Figure 3B).54

**Biological Factors Contributing to Health Disparity**

Transplant outcomes have improved considerably, but significant differences in morbidity and mortality are evident when outcomes are compared across race and ethnicity, especially for late mortality rates.56-60 Many factors contribute to these disparities, including complex interactions of recipient-donor match, pathogenesis of the transplant, access to care, socioeconomic status, and cultural differences that affect healthcare delivery. However, some outcome disparity may be attributed to biological factors conferred by genetic differences. Compared with white PCTx recipients, black patients have a higher burden of genetic variants predisposing to inflammation, which may require more intensive maintenance immuno-suppression.61 In addition, black patients are more likely to be carriers of pharmacogenotypes associated with reduced drug availability and efficacy.62 Thus, it appears rational to prescribe tacrolimus dosing to black transplant recipients based on *CYP3A* genotype rather than an assumed “most likely” genotype based on recipient race. Refinement of predictive models that incorporate clinical and genetic risk factors to personalize therapy holds particular promise to improve outcomes for at-risk populations.

**Future Directions**

Many of the limitations to the evidence base for pharmacogenomics in PCTxs are caused by small sample size. Multicenter consortia such as the Pediatric Heart Transplant Study have the potential to assemble and follow a large, diverse cohort to determine the impact of genetic variants on pharmacokinetics.
and pharmacodynamics through investigation of end points such as drug levels, drug toxicity, transplant rejection, and mortality. Coupling such resources with modern statistical analyses (eg, implementation of propensity score techniques), sophisticated biomarker analyses, and precise phenotyping for covariates and outcomes including adverse drug events will enable incorporation of dense clinical and genetic data to determine independent predictors of drug response and ultimate transplant outcomes. Evaluation of the impact of pharmacogenetic variants in the context of current therapeutic regimens, including induction agents, is ongoing. A key component for these studies is the inclusion and study of patients at the highest risk for poor outcomes, including blacks. Validation of the effects of known variants and discovery of additional pharmacogenes may not only enable personalized care for these at-risk patients but also lead to a better understanding of the mechanisms of rejection and drug action, which could lead to novel therapies.

**Conclusions**

We have the technology to perform rapid, low-cost genotyping, sophisticated data analyses, and personalized CDS based on patient data, clinical parameters, and genotypes. The wide variability in PCTx outcomes, not predicted by clinical factors alone, provides the opportunity for implementation of pharmacogenomic testing and continued research. There is
consistent evidence that genetic variations in CYP3A5 and TPMT alter the pharmacokinetics of tacrolimus and azathioprine, respectively, and the impact of preemptive pharmacogenomic testing for these genes on clinical outcomes in PCTx can only be established through application and evaluation of pharmacogenomic programs. There are many additional genes that encode drug metabolism enzymes, drug transporters, drug targets, and regulatory genes relevant to PCTx recipients. We envision the use of clinical and pharmacogenomic factors for personalizing therapeutic decisions for PCTx. This approach has the potential to minimize rejection events while avoiding the serious, and sometimes fatal, complications of immuno-suppressive therapy.

Sources of Funding
Dr Van Driest is supported by a Pharmaceutical Research and Manufacturers of America Foundation Faculty Development Award in Clinical Pharmacology and by Clinical and Translational Science Award KL2 TR 000446 from the National Center for Advancing Translational Sciences, National Institutes of Health. Dr Webber is supported by S50 HL 074732 from the National Heart, Lung, and Blood Institute, National Institutes of Health.

Disclosures
None.

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Personalizing Pediatric Heart Transplantation


**Key Words:** drugs ■ pediatrics ■ pharmacogenetics ■ transplantation
Pharmacogenomics: Personalizing Pediatric Heart Transplantation
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Circulation. 2015;131:503-512
doi: 10.1161/CIRCULATIONAHA.114.001382
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circ.ahajournals.org/content/131/5/503

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