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Authors

Calabrese, Daniel R
Florez, Rebecca
Dewey, Katherine
et al.

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Genotypes associated with tacrolimus pharmacokinetics impact clinical outcomes in lung transplant recipients

Daniel R. Calabrese[1], **Rebecca Boettger**[2], **Katherine Dewey**[2], **Christine Hui**[2], **Dara Torgerson**[1], **Tiffany Chong**[1], **Hilary Faust**[3], **Raja Rajalingam**[4], **Steven R. Hays**[1], **Jeff A. Golden**[1], **Jasleen Kukreja**[5], **Jonathan P. Singer**[1], **John R. Greenland**[1,6]

[1]Department of Medicine, University of California, San Francisco, CA

[2]Department of Clinical Pharmacy, University of California, San Francisco, CA

[3]Pulmonary and Critical Care Medicine, University of Pennsylvania, Philadelphia, PA

[4]Immunogenetics and Transplantation Laboratory, Department of Surgery, University of California, San Francisco, CA

[5]Department of Surgery, University of California, San Francisco, CA

[6]Medical Service, Veterans Affairs Health Care System, San Francisco, CA.

Abstract

Most lung transplantation immunosuppression regimens include tacrolimus. Single nucleotide polymorphisms (SNPs) in genes important to tacrolimus bioavailability (ABCB1) and clearance (CYP3A4 and CYP3A5) are associated with differences in tacrolimus pharmacokinetics. We hypothesized that polymorphisms in these genes would impact immunosuppression-related clinical outcomes. We categorized ABCB1, CYP3A4, and CYP3A5 SNPs for 321 lung allograft recipients. Genotype effects on time to therapeutic tacrolimus level, interactions with antifungal medications, concentration to dose (C_0/D), acute kidney injury, and rejection were assessed using linear models adjusted for subject characteristics and repeat measures. Compared with CYP3A poor metabolizers (PM), time to therapeutic tacrolimus trough was increased by 5.1 ± 1.6 days for CYP3A extensive metabolizers (EM, p<0.001). In the post-operative period, CYP3A intermediate metabolizers spent 1.2 ± 0.5 days less (p=0.01) and EM spent 2.1 ± 0.5 days less (p<0.001) in goal tacrolimus range than CYP3A PM. Azole antifungals interacted with CYP3A genotype in predicting C_0/D (p<0.001). Increased acute kidney injury rates were observed in subjects with high ABCB1 function (OR 3.0, 95% CI 1.1–8.6, p=0.01). Lower rates of acute cellular rejection were observed in subjects with low ABCB1 function (OR 0.36, 95% CI 0.07–0.94, p=0.02). Recipient genotyping may help inform tacrolimus dosing decisions and risk of adverse clinical outcomes.

Correspondence and reprint requests to Dr. John R. Greenland, San Francisco VA Medical Center, 4150 Clement St, San Francisco, CA 94121, USA; 415-221-4810x22962, john.greenland@ucsf.edu.

DISCLOSURES

The authors of this manuscript have a conflict of interest to disclose. J.R.G. received an unrelated investigator-initiated study grant from Astellas Scientific and Medical Affairs. The authors have no other conflicts of interest to disclose.

Keywords

Lung Transplantation; Genetics; Pharmacokinetics; Tacrolimus

INTRODUCTION

The majority of lung transplant recipients are maintained on an immunosuppressant regimen consisting of a calcineurin inhibitor, a cell-cycle inhibitor, and a corticosteroid. In 2015, 97% of all lung transplant recipients in the United States received an immunosuppression regimen that included the calcineurin inhibitor tacrolimus (1). Importantly, tacrolimus has highly variable pharmacokinetics and a narrow therapeutic window (2–4). Erratic tacrolimus blood levels have been negatively associated with chronic lung allograft dysfunction (CLAD)-free survival suggesting that minimizing time outside of a therapeutic tacrolimus window may improve outcomes (5).

Polymorphisms in ABCB1, CYP3A5, and CYP3A4 genes may explain much of the inter-individual variability in tacrolimus pharmacokinetics in solid organ transplant settings (6, 7). The permeability-glycoprotein (P-gp) efflux pump, which is encoded by the ABCB1 gene, and two cytochrome P450 enzymes, encoded by the CYP3A4 and CYP3A5 genes, are integral to the bioavailability and clearance of tacrolimus. P-gp efflux pumps actively transport exogenous compounds to the intestinal lumen, bile ducts, and renal proximal tubules (8, 9), while cytochrome P450 isoenzymes expressed in the liver, gut, and kidney oxidatively metabolize compounds (10).

Lung allograft recipients may be particularly vulnerable to variability in tacrolimus dosing because of relatively high immunosuppressive medication targets and frequent changes in interacting medications, such as azole-antifungals. There are limited data on the genetic determinants of tacrolimus clearance and bioavailability and resulting clinical outcomes in the lung transplant population (11–14). Moreover, pharmacokinetic profiles among lung transplant recipients differ from other transplant groups, making extrapolation from other clinical contexts difficult (6, 15, 16). We hypothesized that polymorphisms in ABCB1, CYP3A5, and CYP3A4 would impact immunosuppression-related clinical outcomes in lung transplant recipients.

MATERIALS AND METHODS

Study population and standard therapies

This retrospective cohort study included 321 subjects who received single lung, bilateral lung, or heart-lung allografts at University of California, San Francisco (UCSF) between 2000 and 2016. Adults surviving to hospital discharge were included if they had tacrolimus troughs, blood chemistries, and DNA available for genotyping. All subjects provided informed consent. The UCSF institutional review board approved this study under protocols 10–00721 and 13–10738.

Per standard clinical protocols, post-transplant induction regimens included methylprednisolone, and either 20 mg intravenous basiliximab post-operative day (POD) 0

and 4 for single- and double- lung transplant recipients or 1 mg/kg intravenous antithymocyte globulin on POD 0 for heart-lung transplant recipients and for approximately 20% of the lung transplant recipients based on surgeons' preferences (17). Maintenance immunosuppressant therapy included tacrolimus, prednisone, and mycophenolate mofetil. Tacrolimus was started on POD 1 and titrated to a target trough level of 10–14 ng/ml over the first 3 months following transplant. All subjects, with the exception of those with significant renal injury, were started at an equivalent dose of tacrolimus of 1 mg by mouth twice daily. We used a steady stepwise dose increase to avoid side effects that are associated with acute increases in tacrolimus levels. Tacrolimus trough targets were lowered after this initial period to 6–10 ng/ml. All subjects were maintained on 20 mg daily of prednisone following induction therapy. Prednisone was reduced starting at 3 months, if surveillance bronchoscopies were negative for acute rejection, to a target dose of 0.1 mg/kg by 1 year. All subjects were initiated on mycophenolic acid with goal dose of 1000 mg twice daily. Lower doses, alternate formulations, or azathioprine were used if not tolerated due to side effects. Prior to 2006, inhaled amphotericin was used for antifungal prophylaxis for the first 30 days following lung transplantation. After 2006, antifungal prophylaxis with posaconazole or voriconazole was initiated on post-operative day 1 and stopped after 90 days of treatment if bronchoalveolar lavage (BAL) surveillance fungal cultures were negative.

Genotyping and haplotype inference

Single nucleotide polymorphisms (SNPs) were assayed using an Affymetrix gene array designed by the iGeneTRAiN consortium to target HLA, KIR, pharmacogenomics, and metabolic loci (18). Less than 2% of samples were excluded based on 95% call rates, unexpected genetic identity, consistency between genetic and reported sex, levels of heterozygosity, or HLA matching between imputed versus direct HLA typing. Most SNPs in our study were directly sequenced but some unobserved genotypes were imputed through statistical inference using IMPUTE2. These SNPs were in Hardy-Weinberg equilibrium and had a minimum info score of 0.99, indicating high-accuracy imputation.

We grouped ABCB1 polymorphisms into diplotypes based on copies of wild-type (CGC, high efflux capacity) or loss of function (TTT, low efflux capacity) alleles, resulting in the four categories: CGC-CGC, CGC-TTT, TTT-TTT, and a group termed "other," which contained the remaining groupings (19). Consistent with recent studies, we categorized CYP3A genotypes into three different clusters based on functional defects: CYP3A poor metabolizers (CYP3A4*22 carriers and CYP3A5*3/*3), CYP3A intermediate metabolizers (CYP3A4*1/*1 and CYP3A5 *3/*3 or CYP3A4*22 carriers and CYP3A5*1 carriers), and CYP3A extensive metabolizers (CYP3A4 *1/*1 and CYP3A5*1 carriers) (7).

Clinical data and outcomes

Clinical data including tacrolimus trough, creatinine, estimated glomerular filtration rate, transbronchial biopsy findings, spirometry, and survival metrics were abstracted from electronic medical records for all subjects. Because information on route, dose, and timing of immunosuppressant and antifungals required manual chart abstraction, we assessed these in a randomly-sampled subcohort of 142 subjects. This sample size provided 80% power to

detect a 10% increase in C_0/D for CYP3A extensive metabolizers with a two-tailed α of 0.05, as determined by Monte Carlo methods (20).

Our pharmacokinetic outcomes of interest were time to therapeutic tacrolimus trough, time within therapeutic tacrolimus range, and tacrolimus C_0/D. Time to therapeutic tacrolimus trough was calculated as time between post-operative day 2 and the latter of two consecutive troughs at or above the target blood drug level of 10 ng/ml. To account for overexposure as well as underexposure, we counted the number of days subjects were within the range of 10 to 14 ng/ml tacrolimus troughs during the first 14 days following transplant, as time in therapeutic range. Tacrolimus troughs throughout the study period were measured by micro particle enzyme-linked immunoassay (MEIA) in CLIA-certified laboratories. The highest 5% of tacrolimus C_0/D values were excluded from analysis to avoid incorrectly timed medication dosing or laboratory draws.

Our clinical outcomes of interest were acute kidney injury (AKI), chronic kidney disease (CKD), acute and chronic allograft rejection, and survival. We defined acute kidney injury (AKI) as serum creatinine ≥2 times baseline, based on stage 2 of the Kidney Disease Improving Global Outcomes (KIDGO) guidelines, occurring between the transplant surgery and initial hospital discharge (21). Genotype effects on chronic renal disease over time were estimated using the slope of decline in estimated glomerular filtration rate (eGFR), and chronic kidney disease (CKD) was defined as eGFR <90 ml/min/1.73m^2 based on stage 2 of the KDIGO guidelines (22). Bronchoscopy with lavage and transbronchial biopsies were performed as part of routine surveillance at 0.5, 1, 2, 3, 6, 12, 18, and 24 months after transplantation and then annually. Surveillance after the second year was stopped during the study period due to a change in protocol. Additional bronchoscopy procedures were performed when clinically indicated for suspicion of acute infection or rejection. Acute cellular rejection was assessed and graded in clinical transbronchial biopsies by one of two experienced thoracic pathologists using standard nomenclature (23). Chronic lung allograft dysfunction (CLAD) was defined according to established criteria as an unresolving 20% decline in FEV_1 or FVC lasting over 30 days (24) and determined from clinical records as previously described (25, 26). CLAD-free survival was quantified as years of freedom from CLAD or death.

Statistical analysis

Subject characteristics and genotypes were compared using Student’s t-test and chi-square, as appropriate. Wild-type genotypes were used as the referent groups. We visualized time to therapeutic tacrolimus level by Kaplan-Meier plots and, because no subjects were censored, we used generalized linear models to assess median time to therapeutic tacrolimus level and days within tacrolimus target trough range. We used Cox proportional hazards models to determine CLAD-free survival hazard ratios as a function of genotype. Multivariable models comparing clinical endpoints were controlled for repeat measures within subjects and subject characteristics frequently associated with poor transplant outcomes or genetic variability: age at transplantation, recipient sex, recipient ethnicity, lung allocation score diagnostic group, and transplant type (single, double, or heart-lung transplant).

C_0/D Modeling

We investigated the interactions of genotypes with the presence of azole therapy to determine how these would affect C_0/D. We also assessed how much of the variability in C_0/D could be explained by a model including genotype and concomitant azole therapy. Subjects were considered free of azole effects if measured 7 days before or after cessation of azole therapy. C_0/D values for the 142 subjects were assessed in a generalized linear mixed model, adjusted with generalized estimating equations (GEEs) with an exchangeable covariance matrix to account for repeated observations within subjects. Genotype (CYP3A or ABCB1), the use of azole (voriconazole or posaconazole), and the interaction between these variables were considered as predictors of C_0/D. Additional models including baseline subject characteristics (type of transplant, lung allocation score transplant group including cystic fibrosis, recipient sex, age at transplant, and recipient ethnicity) were performed to assess the predictive significance of these variables. Since azole antifungals were instituted in 2006, we also included azole era (before 2006 and after 2006) as an additional predictor.

Statistical analyses were performed in Stata (version 14.4; StataCorp LP, College Station, Texas), and select figures were generated with Prism version 7.0b for Macintosh (GraphPad Software, La Jolla, California).

RESULTS

Study population and polymorphism distributions

Characteristics of the 321 subjects are found in Table 1. Compared with International Society for Heart and Lung Transplantation (ISHLT) registry data (27), our population had a greater proportion of subjects receiving double lung transplantation (89% vs. 76%) and more subjects were transplanted for pulmonary fibrosis (36% vs. 25%). We also transplanted fewer subjects with cystic fibrosis (10% vs. 16%) and chronic obstructive pulmonary disease (COPD) (15% vs. 31%). There were no statistically significant differences between the baseline characteristics of the subjects who were randomly selected for further chart abstraction versus those not selected for chart abstraction.

Frequencies of CYP3A and ABCB1 genotypes in our cohort were similar to other reported studies in transplant populations (7, 11, 19, 28). As shown in Table 2, CYP3A4 and CYP3A5 genotypes were distributed equally ($\chi^2 = 0.005$, p=0.94). ABCB1 diplotypes were also distributed evenly across CYP3A5 ($\chi^2 = 2.5$, p=0.87) and CYP3A4 ($\chi^2 = 9.4$, p=0.15) genotypes.

Tacrolimus trough measurements

All 321 subjects had tacrolimus troughs available for their initial hospitalization. Figure 1 shows the time to therapeutic tacrolimus concentration for the two genotypes. The median time to therapeutic tacrolimus trough was 8.2 days. For CYP3A genotypes, the time to therapeutic tacrolimus trough was increased by 5.1 ± 1.6 days in extensive metabolizers (p<0.001) compared with the reference of 7.6 ± 1.6 days in poor metabolizers. We found no significant difference between CYP3A intermediate metabolizers and CYP3A poor metabolizers in time to therapeutic tacrolimus level. Between the CYP3A genotypes, we

also found significant differences in the number of days within therapeutic tacrolimus range during the first 14 post-operative days. CYP3A intermediate metabolizers had 1.2 ± 0.5 days less (p= 0.01) and extensive metabolizers had 2.1 ± 0.5 days less (p<0.001) than the reference of 2.6 ± 0.7 days in CYP3A poor metabolizers. We found no statistical differences in time to achieve target drug levels or the number of days within therapeutic range between the ABCB1 diplotypes.

Predictors of tacrolimus C_0/D

Subject characteristics for the subcohort undergoing analysis of tacrolimus trough and dose measurements are detailed in supplemental table S1. The antifungal medications posaconazole and voriconazole can increase the tacrolimus C_0/D by inhibiting the cytochrome P450 isoenzymes. Within our cohort of 142 subjects, there were 36,995 troughs included in the analysis with only 511 (1.4%) from the pre-azole era. We found a significant association between posaconazole or voriconazole use and tacrolimus C_0/D across CYP3A genotypes, as shown in Figure 2. While the C_0/D increased in all CYP3A genotypes with concomitant azole antifungal administration, there was also a significant gene-medication interaction (p<0.001). The effect was most marked in CYP3A poor metabolizers increasing from 3.61 ± 0.2 ng/mL per mg with tacrolimus alone to 7.26 ± 0.2 ng/mL per mg with the addition of an antifungal agent (p<0.001). With the addition of these antifungal agents, the C_0/D in CYP3A extensive and intermediate subjects increased but to a lesser extent. Analysis of dose changes by genotype and specific antifungal are found in supplemental table S2.

To assess the proportion of variability in tacrolimus C_0/D over time that is explained by genotype, azole antifungal medication status, and the genotype-azole interaction, we compared observed versus the predicted C_0/D using linear modeling. As shown in Figure 3, 45 percent of the variance of C_0/D (R^2) was explained by this model. However, there were differences as high as 8-fold in measured C_0/D within groups of predicted C_0/D. Inclusion of baseline subject characteristics explained only an additional 4.8 percent of the C_0/D variation (R^2), with decreased C_0/D in subjects with cystic fibrosis (ß −1.5, p=0.03). A model with CYP3A and ABCB1 genotypes without azole antifungals explained only 26 percent of the variance in C_0/D (R^2). Supplemental table S3 shows the comparison of models including the additional covariates. The ABCB1 genotype did not significantly mediate predicted C_0/D in this model, but we found that within the subset of CYP3A4*22 genotypes, subjects also expressing the TTT-TTT diplotype (n = 5) had a 50% reduction in C_0/D from 5.7 ng/mL per mg compared to other ABCB1 genotypes (p=0.03).

Acute and chronic renal dysfunction

We assessed for associations between genotype and acute and chronic kidney disease. Nineteen percent of the subjects (n=61) developed KDIGO stage II or greater AKI in the immediate post-operative period. As shown in Table 3, between the transplant surgery procedure and first hospital discharge, carriers of the ABCB1 CGC-CGC diplotype manifested three-fold greater odds of developing KDIGO stage II or greater acute kidney injury compared to TTT-TTT subjects (95% CI 1.1–8.6, p=0.01). Other ABCB1 genotypes were not associated with a statistical difference in the odds of early acute kidney injury. We

observed no statistical association between ABCB1 or CYP3A genotype and the slope of estimated glomerular filtration rate from time of transplantation through the remainder of available subject data. We also observed no association between genotype and CKD at 6 months, 2 years, and 5 years.

Acute and chronic rejection

Figure 4 shows the odds of rejection in the first year following transplant based on genotype. Our cohort experienced similar rates of overall grade A1 or greater rejection in the first year following transplant as compared with the ISHLT registry data (29% vs. 32%), with the median time to first rejection of 101 days (27). ABCB1 TTT-TTT subjects had reduced odds of developing ≥ A1 rejection compared to other ABCB1 genotypes (OR 0.36, 95% CI 0.07–0.94, p=0.02). Surprisingly, there was no difference in rates of acute allograft rejection across the CYP3A genotypes, although this genotype was the dominant determinant of C_0/D. There were no significant associations between genotype and B grade rejection or ≥ A2 rejection.

CLAD-free survival was assessed using Kaplan-Meier analysis and Cox proportional hazards for all genotypes. Sixty-nine of the subjects died during the study period, surviving a median of five years. Ninety-two subjects had developed CLAD by that time. There were no differences in time to CLAD or death among ABCB1 genotypes (TTT-TTT, HR 0.77, p=0.41; CGC-TTT, HR 0.91, p=0.72; Other, HR 0.75, p=0.26), or CYP3A genotypes (IM, HR 1.2, p=0.44; EM, HR 1.2, p=0.65).

DISCUSSION

This is the largest cohort study to date to investigate genotypes affecting tacrolimus in a lung transplant population. Consistent with what has been reported in solid organ transplant populations, we found that recipients with rapid-metabolizing CYP3A genotypes require higher doses of tacrolimus to maintain therapeutic drug levels compared to other genotypes (7, 11, 28–35). Rapid-metabolizing genotypes were associated with increased time to therapeutic tacrolimus level and decreased days in therapeutic range. Additionally, we found decreased rates of acute cellular rejection in lung allograft recipients with ABCB1 poor efflux activity.

In a multivariable model, only CYP3A genotypes had a significant impact on predicted tacrolimus C_0/D. We found that azole antifungal agents impact tacrolimus concentrations with a significant drug-genotype interaction. The magnitude of this interaction was greater than that reported in a recent study including lung and kidney allograft recipients (12). One potential reason for this difference in outcomes may be that our study was performed solely in lung allograft recipients, reflecting the differences in pharmacokinetics between solid organ transplant groups. Our study also had more statistical power to detect drug-genotype interactions. We found an 8-fold variation in tacrolimus dose-normalized concentration after accounting for genotype and antifungal medications. This wide range of tacrolimus C_0/D is consistent with other studies and may reflect the non-linear kinetics of tacrolimus and variability in tacrolimus measurement (12, 36, 37). C_0/D values were also lower in subjects

with cystic fibrosis, consistent with previous literature on decreased bioavailability of tacrolimus in this population (38).

Although effects of ABCB1 polymorphisms on tacrolimus concentrations vary between studies, the low activity, TTT-TTT P-gp genotype has generally been associated with an increased tacrolimus C_0/D (13, 14, 28, 30, 35, 39, 40). In our cohort, the effect of ABCB1 diplotype on C_0/D was only seen in the CYP3A*22 genotypes. Conclusions from this subgroup analysis are limited by the low number of subjects, but another study demonstrated a similar finding in renal allograft recipients (19). Data from liver transplant subjects show that ABCB1 genetic polymorphisms may influence tacrolimus hepatic concentrations but have little impact on blood drug concentrations (35). It seems plausible that ABCB1-encoded P-gp efflux pump activity might become relevant only in the context of poor hepatic clearance of tacrolimus.

Across solid organ transplants, there is strong evidence of genetic variations affecting tacrolimus pharmacokinetics, but the clinical impacts of these polymorphisms have been less clear. Here, we observed an increased incidence of clinically-relevant acute kidney injury during the initial hospitalization in subjects with the high efflux activity ABCB1 CGC-CGC genotype. Healthy volunteers who carry this genotype have been shown to exhibit worse baseline renal function independent of genetic background (41). Subjects with intact P-gp function may be predisposed to renal injury, or intact P-gp function may lead to increased renal tubule drug or toxic metabolite exposure. We controlled for baseline subject characteristics in our models, but uncontrolled confounders including perioperative parameters or other medication effects could also impact renal outcomes.

A meta-analysis in liver transplant recipients suggested a higher risk of acute rejection and chronic nephrotoxicity in CYP3A5 high metabolizers, for whom higher tacrolimus doses are required to maintain a given target trough (42). However, we did not observe an effect of CYP3A genotype on rates of acute or chronic rejection. It is possible that higher target tacrolimus troughs that are typical for lung allograft recipients could dampen pharmacogenomic effects on acute rejection rates. At the same time, a reduction in acute rejection was observed in subjects with the ABCB1 TTT-TTT diplotype, for whom less tacrolimus would be needed to achieve a therapeutic trough and concentrations throughout the day could be less variable (43). Similar effects of ABCB1 polymorphisms were observed in a renal transplant cohort (39).

The Clinical Pharmacogenetics Implementation Consortium (CPIC) recently recommended tacrolimus adjustment based on CYP3A5 genotype in transplant recipients if genotyping is already available (44). However, there was no specific recommendation that this genotyping be performed and debate regarding its clinical utility based on two subsequent studies where renal allograft recipients were prospectively randomized to receive tacrolimus dosing based on pretransplant CYP3A5 genotyping. In a study of renal allograft recipients, subjects that were randomized to receive genotype-based dosing had more time in therapeutic tacrolimus range and a shorter time to therapeutic blood troughs compared to subjects randomized to a standard of care protocol (45). Conversely, Shuker and colleagues demonstrated no difference in goal therapeutic tacrolimus troughs or acute rejection between a group

randomized to genotype-based dosing versus a group receiving usual care (46). Our study shows that CYP3A5, CYP3A4, and ABCB1 genotyping in lung transplant recipients may prove useful as an adjunct tool for clinicians surrounding dosing decisions in the initial post-transplant period and during azole antifungal medication adjustments. This information would be less likely to impact long-term outcomes substantially. Genotypic information may also prove helpful in considering potentially non-adherent subjects. However, the substantial observed residual variation in C_0/D not explained by genotype and antifungal use implies that genotype-based dosing cannot replace trough-based dosing. Further, there is a risk that higher starting doses in extensive metabolizers would result in overshooting therapeutic targets leading to more toxicity without additional protection from rejection.

These data have limitations: results generated from other centers might vary due to differences in pathologic interpretation, medication dosing regimens, and ethnic distributions, when compared with this single center study. Our modeling of genotype effect on CLAD-free survival may be incomplete as we were unable to control for some known confounders such as primary graft dysfunction, gastroesophageal reflux disease, or graft infections. Tacrolimus dose-adjusted concentration is a limited tool for estimating total drug exposure as it has been shown to poorly correlate with more accurate area-under-the-curve assessments of tacrolimus exposure in lung transplant recipients (47). Genotype effects on clinical outcomes may be more meaningfully assessed in a prospective study measuring area-under-the-curve or peak concentrations. We report a population median of 8.2 days to achieve a therapeutic tacrolimus trough, which is representative of our high target of 10 ng/ml and our incremental dosing strategy designed to avoid toxicity from rapid increases. As shown in supplemental table S1, the time to achieve a tacrolimus trough of 6 ng/ml is 2.9 days. Further, our method of calculating time in therapeutic range may underestimate this value as compared to other methods such as by Rosendaal linear interpolation. Finally, although gene-chip based SNP typing was done on a targeted SNP chip yielding very high information scores, there is likely some risk for misclassification, which might bias results towards the null, when compared with some other SNP typing methods.

In conclusion, CYP3A5, CYP3A4, and ABCB1 genotyping may inform clinical management of lung transplant recipients. Tacrolimus dosing by CYP3A genotype in the early post-transplant period has the potential to decrease time to therapeutic level and time outside of therapeutic tacrolimus range. Genotyping for ABCB1 may be of additional utility in identifying subjects like those with ABCB1 TTT-TTT genotypes, for whom C_0/D are lower. While there was still up to 8-fold variation in C_0/D after accounting for genotype, a sense of target C_0/D by genotype may be useful in informing early tacrolimus dosing and in anticipating dosing requirements following changes in interacting medications. Further study is warranted to determine if genotyping could improve lung allograft recipient satisfaction and decrease costs by reducing time to therapeutic tacrolimus level or rates of acute cellular rejection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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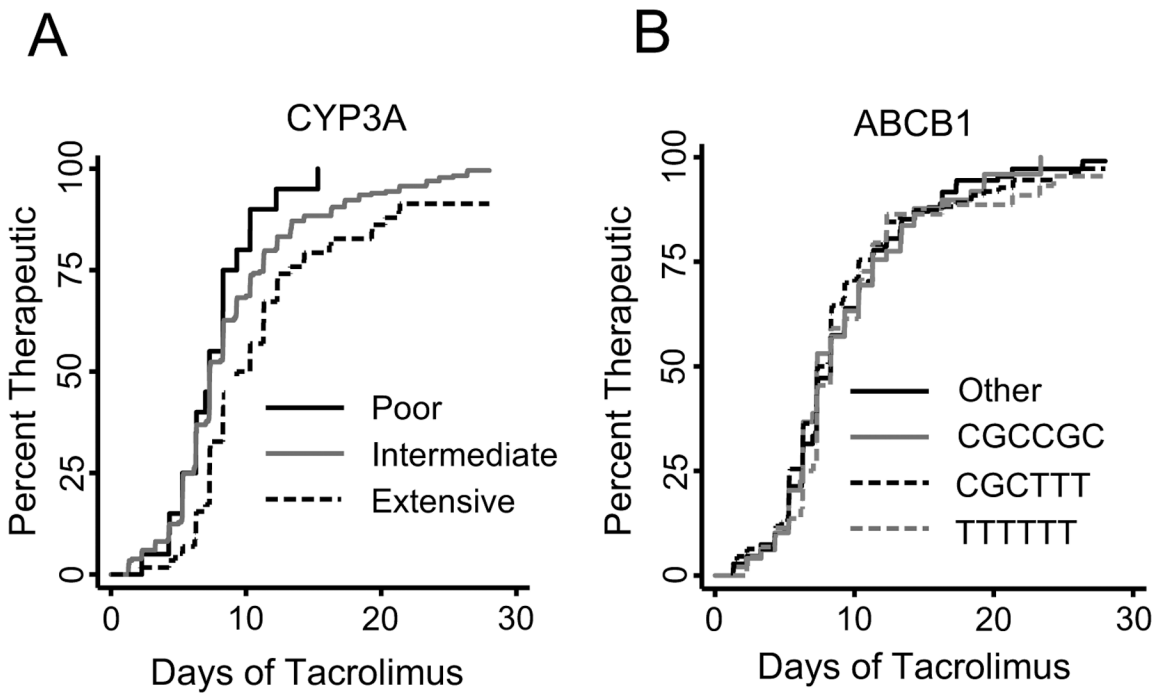

Figure 1: Delayed time to achieve therapeutic tacrolimus trough for CYP3A but not ABCB1 genotypes.

The proportion of subjects with a therapeutic tacrolimus trough, defined as two consecutive troughs at goal, are shown as a function of days post-transplant using Kaplan-Meier curves for subjects stratified by (A) CYP3A and (B) ABCB1 genotype. Increased times to achieve therapeutic tacrolimus troughs were associated with CYP3A genotypes that promote more rapid tacrolimus metabolism. CYP3A extensive metabolizers took 5.1 ± 1.6 days longer (p<0.001) than poor metabolizers to reach therapeutic tacrolimus troughs. We found no differences in time to achieve therapeutic trough in ABCB1 genotypes.

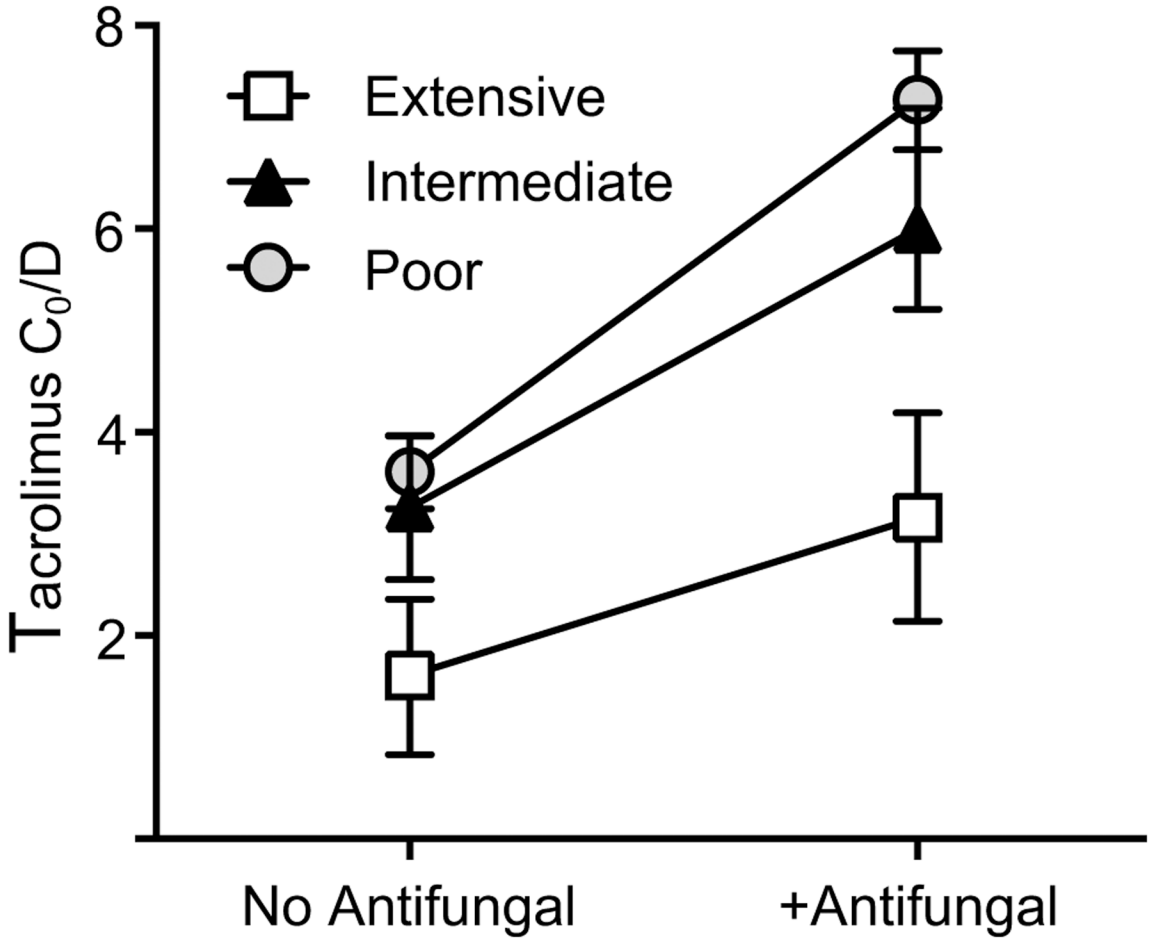

Figure 2: Interaction of CYP3A genotype with the use of tacrolimus in determining C_0/D ratios.
The tacrolimus C_0/D was calculated for a subcohort of 142 lung transplant subjects at times with and without antifungal medications. Across CYP3A genotypes, azole antifungal administration was associated with an increase in C_0/D and subjects with poor CYP3A metabolism had higher C_0/D compared to the other genotypes. The slope of change between genotypes was also different across genotypes, indicating a medication-gene interaction (p<0.001) with a greater magnitude of change observed in the CYP3A poor metabolizers compared to other genotypes.

A

Predictor Variable	β	95% CI
Intercept	1.51	(0.47 - 2.54)
CYP3A		
Poor	0	
Intermediate	1.01	(0.10 - 1.90)
Extensive	1.67	(0.05 - 3.28)
ABCB1	0.09	(-0.24 - 0.42)
Antifungal	1.67	(1.35 - 1.99)
with CYP3A Poor	0	
with CYP3A Intermediate	2.27	(1.91 - 2.64)
with CYP3A Extensive	3.04	(2.45 - 3.63)

B

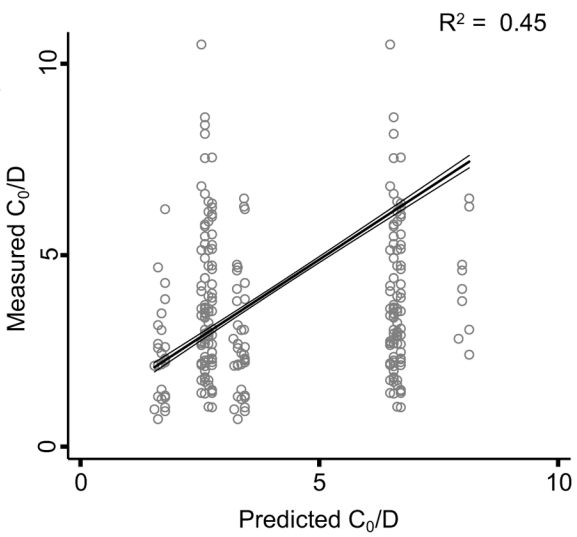

Figure 3: Genotypes and azole antifungals explain some of the variation in tacrolimus C_0/D.

(A) CYP3A and ABCB1 genotypes and the interaction between azole antifungals and the CYP enzymes were used in a GEE-adjusted linear model to predict tacrolimus C_0/D ratio. The table displays the ß values and confidence intervals (CI) for the independent variables with statistically significant variables in bold. (B) The predicted tacrolimus C_0/D is displayed in a scatterplot against the median C_0/D with a line (thick black) and 95% confidence interval (thin black lines) fitted to these plot points. The R^2 was calculated as 45%.

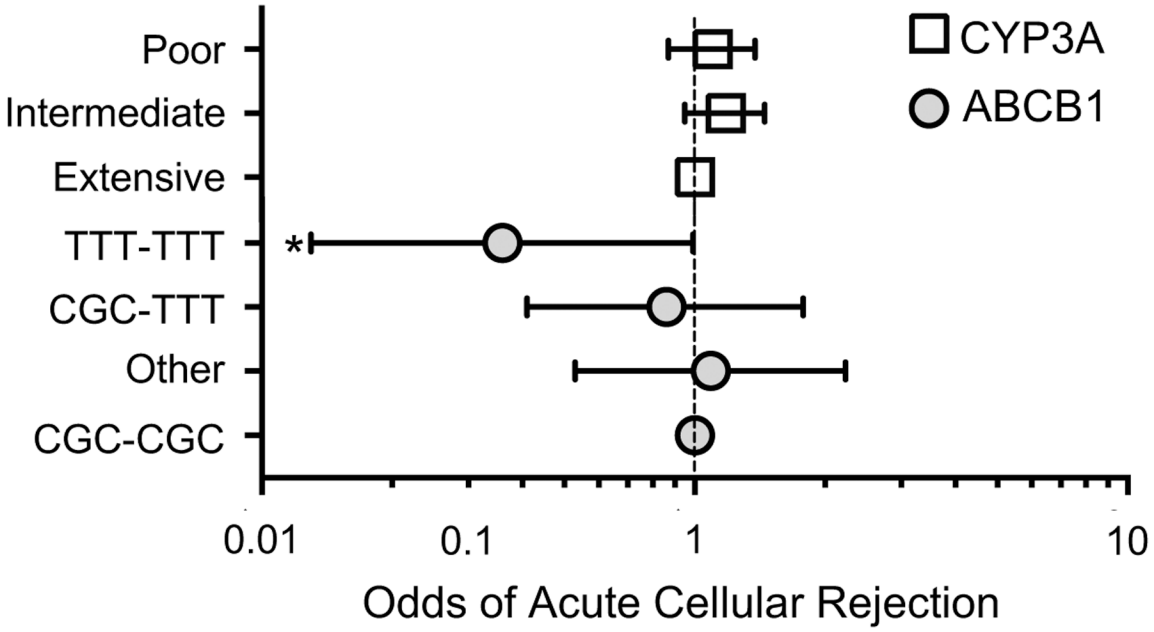

Figure 4: ABCB1 but not CYP3A genotypes were associated with differential risk of acute cellular rejection in the first year following transplantation.

The odds of developing acute rejection are shown in subjects stratified by CYP3A and ABCB1 genotype. Rates of acute cellular rejection were reduced in ABCB1 TTT-TTT subjects (OR 0.36, 95% CI 0.13–0.98). "*" denotes $p<0.05$.

Table 1:

Study Population Characteristics

	Total Cohort	Co/D	*p* value
Subjects (n)	321	142	
Age at transplantation, mean years ± SD	55 ± 12	56 ± 11	0.69
Male gender (%)	54	58	0.27
Transplant type: N (%)			0.71
Double	288 (89.4)	126 (88.7)	
Heart and Lung	6 (1.9)	3 (2.1)	
Single	27 (8.4)	13 (9.2)	
Race/Ethnicity: N (%)			0.28
Caucasian	242 (75.2)	112 (78.9)	
African American	22 (6.9)	10 (7.0)	
Hispanic	36 (11.2)	11(7.8)	
Other	21 (6.5)	9 (6.3)	
Transplant indication group: N (%)			0.60
A (COPD)	66 (20.5)	31 (21.8)	
B (Primary Pulmonary Hypertension)	15 (4.7)	5 (3.5)	
C (Cystic Fibrosis)	31 (9.6)	14 (9.7)	
D (Pulmonary Fibrosis)	210 (65.2)	92 (64.8)	0.88
CYP3A genotype: N (%)			0.22
Extensive Metabolizers	61 (19)	16 (11.2)	
Intermediate Metabolizers	240 (74.8)	116 (81.7)	
Poor Metabolizers	20 (6.2)	10 (7)	
ABCB1 diplotype N (%)			0.64
CGC-CGC	51 (15.8)	24 (16.9)	
CGC-TTT	114 (35.5)	55 (38.7)	
TTT-TTT	46 (14.3)	17 (12.0)	
Other	110 (34.3)	46 (32.4)	

Table 2:

Concurrence of Cytochrome Genotypes

		CYP3A4		
		*1/ *1	*22/	Total
CYP3A5	*1/ *1	61	5	66
	*3/ *3	235	20	255
	Total	296	25	321

Table 3:

Acute and Chronic Kidney Injury

	Acute Kidney Injury		Rate of GFR decline	
	N/Total	**OR [95% CI]**	**Slope (ml/min/year)**	**[95% CI]**
CYP3A genotypes				
Poor Metabolizers	2/20	Ref	−22.1	Ref
Intermediate Metabolizers	43/240	1.1 [0.9 – 1.2]	−38.5	[−63.5 – 30.7]
Extensive Metabolizers	16/61	1.1 [0.9 – 1.3]	−46.8	[−76.9 – 27.5]
ABCB1 diplotype				
TTT-TTT	6/46	Ref	−38.4	Ref
CGC-TTT	20/114	1.4 [0.5 – 3.8]	−38.1	[−84.8 – 25.8]
CGC-CGC	16/51	3.0 [1.1 – 8.6] *	−46.6	[−100 – 24.8]
Other	19/110	1.4 [0.5 – 3.7]	−36.7	[−56.1 – 17.3]