INTRODUCTION
Tacrolimus is an immunosuppressive agent highly dependent on CYP3A4 and CYP3A5 for its metabolism. [1-2] Variability in its pharmacokinetics and narrow therapeutic indices necessitates close monitoring of tacrolimus concentrations. Compared to African Americans, European Americans require lower doses to achieve target concentrations.[2,3]

In our previous study, conducted in 695 kidney transplant recipients, clinical factors and genotypes together explained ~50% of variability in dose normalized trough concentrations.[4] Majority of European Americans (~90-95%) carry the non-functional CYP3A5*3 allele and hence are poor CYP3A5 substrate metabolizers. In our study the CYP3A5*3 was [1,4] the most influential variant. [1,4] After accounting for CYP3A5*3, a large part of the pharmacokinetic variability remained unexplained. We hypothesize that there are additional genetic variants that further influence tacrolimus variability.

The purpose of this study was to perform a genome wide search to find additional genetic variants specific to tacrolimus metabolism in the the European American population.

RESULTS

Figure 1 shows the GWAS Manhattan plots of association of variants towards the tacrolimus dose normalized trough concentrations. In the initial unadjusted analysis, 53 variants were significant (p<10-8, Figure 1A) towards trough concentrations. CYP3A5*3 was the top most significant variant (p=6.88X10-10). With this we then adjusted the analysis for CYP3A5*3 and seven variants remained significant (Figure 1B). The top variant was CYP3A4*2 (p=2.77 X 10-13). We then adjusted the analysis for CYP3A5*3 and CYP3A4*2 and no variants were GWAS significant although CYP3A4*2 was top most although it did not meet genome wide significance (p=0.001, Figure 1C). The allele frequencies are shown in Table 2. The final parameter estimates of clinical and genetic factors obtained after multivariate analysis are in Table 3. Figure 2 shows the plots of dose normalized concentrations vs time by genotypes.

METHODS
Subjects were European American kidney transplant recipients (n=1446) enrolled in our multicenter DEKAF genomics study (NCT00270712) who received tacrolimus maintenance therapy. Tacrolimus trough concentrations were obtained from each subject in the first 6 months (twice each week for the first 2 months and then twice in each month up to 6 months). Trough concentrations were targeted to 8-12 ng/mL for the first 3 months and 6-10 ng/mL for 3-6 months posttransplant. Table 1 shows demographic and clinical characteristics of the subjects. Genotyping DNA was from peripheral blood and genotyped using a custom exome-plus Affymetrix TaArray SNP chip containing 450,150 markers after QC. Data quality control was conducted using PLINK software. Samples were dropped if they had less than 98% call rate, were nonmonomorphic, did not pass Hardy Weinberg equilibrium testing, Hapmap concordance rate > 2%, gender mismatch, or were identical by descent, had minor allele frequency <1%. Principal component analysis and visual inspection was used to confirm European ancestry.

Statistics: Linear mixed effects regression models were used to test for associations between natural log (ln) transformed dose normalized troughs and genotypes. Visual inspection showed that weighted normal trough concentrations initially started low, rose quickly until day 9 post-transplant. Therefore, we initially tested the association of variants from GWAS with the estimated day 9 trough levels from a simple time trend model. For the final model (Table 3), the top variants were adjusted for clinical factors that were identified using backward selection with a retention p-value of 0.10.

CONCLUSION
We identified CYP3A5*3, CYP3A4*2 and CYP3A4*3 as top most variants important towards tacrolimus trough using GWAS. Days post-transplant, recipient age, GRF at time of trough, weight at baseline, primary disease, calcium channel blocker use, ace-inhibitor use and antiviral use were clinical factors significant towards tacrolimus dose-normalized trough concentrations. These variants should be tested in future studies in European Americans.

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