Research Article

Pharmacogenomics

Attempted validation of 44 reported SNPs associated with tacrolimus troughs in a cohort of kidney allograft recipients

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Aim: Multiple genetic variants have been associated with variation in tacrolimus (TAC) trough concentrations. Unfortunately, additional studies do not confirm these associations, leading one to question if a reported association is accurate and reliable. We attempted to validate 44 published variants associated with TAC trough concentrations. **Materials & methods:** Genotypes of the variants in our cohort of 1923 kidney allograft recipients were associated with TAC trough concentrations. **Results:** Only variants in *CYP3A4* and *CYP3A5* were significantly associated with variation in TAC trough concentrations in our validation. **Conclusion:** There is no evidence that common variants outside the *CYP3A4* and *CYP3A5* loci are associated with variation in TAC trough concentrations. In the future rare variants may be important and identified using DNA sequencing.

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Obtaining optimal blood concentrations of the immunosuppressive agent tacrolimus (TAC) in kidney allograft recipients is critical to reducing the risk of acute rejection (AR) and maximizing graft survival, while reducing the risk of TAC-associated adverse effects [1]. Although individuals are given similar doses of TAC, there is significant variation in trough concentrations between individuals, caused in part by differences in TAC clearance [2]. Additionally, dose-adjusted trough concentrations differ significantly between populations. It is well known that African–American (AA) allograft kidney recipients have significantly lower TAC trough concentrations in whole blood when compared with European–American (EA) recipients at equivalent doses of TAC, and these lower concentrations have been associated with increased risk for AR [1,3–5]. Even within populations there is significant variability in TAC trough concentrations, putting some recipients at risk for either AR, due to low TAC concentrations, or adverse effects such as infections, nephrotoxicity, hypertension, hyperglycemia due to excessively high TAC concentrations.

To understand this variation in TAC trough concentrations, numerous studies in the last two decades have been undertaken to identify associated genetic variants in candidate SNPs thought to be involved in TAC pharmacokinetics (see Table 1) [6–56]. Unfortunately, there are additional studies which do not validate these associations. The lack of validation for the initial association is in many cases likely due to underpowered studies [57]. Most of these studies used small, single center cohorts with the majority of the studies having less than 100 recipients. In many studies, multiple genetic variants were analyzed without taking multiple testing into consideration, resulting in inflated type I errors with increased false positives. We and others have shown that variants in *CYP3A4* and *CYP3A5*

Table 1. Variants identified from the literature previously associated with tacrolimus trough concentrations.

rs#	Proxy SNP†	Gene	Chrom.	Position	Common name	Nucleotide	Amino acid	Ref.
rs11265572		*NR1I3*	1	1.61E + 08		g.161243273G>T	Noncoding	[6]
rs1800872		*IL10*	1	2.07E + 08	-592C>A	c.-627A>C	Noncoding	[7,8]
rs1800871		*IL10*	1	2.07E + 08	-819C>T	c.-854T>C	Noncoding	[7,8]
rs1800896		*IL10*	1	2.07E + 08	-1082G>A	c.-1117A>G	Noncoding	[9]
rs4553808	rs16840252	*CTLA4*	2	2.04E + 08		c.-1661A>G	Noncoding	[10]
rs3814055		*NR1I2*	3	1.2E + 08	-25385T>C	c.-1135C>T	Noncoding	[11,12]
rs6785049		*NR1I2*	3	1.2E + 08	7635A/G	c.795-93G>A	Noncoding	[13]
rs2276707	rs3814057	*NR1I2*	3	1.2E + 08	8055C>T	c.938-17C>T	Noncoding	[14]
rs181781	rs657075	*IL3*	5	1.32E + 08		c.-1285G>A	Noncoding	[10]
rs1800796	rs1524107	*IL6*	7	22726627		c.-636G>C	Noncoding	[15]
rs1057868		*POR*	7	75985688	**28*	c.1508C>T	p.Ala503Val	[16–22]
rs1045642	rs2235048	*ABCB1*	7	87509329	3435C/T	c.3435T>C	p.Ile1145=	[12,23–29]
rs2032582	rs4148738	*ABCB1*	7	87531302	2677G/T/A	c.2677T/A/G	p.Ser893Ala/Thr	[12,24,27–30]
rs1128503		*ABCB1*	7	87550285	1236C/T	c.1236T>C	p.Gly412=	[12,24,28–30]
rs2229109	rs117937072	*ABCB1*	7	87550493	1199G/A	c.1199G>A	p.Ser400Asn	[30,31]
rs9282564		*ABCB1*	7	87600124	61A/G	c.61A>G	p.Asn21Asp	[24]
rs3213619		*ABCB1*	7	87600877	-129T/C	c.-129T>C	Noncoding	[13]
rs41303343		*CYP3A5*	7	99652770	**7*	c.1035_1036insT	p.Thr346Tyrfs	[32,33]
rs10264272		*CYP3A5*	7	99665212	**6*	c.624G>A	p.Lys208=	[32,33]
rs4646450		*CYP3A5*	7	99668695		c.319-1630C>T	Noncoding	[34]
rs776746		*CYP3A5*	7	99672916	**3*	c.219-237A>G	Noncoding	[10–12,16,18,19,23,25,26,29,30,32,34–42]
rs2257401		*CYP3A7*	7	99709062	**2*	c.1226C>G	p.Thr409Arg	[11]
rs2242480		*CYP3A4*	7	99763843	**1G*	c.1023 + 12G>A	Noncoding	[7,10–12,29]
rs28371759		*CYP3A4*	7	99764003	**18B*	c.875T>C	p.Leu292Pro	[43,44]
rs4646437		*CYP3A4*	7	99767460		c.671-205C>T	Noncoding	[7,10]
rs35599367		*CYP3A4*	7	99768693	**22*	c.522-191C>T	Noncoding	[16,17,20,21,39,45–47]
rs2740574		*CYP3A4*	7	99784473	**1B*	c.-392G>A	Noncoding	[42,48–51]
rs1927907		*TLR4*	9	1.18E + 08		c.140 + 1757C>T	Noncoding	[52]
rs4986893		*CYP2C19*	10	94780653	**3*	c.636G>A	p.Trp212Ter	[40]
rs4244285	rs12571421	*CYP2C19*	10	94781859	**2*	c.681G>A	p.Pro227=	[37,40,53]
rs2273697		*ABCC2*	10	99804058	1249G>A	c.1249G>A	p.Val417Ile	[42]
rs3740066		*ABCC2*	10	99844450	3972C>T	c.3972C>T	p.Ile1324=	[42]
rs2070673		*CYP2E1*	10	1.34E + 08		c.-333A>T	Noncoding	[37]
rs2237991		*ABCC8*	11	17418682		c.2223-1720T>C	Noncoding	[54]
rs5744247		*IL18*	11	1.12E + 08		c.-8-372C>G	Noncoding	[38]
rs1946518	rs1946519	*IL18*	11	1.12E + 08	A-607C	c.-838A>C	Noncoding	[41]
rs4149117		*SLCO1B3*	12	20858546	T334G	c.335C>A	p.Ser112Ala	[55]
rs7311358		*SLCO1B3*	12	20862826	G699A	c.699G>A	p.Met233Ile	[55]
rs2306283		*SLCO2B1*	12	21176804	A388G	c.388A>G	p.Asn130Asp	[30]
rs4149056		*SLCO2B1*	12	21178615	T521C	c.521T>C	p.Val174Ala	[30]
rs3745274		*CYP2B6*	19	41006936	**6*	c.516G>T	p.Gln172His	[37]
rs2239393		*COMT*	22	19962905		c.289 + 90A>G	Noncoding	[56]
rs4823613	rs4253730	*PPARA*	22	46202410		c.208 + 3819A>G	Noncoding	[19]
rs4253728	rs4253730	*PPARA*	22	46214170		c.209-1003G>A	Noncoding	[19,46]

†SNP was used as a proxy when a variant was not present in the genotyping chip.

Table 2. Recipient characteristics for African–American and European–American recipients.

Characteristics	European–American (n = 1560)	African–American (n = 363)	p-value
Age group (years), n (%)			<0.0001
– 18–34	180 (11.5%)	71 (19.6%)	
– 35–64	1121 (71.9%)	272 (74.9%)	
– 65–84	259 (16.6%)	20 (5.5%)	
Donor age group (years), n (%)			<0.0001
– 0–34	485 (31.1%)	171 (47.1%)	
– 35–64	1031 (66.1%)	184 (50.7%)	
– 65–84	44 (2.8%)	8 (2.2%)	
Living donor status, n (%)	1031 (66.1%)	112 (30.9%)	<0.0001
Female, n (%)	579 (37.1%)	132 (36.4%)	0.81
Diabetes at transplant, n (%)	611 (39.2%)	133 (36.6%)	0.40
SPK, n (%)	130 (8.3%)	18 (5.0%)	0.029
Body mass index, mean (SD)	28.3 (5.5)	28.8 (5.4)	0.075
Antibody induction, n (%)			<0.0001
– Monoclonal	595 (38.1%)	176 (48.5%)	
– Polyclonal	857 (54.9%)	175 (48.2%)	
– None	63 (4.0%)	6 (1.7%)	
– Combination	45 (2.9%)	6 (1.7%)	
Median tacrolimus trough (IQR) in the first 34 days in ng/ml	8.7 (6.6–10.8)	5.4 (3.5–7.9)	<0.0001
Median tacrolimus trough (IQR) after day 34 in ng/ml	8.1 (6.5–9.9)	7.1 (5.4–9.0)	<0.0001
Median daily tacrolimus dose (IQR) in first 24 days in mg	6.0 (4.0–8.0)	6.0 (4.0–8.0)	0.77
Median daily tacrolimus dose (IQR) after day 25 in mg	5.0 (3.5–8.0)	8.0 (6.0–10.0)	<0.0001
Median dose-normalized tacrolimus trough (IQR) in ng/ml per total daily dose in mg	1.52 (1.00–2.33)	0.78 (0.52–1.22)	<0.0001

QR: Interquartile range; SD: Standard deviation.

are major contributors to this variation in TAC troughs and have been estimated to contribute to at least half of the observed variance when combined with clinical factors, leaving approximately half of the observed variance unexplained [32,54,58].

We attempted to validate 44 variants previously reported in the literature to be associated with variation in TAC troughs using DNA from a cohort of kidney allograft recipients on which a genome wide association study (GWAS) had been performed and clinical information from kidney recipients enrolled in the long-term Deterioration of Kidney Allograft Function (DeKAF) Genomics study. This cohort consists of 1923 kidney recipients with 31,906 TAC trough concentrations and doses.

Materials & methods

The design of the DeKAF Genomics study and cohort characteristics has been previously reported [32,59,60]. For this analysis, kidney transplant recipients with genome wide association study data were identified and divided into two sub-cohorts consisting of 1560 EA and 363 AA kidney allograft recipients and tested separately (Table 2). Though, self-reported race was available in the clinical information, subjects were separated into EA and AA cohorts using principal components using the GWAS. Subjects were aged 18 years and older, received TAC for maintenance immunosuppression and had TAC troughs and doses available in the first 6 months post-transplant. Subjects were enrolled at time of transplant and signed informed consents were approved by the Institutional Review Boards of the enrolling centers. This study is registered at www.clinicaltrials.gov (NCT01714440).

Clinical information was obtained through the DeKAF Genomics study and obtained from the respective medical records [59,60]. Participants received oral immediate release TAC therapy with mycophenolate maintenance with varying durations of steroid per transplant center standard-of-care protocols. Induction therapy was administered as per transplant center preference but mainly consisted of rabbit antithymocyte globulin, basiliximab or Campath-1H. Immunologically, high risk patients were more likely to receive rabbit antithymocyte globulin, such as those with donor specific antibody, pregnancies or repeat transplants. TAC troughs were clinically measured at each

site and were analyzed in a clinical laboratory improvement amendments approved laboratory and >95% were measured from whole blood by liquid chromatography-mass spectrometry. When available, two measurements were obtained in the first 8 weeks, and two levels per month in months 3, 4, 5 and 6 for a maximum of 24 trough concentrations per patient. TAC doses were adjusted based on trough concentrations to reach institution-specific trough goals based on time post-transplant (generally 8–12 ng/ml in months 0–3 and 6–10 ng/ml in months 4–6). Doses were also adjusted for toxicity (e.g., nephrotoxicity) by center specific preferences. Trough values were normalized for dose (ng/ml per total daily dose in mg) prior to statistical analysis.

An extensive review of the literature was conducted using PubMed and published variants that were reported to be significantly associated with variation in TAC trough concentrations in solid organ transplantation patients were identified. Only variants which were shown to have a statistically significant association ($p < 0.05$ as stated in the published report) were included in this study. Most studies analyzed TAC concentrations in kidney recipients. The variants which were chosen from the literature for validation in this report are shown in Table 1. Genotype information for this study was extracted from our previous study using an Affymetrix TxArray GWAS chip created specifically for analysis of allograft recipients [32,61]. Polymorphisms capturing Ancestry informative markers and 7500 drug adsorption, metabolism, excretion and toxicity markers (ADME; n = approximately 7500) including SNPs from PharmGKB were added to this chip [61,63]. Also included were additional SNPs extracted from the Affymetrix-Biobank to increase coverage of African and other populations [61]. A total of 644,224 SNPs were available for genotyping data. A total of 44 variants in 22 genes were identified in the literature and analyzed using the genotypes from this chip for each individual. For those variants which were not part of the GWAS chip, a proxy SNP was selected which was present on the chip and genotypes available. In all cases, the r^2 between the reported variant and the proxy SNP was 1.0 as determined by the SNP Annotation and Proxy Search program (SNAP; 62), with the exception of rs181781 and rs2276707 ($r^2 = 0.938$), rs4823613 and rs4253730 ($r^2 = 0.959$) and rs4253728 and rs4253730 ($r^2 = 0.920$).

Statistical analysis for validation of TAC trough concentration associated variants

A linear mixed effects model was used to test for the association between genotypes of each SNP and the longitudinal dose-normalized TAC trough concentrations in each cohort. To achieve a better normality approximation, we log transformed the dose-normalized TAC concentrations. A spline model with a change of slope at day 9 post-transplant was used to model the varying time effect of trough concentrations following previous approaches [32,56]. We included a random intercept and slope for days post-transplant and modeled the additive effects of genotypes, adjusting for age, gender and transplant center. To adjust for potential population stratification, the first ancestry principal component was incorporated in the regression models instead of their reported race. The threshold for significance was set at $p < 0.001$ after taking multiple testing into consideration (n = 44 tests).

For comparison between the two cohorts, Fisher Exact test was used for categorical variables and t-test for continuous variables. TAC total daily dose-normalized trough doses and troughs were compared using simple linear mixed effects longitudinal models with an effect for days post-transplant and a random intercept in each cohort. The data were visually inspected to determine the point of divergence post-transplant of troughs (day 24) and doses (day 34) and used for the time points for comparison between the two cohorts.

Results

Characteristics of the two cohorts are shown in Table 2. Significant differences between the EA and AA cohorts included recipient age ($p < 0.0001$) where recipients in the EA cohort were older, donor age ($p < 0.0001$) where the EA recipients received older donor allografts, living donor status ($p < 0.0001$) where EA recipients had a higher percentage of living donors, and type of antibody induction ($p < 0.0001$). Compared with AA recipients, the median TAC trough was significantly higher in EA recipients $p < 0.0001$) in the first 34 days post-transplant as was the median dose-normalized TAC trough ($p < 0.0001$). Median daily TAC doses were similar in the two cohorts until day 24 when after day 25, the doses were significantly higher in AA subjects ($p < 0.0001$) compared with the EA subjects.

The significance of association for each published variant tested for validation in the DeKAF cohort is shown in Table 3. We were able to validate seven of the previously reported variants in the EA cohort and all were in the *CYP3A* locus region ($p < 0.001$). All other variants were below the threshold of significance. All significant variants, with the exception of *CYP3A4*22* (rs35599367), were found to be in linkage disequilibrium (LD) with rs776746 (*CYP3A5*3*), using the CEU population panel for LD testing (Table 4). CEU is defined by 1000 genomes as Utah

Table 3. Validation of published variants for tacrolimus trough concentrations in European–American and African–American cohorts in the Deterioration of Kidney Allograft Function genomics study.

rs_id	Gene	Variant	EA	AA	EA	AA		
			Freq.†	Freq.†	Beta	p-value	Beta	p-value
rs11265572	*NR1I3*	g.161243273G>T	0.001	0.004	NA	NA	NA	NA
rs1800872	*IL10*	-592C>A	0.236	0.398	-0.039	0.066	-0.014	0.735
rs1800871	*IL10*	-819C>T	0.236	0.398	-0.039	0.066	-0.014	0.735
rs1800896	*IL10*	-1082G>A	0.498	0.356	0.022	0.218	0.016	0.707
rs16840252‡	*CTLA4*		0.191	0.171	0.01	0.655	-0.113	0.032
rs3814055	*NR1I2*	-25385T>C	0.383	0.283	-0.017	0.352	-0.026	0.558
rs6785049	*NR1I2*	7635A/G	0.389	0.095	0.023	0.218	0.06	0.384
rs3814057‡	*NR1I2*	8055C>T	0.19	0.441	0.016	0.478	0.03	0.467
rs657075‡	*IL3*		0.099	0.031	0.054	0.075	0.027	0.802
rs1524107‡	*IL6*	c.-636G>C	0.052	0.066	-0.074	0.075	-0.035	0.656
rs1057868	*POR*	**28*	0.277	0.201	0.009	0.657	0.044	0.375
rs2235048‡	*ABCB1*	3435C/T	0.458	0.202	0.006	0.758	0.075	0.134
rs4148738‡	*ABCB1*	2677G/T/A	0.442	0.235	0.01	0.587	0.062	0.201
rs117937072‡	*ABCB1*	1199G/A	0.041	0.009	-0.01	0.817	NA	NA
rs1128503	*ABCB1*	1236C/T	0.427	0.202	0.014	0.455	0.066	0.19
rs9282564	*ABCB1*	61A/G	0.108	0.004	0.001	0.968	NA	NA
rs3213619	*ABCB1*	-129T/C	0.036	0.076	-0.036	0.462	-0.053	0.466
rs41303343	*CYP3A5*	**7*	0	0.109	NA	NA	0.361	1.18E-09
rs10264272	*CYP3A5*	**6*	0.001	0.123	NA	NA	0.067	0.269
rs4646450	*CYP3A5*		0.156	0.133	-0.305	3.46E-38	0.271	4.91E-07
rs776746	*CYP3A5*	**3*	0.068	0.29	-0.657	6.08E-98	0.299	2.34E-14
rs2257401	*CYP3A7*	**2*	0.087	0.46	-0.538	1.46E-79	0.094	0.015
rs2242480	*CYP3A4*	**1G*	0.094	0.254	-0.487	8.55E-68	0.236	1.91E-08
rs28371759	*CYP3A4*	**18B*	0	0.006	NA	NA	NA	NA
rs4646437	*CYP3A4*		0.11	0.263	-0.405	5.63E-53	0.223	8.86E-08
rs35599367	*CYP3A4*	**22*	0.057	0.004	0.339	4.81E-19	NA	NA
rs2740574	*CYP3A4*	**1B*	0.037	0.346	-0.464	1.74E-24	0.026	0.502
rs1927907	*TLR4*	c.140 + 1757C>T	0.13	0.226	0.034	0.209	-0.064	0.172
rs4986893	*CYP2C19*	**3*	0	0.001	NA	NA	NA	NA
rs12571421‡	*CYP2C19*	**2*	0.153	0.177	0.02	0.428	0.026	0.603
rs2273697	*ABCC2*	1249G>A	0.214	0.153	0.023	0.286	0.024	0.656
rs3740066	*ABCC2*	3972C>T	0.359	0.257	-0.018	0.331	-0.057	0.197
rs2070673	*CYP2E1*	c.-333A>T	0.145	0.315	0.012	0.631	-0.032	0.46
rs2237991	*ABCC8*	c.2223–1720T>C	0.252	0.332	0.027	0.195	-0.039	0.349
rs5744247	*IL18*	c.-8–372C>G	0.108	0.023	-0.002	0.933	-0.171	0.197
rs1946519‡	*IL18*	c.-838A>C	0.399	0.332	-0.003	0.861	-0.038	0.378
rs4149117	*SLCO1B3*	T334G	0.147	0.387	-0.014	0.591	0.093	0.022
rs7311358	*SLCO1B3*	G699A	0.145	0.393	-0.012	0.632	0.084	0.037
rs2306283	*SLCO1B1*	A388G	0.406	0.206	-0.007	0.708	0	0.994
rs4149056	*SLCO1B1*	T521C	0.156	0.024	-0.022	0.371	-0.052	0.684
rs3745274	*CYP2B6*	**6*	0.247	0.374	-0.001	0.945	-0.014	0.732
rs2239393	*COMT*	c.289 + 90A>G	0.39	0.409	-0.018	0.326	0.005	0.891
rs4253730‡	*PPARA*	c.209–1003G>A	0.275	0.47	0.015	0.44	0.027	0.499

†MAF based on the cohort genotypes. Minor alleles are those which differ from the reference sequence.
‡Proxy variant used (see Table 1).
AA: African–American; EA: European–American; MAF: Minor allele frequency; NA: Not analyzed due to low minor allele frequency.

Table 4. Linkage disequilibrium between significant SNPs in the *CYP3A* locus and rs776746 (*CYP3A5*3*) in European–American (CEU) and African (YRI) populations.

rs#	Gene	Variant	r^2 (CEU)	r^2 (YRI)
rs776746	*CYP3A5*	**3*	1	1
rs2257401	*CYP3A7*	**2*	0.87	0.34
rs2242480	*CYP3A4*	**1G*	0.68	0.39
rs4646437	*CYP3A4*		0.56	0.27
rs4646450	*CYP3A5*		0.28	0
rs2740574	*CYP3A4*	**1B*	0.41	0.1
rs35599367	*CYP3A4*	**22*	0.03	0

Residents (CEPH) with northern and western European ancestry.

For the AA cohort, we were able to validate five variants (Table 3) and as in the EA cohort, only variants in the *CYP3A* locus region were statistically significant. Out of these five variants, two were in LD with rs776746 using the YRI population panel for LD testing, *CYP3A4*1G* (rs2242480; $r^2 = 0.387$) and rs4646437 ($r^2 = 0.273$). The LoF variant *CYP3A5*7* (rs41303343) was not in LD with *CYP3A5*3*. The variant rs4646450 was not in LD with rs776746 in the YRI population panel, but was when the CEU panel was used. YRI is defined by 1000 genomes as Yoruba in Ibadan, Nigeria. The LoF variant *CYP3A5*6* was not statistically significant in this analysis, but is likely due to the low number of individuals with this variant in the AA cohort.

There were also a few variants that presented with suggestive significance. In the EA cohort four variants in three interleukin genes: *IL10* (rs1800871; 0.066 and rs1800872; $p = 0.066$), *IL3* (rs181781; $p = 0.075$) and *IL6* (rs1800796; $p = 0.075$) had p-values below 0.1. The two *IL10* variants are in complete LD ($r^2 = 1.000$). In the AA cohort, two variants in the solute carrier organic anion transporter family member 1B3 (*SLCO1B3*) presented with suggestive significance (rs4149117; $p = 0.022$ and rs7311358; $p = 0.037$). These two variants in *SLCO1B3* are in complete LD ($r^2 = 1.000$). Additionally, a variant in the *CTLA4* gene (rs16840252, a proxy for rs4553808, was also suggestive ($p = 0.032$). In all of these variants, significance was below the threshold after multiple testing is taken into consideration.

Discussion

Optimizing TAC blood concentrations is critical to maximizing graft survival for kidney allograft recipients. Unfortunately, trough levels can vary widely between recipients, even when taking the same dose. One reason for this variation is difference in the pharmacokinetics of TAC between individuals in part due to genetic variation in critical TAC metabolizing enzymes. Identification of these genetic variants would help in explaining this variation in drug concentrations and provide a tool to personalize dosing.

Numerous studies have reported genetic variants with a statistically significant association with TAC trough concentrations (Table 1). The genes containing these variants include drug metabolism enzymes (e.g., CYP3A4 and CYP3A5), drug transporters (e.g., ABCB1 and SLCO1B3), transcriptional factors which effect *CYP3A4* expression (e.g., NR1I2 and NR1I3) and members of the interleukin family of genes (e.g., *IL3* and *IL6*) as well as others. In this analysis, only LoF alleles in *CYP3A5*, and the *CYP3A4*22* allele in the EA cohort, were found to be statistically significant for association with TAC troughs. Some of the variants we tested were shown to be statistically significant in numerous previous reports including *POR*28* (rs1057868) and *ABCB1* variants c.3435T>C (rs1045642), c.2677T/A/G (rs2032582) and c.1236T>C (rs1128503). Though, subsequent reports appear to validate the original findings for these four variants, our larger cohort did not identify a significant association with these variants

In this analysis, only variants within the *CYP3A4* and *CPY3A5* genes were identified, and most of those variants were in LD with a known functional variant leaving only three variants which were associated with variation in TAC trough levels in this study, *CYP3A5*3*, *CYP3A5*7* and *CYP3A4*22*. This is not surprising because the CYP3A4 and CYP3A5 enzymes are the major metabolizing enzymes for TAC.

There are several reasons for the lack of validation of the majority of the variants tested. For the most part, the original reports utilized small underpowered cohorts for the initial association. Also, most studies did not follow-up with an additional cohort to confirm their findings. Additional reasons for the lack of validation include possible center effects when recipients are from multiple centers. Clinical differences in practice and concomitant

medications that may influence clearance, age of the recipient, time post-transplant, adherence, use of self-reported race instead of principal components analysis resulting in a combination of racial groups are additional sources of error.

This study focused on common (high minor allele frequency) variants. We have shown that only variants within the *CYP3A4* and *CYP3A5* genes, along with clinical factors, account for approximately 40% of the variance [58]. This leaves a significant percentage of unexplained variance associated with TAC trough concentrations [58]. Some of this missing unexplained variance may be due to additional genetic variance (missing heritability) or it may be a result of an over estimation of the heritability which exists. This analysis may also lack additional clinical variables not adjusted for, such as non-adherence. Any additional heritability which still exists most likely is not due to variants with a high minor allele frequency (MAF>0.01). We hypothesize that the source of any missing heritability will be low allele frequency variants in genes influencing TAC pharmacokinetics. DNA sequencing of these genes will reveal if these variants exist and these studies are on-going in our cohort.

Conclusion

Validation of identified variants associated with a specific outcome is necessary before this information can be translated into clinical care. A large number of genetic variants have been previously reported to be associated with variability in TAC trough concentrations. Unfortunately, most of these studies were underpowered. In our large cohort of kidney allograft recipients, we found that only functional variants in *CYP3A4* and *CYP3A5* could be validated.

Future perspective

Optimized serum concentrations of TAC in allograft recipients are critical for graft survival. Identifying which variants are important for individualized immunosuppression will allow for the development of dosing equations utilizing the proper genomic data. In this report, we were able to validate those common genetic variants which are associated with TAC trough concentrations, while eliminating those that have been previously reported as associated with this variation. We are using genetic information, along with clinical variables, to optimize dosing equations for TAC, to better estimate the initial dose for kidney allograft recipients.

Summary points

- We attempted to validate 44 common variants in 22 loci previously reported to be associated with variation in tacrolimus trough concentrations.
- We used DNA samples, clinical information and tacrolimus trough concentrations from 1560 EA and 363 AA kidney allograft recipients.
- Common functional variants in *CYP3A4* and *CYP3A5* were associated with variation in tacrolimus trough concentrations.
- All other variants tested were not validated.
- This information may improve the precision of genotype-guided dosing.

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