Donors *FMO3* polymorphisms affect tacrolimus elimination in Chinese liver transplant patients

Aim: Flavin-containing monooxygenase (FMO) variants were potentially involved in tacrolimus metabolism in kidney transplantation. The influences of *FMO3* genotypes on tacrolimus elimination in Chinese liver transplant patients remained unclear. **Patients & methods:** *FMO3* SNPs and *CYP3A5* rs776746 were analyzed in 110 Chinese patients. **Results:** Donor *FMO3* rs1800822 allele T and rs909530 allele T were associated with fast tacrolimus elimination. Combination of polymorphisms of donor *FMO3* rs1800822 and rs909530 genotype impacted on tacrolimus elimination ($p = 0.0221$). The number of donor rs1800822 allele T and rs909530 allele T was confirmed to be an independent predictor of the tacrolimus concentration-to-dose ratios for weeks 2, 3 and 4 in the multivariate analysis. **Conclusion:** Donor's *FMO3* polymorphisms might affect tacrolimus elimination.

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Tacrolimus is an immunosuppressant drug belonging to the calcineurin inhibitor class and is widely used in liver transplantation to prevent allograft rejection. It is characterized by a narrow therapeutic index and large inter-patient variabilities in its pharmacokinetic and pharmacodynamic profiles. Therefore, tacrolimus-based immunosuppressive therapy can easily lead to either insufficient or excessive immunosuppression in liver transplant patients. Insufficient immunosuppression results in graft rejection while excessive immunosuppressive therapy can cause drug intoxication [1–3]. Such variability in drug response can result from genetic polymorphisms in drug-metabolizing enzymes, transport proteins and to a certain extent the target protein [4]. Pharmacogenetic studies, focusing on the effects of genetic polymorphisms on drug response, could provide useful clinical tools for tailoring immunosuppressive therapy to the genetics of an individual patient and their donor [5–7].

CYP3A5 is the main catalyst of tacrolimus. The genotype of rs776746 (*CYP3A5* 6986A>G) has a well-established influence on the pharmacokinetics of tacrolimus regardless

of the population studied and is the best characterized pharmacogenetic marker of tacrolimus therapy. Almost all studies report that recipients with the *CYP3A5**3/*3 genotype (nonexpressers) exhibit higher dose-adjusted tacrolimus exposure and a lower dose requirement than in *CYP3A5**1/*1 or *1/*3 carriers (expressers) [8,9]. It is usually only possible to classify patients into one of these two categories; the proportion in the Han Chinese population is close to 50% [10]. However, residual interindividual differences in tacrolimus pharmacokinetics within the expressers and nonexpressers exist, demonstrating that different *CYP3A5* genotypes cannot fully account for the drug response variations [11]. This is consistent with the fact that many drugs are subjected to multipathway metabolism. This has limited the clinical use of *CYP3A5* rs776746 as a biomarker in individual immunosuppression therapy. In addition to *CYP3A5* genetic variations, other genes involved in the metabolism and clearance pathway of tacrolimus may also be involved. It has been shown that *CYP-450* 3A4, the *ABCB1* gene that encodes the P-glycoprotein ATP-binding cassette transporter, the

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immunosuppressor IL-10 and the *SLCO1B3* gene that encodes the organic anion-transporting polypeptide 1B3 may also contribute to interindividual differences in tacrolimus disposition [12–15]. But their influence on the pharmacokinetics of tacrolimus appears limited. This has raised the question as to whether other genetic determinants influencing tacrolimus oxidative metabolism could help in dose individualization.

Flavin-containing monooxygenases (FMOs) represent the second most important group of human Phase I drug-metabolizing enzymes, after cytochrome P450. Several drugs are metabolized by both FMOs and CYPs. However, their role in drug metabolism has been underestimated compared with that of cytochrome P450 [16,17]. In humans, the FMOs' system includes five genes (*FMO1–FMO5*) and six pseudogenes (*FMO6P–FMO11P*). FMOs 1, 2, 3, 4 and 6P are located within a cluster spanning approximately 245 kb, in the region q24.3. FMOs 7P–11P are located in the region q24.2. The *FMO5* gene is closer to the centromere, in the region 1q21.1. To date, only FMOs 1, 2 and 3 have been shown to play a significant role in drug metabolism [16,18]. There are striking tissue-specific differences in *FMO* gene expression. Expression of *FMO1* is switched off in the liver shortly after birth and continues to be expressed in the adult kidney through the use of an alternative downstream promoter. Functional *FMO2* protein is present in only a small percentage of the overall human population. *FMO3* is expressed primarily in the liver after birth and is the major FMO isoform that contributes to hepatic drug metabolism [18,19]. The *FMO3* gene is very polymorphic. *FMO3* variants have also been shown to be associated to drug responses of sulindac (NSAID), ranitidine (histamine 2 receptor blockers), benzydamine (analgesic), amphetamine and methamphetamine (psycho-stimulant drugs) [17,20].

Little is known about the effect of *FMO3* on common drugs used in transplantation. *FMO3* SNP, rs1800822, was identified to be associated with tacrolimus troughs in non-African–American kidney transplant patients in a multicenter kidney transplant consortium study [21]. The influence of *FMO3* genetic polymorphisms on the pharmacokinetic parameters of tacrolimus in Chinese liver transplant patients remains unclear.

In this study, we examined the *FMO3* genotypes of liver transplant recipients and donors to clarify the influence of these genetic variants on tacrolimus dose requirements and pharmacokinetics post-transplantation.

Patients & methods

Study subjects

In this retrospective observational study, 110 patients were enrolled who underwent liver transplantation at

Shanghai Jiao Tong University affiliated First People's Hospital from July 2007 to March 2012. The population, including 18 females and 92 males, were all Han Chinese. The average age was 47.5 ± 8.8 years and the average weight was 62.9 ± 104.0 kg. All patients received tacrolimus-based immunosuppressive regimens. The oral administration of tacrolimus was introduced in our previous study [7].

Ethics statement

This research was approved by the Ethics Committee of Shanghai Jiao Tong University. Informed written consent was obtained according to the Declaration of Helsinki.

Data collection

We used the tacrolimus serum concentration-to-dose ratios (C/D ratio) for 1 month post-transplantation as an index of tacrolimus pharmacokinetics as used in previous studies [7]. Trough tacrolimus blood concentration (ng/ml) was measured by PRO-TracTMII Tacrolimus ELISA kit (Diasorin, MN, USA) with microparticle enzyme immunoassay (ELx800NB analyzer, BioTek, USA). Tacrolimus daily dose (mg) was recorded and weight-adjusted dosage (mg/kg per day) was calculated. Tacrolimus C/D ratio was calculated by dividing tacrolimus trough concentration by the corresponding weight-adjusted dosage. Results of laboratory tests were also recorded. The average clinical data in different periods were calculated to represent the corresponding clinical status.

Genotyping

All the 27 *FMO3* SNPs in the drug-metabolizing enzyme and transporter array, a standard platform for pharmacogenomics study, were selected for genotyping in this study. Both donor and recipient genomic DNA were extracted from liver tissues (stored at -80°C) using an AllPrep DNA/RNA Mini Kit (Qiagen, Hilden, Germany). SNPs were genotyped using Sequenom MassARRAY SNP-genotyping platform (Sequenom, CA, USA). The protocols included PCR amplification, shrimp alkaline phosphatase treatment, single-base extension reaction, resin cleanup, nanodispensing on SpectroCHIP and data acquisition.

Statistical analysis

Chi-square test and Fisher's exact test were used to compare the distributions of genotypes and alleles of recipients and donors. Mann–Whitney U and Kruskal–Wallis tests were used to assess the statistical significance of differences in C/D ratios for comparisons of all groups. Correlation between gene polymorphism and C/D ratios was studied with univariate

and multivariate linear regression analysis. Statistical analysis was performed using SPSS version 17.0 (SPSS Inc., IL, USA). Hardy–Weinberg equilibrium, allele frequency, linkage disequilibrium and haplotype analysis were analyzed using SHEsis software [22]. $p < 0.05$ was considered statistically significant. The Statistical graphs were made by Graphpad Prism version 5.00 software [23].

Results

Gene polymorphisms

The genotypes of the 27 *FMO3* variants were shown in [Supplementary Table 1](#). Rs2266782 (E158K), rs1736557 (V257M) and rs2266780 (E308G) that were previous found in various ethnic groups have a significant population distribution in the studied subjects. Rs1800822 (S147S) and rs909530 (N285N) that were rarely referred in other studies were found in the present study. Twenty-two SNPs were excluded for further analysis. Allele and genotype frequency of the remaining five SNPs and *CYP3A5* rs776746 are shown in [Table 1](#). There was no significance between donor and recipient genotype frequency ($p > 0.05$). All six SNPs were in accordance with Hardy–Weinberg equilibrium ($p > 0.05$). Rs2266782 and rs2266780 were in strong linkage disequilibrium ($r^2 = 0.848$; [Figure 1](#)).

Effect of single SNP on tacrolimus C/D ratios

The effects of single-donor SNP on tacrolimus C/D ratios are shown in [Table 2](#). Tacrolimus C/D ratios of donors with *FMO3* SNP rs1800822 CC genotype were 131.9 ± 123.9 , 115.6 ± 97.8 , 128.3 ± 93.8 and 139.0 ± 89.8 at weeks 2, 3, 4 and month 1, respectively. For TC and TT genotype carriers, the corresponding tacrolimus C/D ratios at each time point were 94.3 ± 71.2 , 69.4 ± 63.0 , 85.6 ± 61.9 and 99.0 ± 82.5 . The differences were significant ($p = 0.016$, 0.002 , 0.026 and 0.008 , respectively). Donor *FMO3* rs909530 polymorphisms were associated with tacrolimus C/D ratios at weeks 2, 3 and month 1 ($p = 0.001$, 0.003 and 0.001 , respectively). Tacrolimus C/D ratios of donor rs909530 TC and TT carriers were very close and were significantly lower than those of CC genotype carriers. Tacrolimus C/D ratio of donor *FMO3* rs2266780 AA genotype carriers was 131.4 ± 117.6 at week 2, and GA and GG genotype carriers were 92.8 ± 64.7 . The difference was significant ($p = 0.025$). Tacrolimus C/D ratios of donor *CYP3A5* rs776746 GG genotype carriers were 278.3 ± 396.9 , 130.8 ± 103.2 , 119.3 ± 79.1 , 144.7 ± 123.2 and 145.0 ± 89.2 at weeks 1, 2, 3, 4 and month 1 respectively. For AG and AA genotype carriers, the corresponding tacrolimus C/D ratios were 176.0 ± 197.8 , 102.4 ± 101.3 , 82.8 ± 89.3 , 83.0 ± 73.0 and 101.5 ± 85.7 , respectively. The differences were

Table 1. Genotype and allele frequency of *FMO3* and *CYP3A5* polymorphism in liver transplant donors ($n = 110$) and recipients ($n = 110$).

Gene	SNP	Genotype frequency, % (n)			Allele frequency, % (n)	
FMO3	rs1800822	CC	TC	TT	C	T
	Donors	0.69 (76)	0.29 (32)	0.02 (2)	0.84 (184)	0.16 (36)
	Recipients	0.68 (75)	0.26 (29)	0.06 (6)	0.81 (179)	0.19 (41)
	rs2266782	GG	AG	AA	G	A
	Donors	0.63 (69)	0.30 (33)	0.07 (8)	0.78 (171)	0.22 (49)
	Recipients	0.58 (64)	0.36 (40)	0.06 (6)	0.76 (168)	0.24 (52)
	rs1736557	GG	AG	AA	G	A
	Donors	0.63 (69)	0.33 (36)	0.04 (5)	0.79 (174)	0.21 (46)
	Recipients	0.66 (73)	0.31 (34)	0.03 (3)	0.82 (180)	0.18 (40)
	rs909530	CC	TC	TT	C	T
	Donors	0.43 (47)	0.44 (49)	0.13 (14)	0.65 (143)	0.35 (77)
	Recipients	0.38 (42)	0.46 (51)	0.16 (17)	0.61 (135)	0.39 (85)
	rs2266780	AA	GA	GG	A	G
	Donors	0.67 (74)	0.26 (29)	0.06 (7)	0.80 (177)	0.20 (43)
	Recipients	0.60 (66)	0.34 (38)	0.06 (6)	0.77 (170)	0.23 (50)
CYP3A5	rs776746	GG	AG	AA	G	A
	Donors	0.46 (51)	0.46 (50)	0.08 (9)	0.69 (152)	0.31 (68)
	Recipients	0.53 (58)	0.41 (45)	0.06 (7)	0.73 (161)	0.27 (59)

Figure 1. Linkage disequilibrium tests for five *FMO3* variants in our study subjects (n = 220).

significant at weeks 1, 3, 4 and month 1 ($p = 0.002$, 0.004 , <0.001 and 0.002 , respectively), and were nearly significant at week 2 ($p = 0.053$).

The effects of single-recipient SNP on tacrolimus C/D ratios are shown in Table 3. The associations between tacrolimus C/D ratios and recipient *FMO3* gene polymorphisms were not significant except for rs1736557 polymorphisms at week 3 ($p = 0.030$). Tacrolimus C/D ratios of recipient *CYP3A5* rs776746 GG genotype carriers were 285.4 ± 265.6 , 132.8 ± 104.6 , 116.1 ± 99.6 , 130.7 ± 97.0 and 137.1 ± 92.4 at weeks 1, 2, 3, 4 and month 1, respectively, and 158.6 ± 201.0 , 100.6 ± 93.5 , 86.8 ± 84.3 , 90.4 ± 81.9 and 100.0 ± 91.5 for AG and AA genotype carriers. The differences were significant at every time point ($p = 0.002$, 0.029 , 0.010 , 0.013 and 0.007 , respectively).

Donor *FMO3* rs1800822 and rs909530 were shown to be associated with tacrolimus elimination rate as stated above. Therefore, the two genotypes were further investigated in a combination analysis. Eight kinds of genotype combination were found in our study subjects. The percentages of CC+CC (*FMO3* rs1800822 CC and rs909530 CC), CC+TC (*FMO3* rs1800822 CC and rs909530 TC), TC+TC (*FMO3* rs1800822 TC and rs909530 TC) were 43, 20 and

24%. The three genotype combinations represent the overwhelming majority (87%) of *FMO3* genotype population distribution (Figure 2A). Tacrolimus C/D ratios of donor CC+CC, CC+TC and TC+TC have significant differences in weeks 2, 3, 4 and month 1 ($p = 0.0022$, 0.0051 , 0.0466 and 0.0221 , respectively; Figure 2C–F).

Effects of *FMO3* polymorphisms on tacrolimus C/D ratios in subjects with different *CYP3A5* genotype backgrounds

To eliminate the potential contribution of *CYP3A5* polymorphisms on detected differences of tacrolimus C/D ratios as results of *FMO3* SNP types, we stratified patients by donor and recipient *CYP3A5* expresser status and tested the effects of combined donor *FMO3* rs1800822 and rs909530 SNPs on tacrolimus C/D ratios in each group. As shown in Table 4, for both donor and recipient *CYP3A5**3/*3 (nonexpresser) groups, donor *FMO3* polymorphisms did not seem to have effect on tacrolimus C/D ratios. The only significant differences were observed at week 2 in the donor nonexpresser group. On the other side, for both donor and recipient *CYP3A5**1 (expresser) groups, the associations between tacrolimus C/D ratios and donor

Table 2. Comparison of tacrolimus concentration-to-dose ratios in different groups of donor <i>FMO3</i> and <i>CYP3A5</i> polymorphisms at different times after transplantation.											
Gene	Locus	Geno-type	Week 1		Week 2		Week 3		Week 4		Month 1
			C/D ratios	p-value	C/D ratios	p-value	C/D ratios	p-value	C/D ratios	p-value	C/D ratios
<i>FMO3</i>	rs1800822	CC	241.6 ± 251.2	0.119	131.9 ± 123.9	0.016	115.6 ± 97.8	0.002	128.3 ± 93.8	0.026	139.0 ± 89.8
		TC+TT	174.2 ± 265.6		94.3 ± 71.2		69.4 ± 63.0		85.6 ± 61.9		99.0 ± 82.5
	rs2266782	GG	228.5 ± 268.3	0.958	130.8 ± 115.4	0.053	108.4 ± 111.0	0.229	117.5 ± 101.6	0.832	140.1 ± 110.0
		AG+AA	200.6 ± 249.2		94.3 ± 66.5		99.6 ± 65.5		98.4 ± 93.3		104.2 ± 64.1
	rs1736557	GG	240.9 ± 262.2	0.625	111.7 ± 106.1	0.448	99.9 ± 79.1	0.344	116.3 ± 103.8	0.983	123.9 ± 87.9
		AG+AA	208.7 ± 266.4		124.8 ± 111.4		106.1 ± 106.4		115.5 ± 86.8		137.8 ± 96.6
	rs909530	CC	251.4 ± 236.9	0.292	152.7 ± 129.4	0.001	142.8 ± 118.3	0.003	135.7 ± 105.1	0.188	156.0 ± 101.5
		TC	186.9 ± 311.7		93.2 ± 78.6		96.7 ± 61.3		96.0 ± 97.8		100.2 ± 71.2
	rs2266780	TT	174.2 ± 180.2		99.2 ± 61.3		95.5 ± 65.4		83.5 ± 90.0		99.2 ± 69.2
		AA	224.8 ± 261.1	0.634	131.4 ± 117.6	0.025	106.1 ± 110.8	0.320	117.5 ± 100.0	0.855	134.0 ± 103.3
<i>CYP3A5</i>	rs776746	GA+GG	202.2 ± 286.6		92.8 ± 64.7		99.9 ± 56.5		98.4 ± 98.2		103.4 ± 64.2
		GG	278.3 ± 396.9	0.002	130.8 ± 103.2	0.053	119.3 ± 79.1	0.004	144.7 ± 123.2	<0.001	145.0 ± 89.2
		AG+AA	176.0 ± 197.8		102.4 ± 101.3		82.8 ± 89.3		83.0 ± 73.0		101.5 ± 85.7
C/D: Concentration-to-dose.											

Table 3. Comparison of tacrolimus concentration-to-dose ratios in different groups of recipient *FMO3* and *CYP3A5* polymorphisms at different times after transplantation.

Gene	Locus	Geno-type	Week 1			Week 2			Week 3			Week 4			Month 1		
			C/D ratios	p-value	C/D ratios	C/D ratios	p-value	C/D ratios	p-value	C/D ratios	p-value	C/D ratios	p-value	C/D ratios	p-value	C/D ratios	p-value
<i>FMO3</i>	rs1800822	CC	240.9 ± 278.7	0.444	117.2 ± 103.5	0.951	103.1 ± 81.5	0.414	114.0 ± 103.2	0.483	136.5 ± 94.6	0.656					
		TC+TT	175.1 ± 252.2		119.0 ± 91.0		113.6 ± 96.8		127.4 ± 103.8		123.9 ± 70.4						
	rs2266782	GG	210.9 ± 235.7	0.504	111.7 ± 103.4	0.248	98.4 ± 91.6	0.225	120.0 ± 106.0	0.572	117.4 ± 88.4	0.270					
		AG+AA	235.4 ± 298.6		120.8 ± 97.3		120.2 ± 127.6		97.9 ± 94.1		134.5 ± 102.1						
	rs1736557	GG	223.6 ± 259.6	0.617	126.1 ± 94.9	0.094	113.6 ± 104.5	0.030	118.2 ± 96.8	0.473	135.2 ± 89.2	0.080					
		AG+AA	211.7 ± 266.3		93.5 ± 115.6		97.3 ± 64.5		110.9 ± 97.7		105.4 ± 82.3						
	rs909530	CC	197.6 ± 231.0	0.604	97.0 ± 108.9	0.223	98.7 ± 70.5	0.292	119.5 ± 119.4	0.927	117.4 ± 83.1	0.559					
		TC	222.4 ± 268.9		122.3 ± 114.6		109.6 ± 89.0		100.8 ± 74.0		133.9 ± 99.1						
	rs2266780	TT	260.4 ± 279.9		135.5 ± 152.9		134.2 ± 112.4		107.6 ± 100.8		130.7 ± 79.6						
		AA	202.2 ± 234.1	0.304	100.6 ± 97.1	0.093	97.4 ± 91.6	0.088	120.0 ± 104.5	0.657	106.7 ± 85.3	0.105					
<i>CYP3A5</i>	rs776746	GA+GG	242.4 ± 302.7		127.4 ± 102.1		121.8 ± 132.0		97.9 ± 88.3		135.8 ± 99.4						
		GG	285.4 ± 265.6	0.002	132.8 ± 104.6	0.029	116.1 ± 99.6	0.010	130.7 ± 97.0	0.013	137.1 ± 92.4	0.007					
		AG+AA	158.6 ± 201.0		100.6 ± 93.5		86.8 ± 84.3		90.4 ± 81.9		100.0 ± 91.5						
C/D: Concentration-to-dose.																	

FMO3 gene polymorphisms were significant at every time point tested. These results indicate that although *CYP3A5* is the major enzyme responsible for tacrolimus metabolism, in an rs776746 (expresser) genotype background when *CYP3A5* expression is hindered, *FMO3* might be a complementary pathway for tacrolimus metabolism. Therefore, the genotypes of *FMO3* affect the pharmacokinetics of tacrolimus.

Multivariate analysis

Multivariate linear regression analysis was used to screen the influence factors of tacrolimus pharmacokinetics. The incorporated cofactors included daily drug dose, donor and recipient *CYP3A5* rs776746 genotypes, serum albumin concentration, red blood cell count, as well as liver function indexes (glutamate pyruvate transaminase, glutaminoxalacetic transaminase, total bilirubin, direct bilirubin), all reported to have the potential effects on tacrolimus pharmacokinetics, as well as the number of alleles associated with fast metabolism patients carrying in donor rs1800822 and rs909530 sites. The results (Table 5) have shown that daily dose and donor and recipient *CYP3A5* rs776746 genotypes are the consistent predictors except for week 2. The number of donor rs1800822 allele T and rs909530 allele T was confirmed to be an independent predictor of C/D ratios for weeks 2, 3 and 4.

Discussion

FMOs play an important role in the metabolism of a wide variety of foreign chemicals, including therapeutic drugs, dietary-derived compounds and pesticides [18]. However, in comparison with the CYPs, their role has largely been overlooked. Several reasons probably contribute to a lack of consideration of FMOs when the metabolism of a drug is investigated. FMOs are generally not inducible, they have no known specific inhibitors, the FMO proteins are thermally labile and it can be difficult to identify and quantify the products of FMO-catalyzed reactions [17,24]. *FMO3* is a major FMOs enzyme in the human liver. Polymorphism of the *FMO3* gene is known to influence the functionality of the *FMO3* enzymes [18,25]. *FMO3* rs1800822 was identified to be associated with tacrolimus troughs in non-African–American kidney transplant patients in a multicenter kidney transplant consortium study. In the present study, we have explored the association between *FMO3* polymorphisms and tacrolimus elimination in Chinese liver transplant patients.

Among 27 *FMO3* SNPs analyzed, rs1800822 (S147S), rs2266782 (E158K), rs1736557 (V257M), rs909530 (N285N) and rs2266780 (E308G) variants have a relatively high population distribution in

Figure 2. Combined analysis of donor *FMO3* rs1800822 and rs909530 genotype on tacrolimus concentration-to-dose ratios. (A) *FMO3* genotype population distribution, (B–F) The differences of tacrolimus C/D ratios of donor CC+CC, CC+TC and TC+TC in weeks 1, 2, 3, 4 and month 1. CC+CC represents *FMO3* rs1800822 CC and rs909530 CC, CC+TC represents *FMO3* rs1800822 CC and rs909530 TC, TC+TC represents *FMO3* rs1800822 TC and rs909530 TC, then the rest (CC+TT, TC+CC, TC+TT, TT+CC and TT+TC) and so on.

the studied subjects. No statistically significant differences in genotype and allele frequencies were observed between recipients and donors for any of the above genetic polymorphisms. E158K, V257M and E308G variants have been identified in all populations exam-

ined so far including Caucasians, Asians and African-Americans, with marked variability in frequency between major ethnicities [26,27]. In our data, the minor allele frequencies were 0.230 (E158K), 0.195 (V257M) and 0.211 (E308G). *Cis*-linkage between K158 and

Table 4. The effects of donor <i>FMO3</i> polymorphisms on tacrolimus concentration-to-dose ratios in different <i>CYP3A5</i> genotype groups.												
Genotype	Week 1			Week 2			Week 3			Week 4		
	rs1800822	rs909530	C/D ratio	p-value	C/D ratio	p-value	C/D ratio	p-value	C/D ratio	p-value	C/D ratio	p-value
Donor <i>CYP3A5</i> *1	rs776746	rs909530										
	CC	CC	221.2 ± 221.2	0.0188	135.0 ± 134.0	0.0249	142.8 ± 149.5	0.0289	124.7 ± 106.1	0.0457	177.5 ± 147.2	0.0021
	CC	TC	143.5 ± 154.4		86.3 ± 84.1		90.6 ± 91.9		93.9 ± 60.5		122.8 ± 96.9	
	TC	TC	82.7 ± 132.6		68.5 ± 75.6		65.5 ± 30.1		62.3 ± 47.1		75.9 ± 65.6	
Donor <i>CYP3A5</i> *3/*3	CC	CC	303.7 ± 255.3	0.9606	160.3 ± 116.2	0.0124	157.2 ± 88.8	0.1123	147.9 ± 99.0	0.1043	228.1 ± 162.8	0.6492
	CC	TC	326.8 ± 408.8		96.6 ± 72.6		112.0 ± 110.0		178.4 ± 247.5		240.1 ± 182.1	
	TC	TC	280.5 ± 978.9		126.1 ± 88.7		101.1 ± 51.3		114.5 ± 117.9		163.1 ± 270.9	
Recipient <i>CYP3A5</i> *1	CC	CC	242.4 ± 205.2	0.0044	143.6 ± 119.7	0.0007	141.6 ± 105.8	0.0007	115.7 ± 103.6	0.0212	191.0 ± 145.7	0.0012
	TC	TC	83.1 ± 137.5		74.6 ± 68.6		65.0 ± 33.0		68.6 ± 58.4		75.9 ± 74.3	
Recipient <i>CYP3A5</i> *3/*3	CC	CC	258.4 ± 260.8	0.0832	160.3 ± 176.5	0.7884	163.4 ± 214.1	0.9826	148.2 ± 94.7	0.6543	203.7 ± 198.5	0.8447
	TC	TC	403.1 ± 785.9		160.2 ± 77.5		120.6 ± 118.7		119.0 ± 208.1		182.2 ± 304.1	
C/D: Concentration-to-dose.												

G308 has constantly appeared in all populations, with considerable variation in frequencies, of the inferred haplotype K158–G308 [28]. Among the five *FMO3* polymorphisms, only sites 158 and 308 were also in significant linkage disequilibrium. The three variants can result in decreased *FMO3* enzyme activity in a substrate-dependent manner. Trimethylaminuria is caused by a decreased ability of the *FMO3* enzyme to oxidize trimethylamine. Previously, E158K, V257M and E308G inherited in particular combinations were shown to result in decreasing in trimethylamine oxidation and may predispose people to mild trimethylaminuria [29,30]. While E158K and E308G loci reduce activity in catabolizing sulindac, an NSAID, used as a chemo-preventive agent in patients with familial adenomatous polyposis, resulting in an increased efficacy to prevent polyposis in familial adenomatous polyposis [31,32]. However, our data did not show an association between tacrolimus elimination with E158K, V257M or E308G.

The other two synonymous exon variants, rs1800822 and rs909530, were discovered by resequencing technology. Rs1800822 and rs909530 occurred at relatively high frequencies that differed among Hispanics (Mexican descent), African-Americans and non-Latino whites with significant ethnic differences [33]. In this study, we are the first to provide the allele and genotype frequency of the two *FMO3* polymorphisms in the Han Chinese population. Rs909530 has been previously reported to be associated with toxicity due to docetaxel treatment including febrile neutropenia, reduced absolute neutrophil count and hemoglobin reduction [34]. The function of rs1800822 and rs909530 remains unclear. Rs1800822 and rs909530 were synonymous mutations, which are considered to be ‘silent’ as they do not affect the protein sequence. However, a ‘silent’ mutation may still affect protein expression through various mechanisms [35–37]. Our data showed that donor *FMO3* rs1800822 allele T and rs909530 allele T were associated with fast tacrolimus elimination. To our knowledge, the present study was the first analysis of the full extent of *FMO3* genetic polymorphisms involving a drug-metabolizing enzyme and transporter array in a Han Chinese population.

The main metabolic organs of tacrolimus are the liver and intestine. A liver transplant patient is a good model for research on tacrolimus metabolism because the liver graft comes from the donor and intestine belongs to the recipient. In our data, donor but not recipient *FMO3* polymorphisms were associated with tacrolimus elimination. This finding was consistent with *FMO3* liver-specific expression in adults.

In our data, the pharmacokinetic influence of *FMO3* polymorphisms was limited in the subjects

Table 5. Multiple linear regression model for log-transformed tacrolimus concentration/dose ratios.

Model		Unstandardized coefficients		Standardized coefficients	t	p-value
		B	Std. error	Beta		
Week 1	(Constant)	3.087	0.145		21.302	0.000
	Donor rs776746	-0.266	0.064	-0.371	-4.158	0.000
	Recipient rs776746	-0.239	0.064	-0.334	-3.744	0.000
Week 2	(Constant)	2.077	0.038		54.115	0.000
	Total bilirubin	0.002	0.000	0.368	4.294	0.000
	No. of <i>FMO3</i> alleles*	-0.083	0.022	-0.323	-3.772	0.000
Week 3	(Constant)	2.597	0.115		22.586	0.000
	No. of <i>FMO3</i> alleles*	-0.088	0.025	-0.314	-3.568	0.001
	Donor rs776746	-0.165	0.051	-0.287	-3.252	0.002
	Recipient rs776746	-0.138	0.051	-0.241	-2.734	0.007
Week 4	(Constant)	2.563	0.114		22.429	0.000
	No. of <i>FMO3</i> alleles*	-0.087	0.024	-0.312	-3.600	0.001
	Donor rs776746	-0.176	0.050	-0.307	-3.513	0.001
	Recipient rs776746	-0.137	0.050	-0.239	-2.756	0.007
	Total bilirubin	0.002	0.001	0.179	2.058	0.042

No. of *FMO3* alleles* indicates the number of alleles of donor rs1800822 and rs909530 associated with fast metabolism patients carrying.

carrying *CYP3A5**1 but not *CYP3A5**3/*3. This discrepancy come from the allele frequency of *FMO3* rs1800822 and rs909530. The T allele frequencies of rs1800822, rs909530 were 16 and 35%, respectively. *FMO3* rs1800822 allele T and rs909530 allele T were associated with fast tacrolimus elimination. The patients with fast tacrolimus elimination were prone to be screened using the genotype *FMO3* rs1800822 and rs909530.

Many different factors were reported to have the potential effects on tacrolimus pharmacokinetics and may bias the results. The number of donor rs1800822 allele T and rs909530 allele T was confirmed to be an independent predictor of C/D ratios for weeks 2, 3 and 4 in the multivariate analysis.

Our study has several limitations. First, study population is relatively small and this limits the strength of conclusions. Due to the limited number of transplant operation, it is difficult to conduct the large sample pharmacogenomics research of tacrolimus. To our knowledge, the sample size of this study (n = 110) exceeded that of many pharmacogenomics researches of tacrolimus [7–10]. However, the association of *FMO3* polymorphisms and tacrolimus elimination should be confirmed in further multicenter study with large sample size. Second, tacrolimus C/D ratios beyond month 1 post-transplant (e.g., 6 and 12 months), in a more stable setting, were not observed in this study.

According to our clinical practice, individualized administration of tacrolimus in 1 month after liver transplantation had great impact on prognosis. We will concern the influence of *FMO3* polymorphisms on tacrolimus C/D ratios beyond month 1 post-transplant in further study.

Conclusion & future perspective

In the present study, we conducted systematic genetic association analysis with tacrolimus elimination in Chinese liver transplant patients and first found that donor *FMO3* rs1800822 allele T and rs909530 allele T were associated with fast tacrolimus elimination. These results provided critical information for the development of personalized medicine with tacrolimus for Chinese liver transplant patients and may lead to optimal dosing recommendations reduced adverse drug reactions and improved drug efficacy.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/full/10.2217/pgs-2016-0098

Author contributions

J Fan, L Ren, M Teng and T Zhang carried out the studies, participated in collecting data and drafted the manuscript. S Qin and J Fan performed the statistical analysis and participated in

its design. B Sun and X Zhang helped to draft the manuscript. All authors read and approved the final manuscript.

Financial & competing interests disclosure

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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Executive summary

Background

- The *FMO3* gene is very polymorphic and *FMO3* variants have also been shown to be associated to many drug responses.
- *FMO3* variants were potentially involved in tacrolimus metabolism in kidney transplantation. The influences of *FMO3* genotypes on tacrolimus elimination in Chinese liver transplant patients remained unclear.

Results

- Donor *FMO3* rs1800822 allele T and rs909530 allele T were associated with fast tacrolimus elimination.
- The number of donor rs1800822 allele T and rs909530 allele T was confirmed to be an independent predictor of the tacrolimus concentration-to-dose ratios for weeks 2, 3 and 4 in the multivariate analysis.

References

- Starzl TE, Fung JJ. Themes of liver transplantation. *Hepatology* 51, 1869–1884 (2010).
- Beckebaum S, Cicinnati VR, Radtke A, Kabar I. Calcineurin inhibitors in liver transplantation – still champions or threatened by serious competitors? *Liver Int.* 33, 656–665 (2013).
- Arkas SA, Schnitzbauer AA, Kirchner G, Obed A, Banas B, Schlitt HJ. Calcineurin inhibitor minimization protocols in liver transplantation. *Transpl. Int.* 22, 49–60 (2009).
- Wang L, McLeod HL, Weinshilboum RM. Genomics and drug response. *N. Engl. J. Med.* 364, 1144–1153 (2011).
- Chen P, Lin JJ, Lu CS *et al.* Carbamazepine-induced toxic effects and *HLA-B*1502* screening in Taiwan. *N. Engl. J. Med.* 364, 1126–1133 (2011).
- Kurawski M, Drożdżik M. Pharmacogenetics in solid organ transplantation: genes involved in mechanism of action and pharmacokinetics of immunosuppressive drugs. *Pharmacogenomics* 14, 1099–1118 (2013).
- Zhang X, Wang Z, Fan J, Liu G, Peng Z. Impact of interleukin-10 gene polymorphisms on tacrolimus dosing requirements in Chinese liver transplant patients during the early posttransplantation period. *Eur. J. Clin. Pharmacol.* 67, 803–813 (2011).
- Rojas LE, Herrero MJ, Bosó V *et al.* Meta-analysis and systematic review of the effect of the donor and recipient *CYP3A5* 6986A>G genotype on tacrolimus dose requirements in liver transplantation. *Pharmacogenet. Genomics* 23, 509–517 (2013).
- Ji E, Choi L, Suh KS, Cho JY, Han N, Oh JM. Combinational effect of intestinal and hepatic *CYP3A5* genotypes on tacrolimus pharmacokinetics in recipients of living donor liver transplantation. *Transplantation* 94, 866–872 (2012).
- Yu S, Wu L, Jin J *et al.* Influence of *CYP3A5* gene polymorphisms of donor rather than recipient to tacrolimus individual dose requirement in liver transplantation. *Transplantation* 81, 46–51 (2006).
- de Jonge H, Kuypers DR. Pharmacogenetics in solid organ transplantation: current status and future directions. *Transplant. Rev. (Orlando)* 22, 6–20 (2008).
- Elens L, Capron A, van Schaik RH *et al.* Impact of *CYP3A4*22* allele on tacrolimus pharmacokinetics in early period after renal transplantation: toward updated genotype-based dosage guidelines. *Ther. Drug Monit.* 35, 608–616 (2013).
- Yoon SH, Cho JH, Kwon O *et al.* *CYP3A* and *ABCB1* genetic polymorphisms on the pharmacokinetics and pharmacodynamics of tacrolimus and its metabolites (M-I and M-III). *Transplantation* 95, 828–834 (2013).
- Li D, Lu W, Zhu JY, Gao J, Lou YQ, Zhang GL. Population pharmacokinetics of tacrolimus and *CYP3A5*, *MDR1* and *IL-10* polymorphisms in adult liver transplant patients. *J. Clin. Pharm. Ther.* 32, 505–515 (2007).
- Boivin AA, Cardinal H, Barama A *et al.* Influence of *SLCO1B3* genetic variations on tacrolimus pharmacokinetics

- in renal transplant recipients. *Drug Metab. Pharmacokinet.* 28, 274–277 (2013).
- 16 Hines RN, McCarver DG. The ontogeny of human drug-metabolizing enzymes: Phase I oxidative enzymes. *J. Pharmacol. Exp. Ther.* 300, 355–360 (2002).
- 17 Shephard EA, Phillips IR. The potential of knockout mouse lines in defining the role of flavin-containing monooxygenases in drug metabolism. *Expert Opin. Drug Metab. Toxicol.* 6, 1083–1094 (2010).
- 18 Phillips IR, Shephard EA. Flavin-containing monooxygenases: mutations, disease and drug response. *Trends Pharmacol. Sci.* 29, 294–301 (2008).
- 19 Hines RN. Developmental and tissue-specific expression of human flavin-containing monooxygenases 1 and 3. *Expert Opin. Drug Metab. Toxicol.* 2, 41–49 (2006).
- 20 Hisamuddin IM, Yang VW. Genetic polymorphisms of human flavin-containing monooxygenase 3: implications for drug metabolism and clinical perspectives. *Pharmacogenomics* 8, 635–643 (2007).
- 21 Jacobson PA, Oetting WS, Brearley AM *et al.* Novel polymorphisms associated with tacrolimus trough concentrations: results from a multicenter kidney transplant consortium. *Transplantation* 91(3), 300–308 (2011).
- 22 SHEsis.
<http://analysis2.bio-x.cn/myAnalysis.php>
- 23 GraphPad Software.
www.graphpad.com
- 24 Krueger SK, Williams DE. Mammalian flavin-containing monooxygenases: structure/function, genetic polymorphisms and role in drug metabolism. *Pharmacol. Ther.* 106, 357–387 (2005).
- 25 Zhou J, Shephard EA. Mutation, polymorphism and perspectives for the future of human flavin-containing monooxygenase 3. *Mutat. Res.* 612, 165–171 (2006).
- 26 Hao D, Sun J, Furnes B *et al.* Allele and genotype frequencies of polymorphic *FMO3* gene in two genetically distinct populations. *Cell Biochem. Funct.* 25, 443–453 (2007).
- 27 Hao DC, Sun J, Furnes B *et al.* Haplotype frequency distribution and linkage disequilibrium analysis of single nucleotide polymorphisms at the human *FMO3* gene locus. *Biochem. Genet.* 44, 391–407 (2006).
- 28 Mao M, Matimba A, Scordo MG *et al.* Flavin-containing monooxygenase 3 polymorphisms in 13 ethnic populations from Europe, east Asia and sub-Saharan Africa: frequency and linkage analysis. *Pharmacogenomics* 10, 1447–1455 (2009).
- 29 Yeung CK, Adman ET, Rettie AE. Functional characterization of genetic variants of human *FMO3* associated with trimethylaminuria. *Arch. Biochem. Biophys.* 464, 251–259 (2007).
- 30 Lambert DM, Mamer OA, Akerman BR *et al.* *In vivo* variability of TMA oxidation is partially mediated by polymorphisms of the *FMO3* gene. *Mol. Genet. Metab.* 73, 224–229 (2001).
- 31 Hisamuddin IM, Wehbi MA, Chao A *et al.* Genetic polymorphisms of human flavin monooxygenase 3 in sulindac-mediated primary chemoprevention of familial adenomatous polyposis. *Clin. Cancer Res.* 10, 8357–8362 (2004).
- 32 Hisamuddin IM, Wehbi MA, Schmotzer B *et al.* Genetic polymorphisms of flavin monooxygenase 3 in sulindac-induced regression of colorectal adenomas in familial adenomatous polyposis. *Cancer Epidemiol. Biomarkers Prev.* 14, 2366–2369 (2005).
- 33 Koukouritaki SB, Poch MT, Henderson MC *et al.* Identification and functional analysis of common human flavin-containing monooxygenase 3 genetic variants. *J. Pharmacol. Exp. Ther.* 320, 266–273 (2007).
- 34 Awada Z, Haider S, Tfayli A *et al.* Pharmacogenomics variation in drug metabolizing enzymes and transporters in relation to docetaxel toxicity in Lebanese breast cancer patients: paving the way for OMICs in low and middle income countries. *OMICS* 17, 353–367 (2013).
- 35 Waldman YY, Tuller T, Keinan A, Ruppin E. Selection for translation efficiency on synonymous polymorphisms in recent human evolution. *Genome Biol. Evol.* 3, 749–761 (2011).
- 36 Okamoto K, Iwasaki N, Nishimura C *et al.* Identification of *KCNJ15* as a susceptibility gene in Asian patients with Type 2 diabetes mellitus. *Am. J. Hum. Genet.* 86, 54–64 (2010).
- 37 Dunn PP, Hammond L, Coates E, Street J, Griner L, Darke C. “Silent” nucleotide substitution in exon 4 is responsible for the “alternative expression” of *HLA-A*01:01:38L* through aberrant splicing. *Hum. Immunol.* 72, 717–722 (2011).