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ARTICLE

The effect of genetic variants in the transcription factor TSPYL family on the CYP3A4 mediated cyclosporine metabolism in kidney transplant patients

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Abstract

CYP3A4 activity shows considerable interindividual variability. Although studies indicate 60%–80% is heritable, common single nucleotide variants (SNVs) in *CYP3A4* together only explain ~10%. Transcriptional factors, such as the testis-specific Y-encoded-like proteins (TSPYLs) family, have been reported to regulate the expression of CYP enzymes including CYP3A4 in vitro. Here, we investigated the effect of genetic variants in *TSPYL* on CYP3A4 activity using data from a clinical study and a human liver bank. Five SNVs (rs3828743, rs10223646, rs6909133, rs1204807, and rs1204811) in *TSPYL* were selected because of a reported effect on *CYP3A4* expression in vitro or suggested clinical effect. For the clinical study, whole blood concentrations, clinical data, and DNA were available from 295 kidney transplant recipients participating in the prospective MECANO study. A multivariate pharmacokinetic model adjusted for body weight, steroid treatment, and *CYP3A4* genotype was used to assess the effect of the genetic variants on cyclosporine clearance. In multivariate analysis, homozygous carriers of rs3828743 had a 18% lower cyclosporin clearance compared to the wild-type and heterozygous patients (28.72 vs. 35.03 L/h, $p = 0.018$) indicating a lower CYP3A4 activity and an opposite direction of effect compared to the previously reported increased CYP3A4 expression. To validate, we tested associations between rs3828743 and CYP3A4 mRNA and protein expression as well as enzyme activity with data from a liver bank ($n = 150$). No association with any of these end points was observed. In conclusion, the totality of evidence is not in support of a significant role for *TSPYL* SNV rs3828743 in explaining variability in CYP3A4 activity.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Whereas 60%–80% of the variability in CYP3A4 enzyme activity results from genetic variation, single nucleotide variants (SNVs) in *CYP3A4* only explain ~20% of the observed interindividual variability. The *TSPYL* gene family serves as transcriptional factors that regulate CYP3A4 expression.

WHAT QUESTION DID THIS STUDY ADDRESS?

Whether genetic variants located in *TSPYL* genes are associated with cyclosporine clearance in vivo in kidney transplant patients and can be used to explain variability in CYP3A4 activity.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

The TSPYL1 missense variant, rs3828743, was associated with a decreased cyclosporine clearance suggesting a reduced CYP3A4 activity in vivo. However, these data could not be confirmed with data from a large liver bank indicating that TSPYL SNV rs3828743 does not have an important effect on CYP3A4 activity.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Genetic variation underlying variability in CYP3A4 activity remains to be elucidated. Our data show that transcriptional regulation of CYP3A4 through TSPYL does not play an important role.

INTRODUCTION

The cytochrome P450 3A (CYP3A) subfamily is the most abundant type of CYP enzyme in the human liver and small intestine.[1] It is involved in the metabolism of 30%–50% of prescription drugs.[2] Cyclosporine, one of the cornerstone immunosuppressants used after solid organ transplantation, is extensively metabolized by CYP3A enzymes. Cyclosporine is characterized by considerable interpatient variability in pharmacokinetics (PKs) which could be explained by variability of CYP3A enzyme activity.[3] Notably, the hepatic expression of CYP3A varies 20-fold between individuals.[4] Within the CYP3A subfamily, *CYP3A4* and *CYP3A5* are the predominant isoforms. Indeed, several common variant alleles, such as *CYP3A4**22 and *CYP3A5**3 have been consistently linked with altered CYP3A activity and its substrate clearance.[5,6] These variants are used in clinical practice to tailor the dosage of drugs metabolized by CYP3A enzymes.[7] However, although it is estimated that 60%–80% of the variability of the enzyme activity is heritable, single nucleotide variants (SNVs) in the *CYP3A4* gene only explain ~10% of the observed interindividual variability in CYP3A4 activity.[8,9] It has been suggested that transcriptional regulators, such as *HNF3γ* and *PXR*, contribute to the observed variability in CYP3A4 activity.[2,10,11]

More recently, associations between variants in the testis-specific Y-encoded-like proteins (TSPYLs) family and CYP3A activity have been reported. This family consists of TSPYL1 to TSPYL6, all of which are involved in many bio-functions, including chromatin remodeling[12] and transcriptional regulation.[13] Associations between *TSPYL* variants and multiple diseases, such as chronic obstructive pulmonary disease and endometrial cancer, have been reported.[14,15] A genomewide association study identified an association between rs1864729, an SNV in *TSPYL5*, with the regulation of CYP expression via transcriptional activation[15] showing the potential capability of genetic variants in TSPYL to regulate CYP enzymes expression and activity. Moreover, TSPYL1, TSPYL2, and TSPYL4 were observed to regulate the expression of several CYP enzymes, including *CYP2C9*, *CYP2C19*, *CYP17A1*, and *CYP3A4* in an in vitro setting by acting as a transcriptional factor. In vitro, increased expression of *CYP3A4* and *CYP2C19* was observed when the TSPYL1, TSPYL2, and TSPYL4 were silenced and this tendency was reversed in an overexpressed system.[16] By contrast, no transcriptional regulation effect was identified for *CYP3A5*.[16] Remarkably, *TSPYL1* rs3828743, a common missense variant, resulted in a loss of DNA binding ability to the promoters of *CYP2C19* and *CYP3A4*, thereby abolishing TSPYL1 suppression of *CYP2C19* and *CYP3A4* transcription resulting in an enhanced enzyme activity and substrate clearance.[16] Interestingly the effect of rs3828743 on the CYP3A4 expression was only investigated in prostate cancer cell-lines, which warrants further study to

determine if it has any gender-specific or disease-specific effect.

Here, we investigate associations between SNVs in *TSPYL* and *CYP3A4* activity using the clearance of the CYP3A4 substrate cyclosporine as a proxy.

METHODS

Sample and data collection

Whole blood concentrations, clinical data, and DNA were used from kidney transplant recipients who participated in the MECANO study (trial registration: NTR1615), a prospective randomized clinical trial.[17] All patients received orally administered cyclosporine therapy for the first 6 months after transplantation, of which the initial dosage was 4 mg/kg twice daily. Therapeutic drug monitoring was conducted for all patients at 0, 1, 2, 3, 4, 5, and 6 h after dose administration, and cyclosporine dosages were adjusted based on area under the cyclosporin concentration-time curve (AUC). Cyclosporine blood concentration was determined by fluorescence polarization immunoassay. For patients with data from multiple AUCs, available data of the first occasion (1 week after transplantation) were used for the analysis. Detailed information on the PK analysis and cyclosporine measurement can be found in the previous publications on this MECANO study.[5,17]

Genotyping

TSPYL variants were selected based on the following two criteria (1) a reported in vitro regulatory effect on CYP3A4 expression or (2) a described association with baseline severity of depression in patients with major depressive disorder. Allele-specific probes for each selected *TSPYL* SNV were ordered from Thermo Fisher (Thermo Fisher Scientific) as part of an openArray design. The QuantStudio 12K Flex OpenArray Genotyping system was used for genotyping together with TaqMan Genotyper Software (version 1.3) according to the protocols provided by the manufacturer. *CYP3A4* and *CYP3A5* genotypes were available from a previous study.[5] SNVs with a call rate of less than 95% were excluded from the analyses. Samples with a poor call rate (≥2 SNV calls missing) were also excluded. Finally, SNVs that were not in Hardy–Weinberg equilibrium ($p < 0.05$) were excluded from the analysis.

PK modeling and statistical analysis

Cyclosporine is mainly metabolized by CYP3A4 and was used as a proxy of CYP3A4 activity.[18] The influence of *TSPYL* variants on cyclosporine clearance (L/h) was analyzed based on a multivariate PK model. First, a population PK base model without covariates was developed using PK data from all patients. Patients with unknown CYP3A4*22 genotype ($n = 11$) were removed. For the patients with available genotyping data for both the TSPYLs as well as CYP3A4 variants, SNVs were included for association with the estimated individual cyclosporine clearance. Second, a multivariate model, which included established covariates body weight, steroid usage, and *CYP3A4*22* genotype for cyclosporine clearance was developed[5] and parameters of the model are shown in Table S1. Covariates were assessed via a forward inclusion and backward elimination stepwise process. Only the covariates that showed a significant influence on cyclosporine clearance in the univariate analysis were included in the multivariate model. Finally, the *TSPYL* variants that showed a significant effect on the cyclosporine clearance in the base model were analyzed with the multivariate model. A recessive genetic model was used (homozygous vs. heterozygous + wild-type), because this model showed statistical significance in a previous study.[16] Previous studies only included male samples (prostate cancer) and therefore a gender stratified analysis was performed.[16] The apparent cyclosporine clearance with fixed bioavailability was used as a proxy for CYP3A4 enzyme activity. Differences in cyclosporine clearance between each genotype group were evaluated via the Mann–Whitney *U* test. Results with *p* value < 0.05 were considered statistically significant. NONMEM version 7.4.4 was used for modeling assessment. Statistical analyses were carried out with R studio RStudio 2022.02.3.

Validation using a liver tissue cohort

The liver bank contained 150 liver tissue samples from patients undergoing liver surgery. More collection details have been described previously.[19–21] The study protocol was approved by the ethics committees of University Medical Center, Charite, Humboldt University, Berlin, and the University of Tübingen, Tübingen, Germany. Genotypes of TSPYL_rs3828743 were not directly tested and, therefore, estimated by imputation as described.[22] Associations between TSPYL variant and the CYP3A4 mRNA expression, the amount of microsomal CYP3A4 protein, and microsomal CYP3A4 enzyme activities quantified with 3 different CYP3A4 substrates (atorvastatin-hydroxylation,[19] verapamil-N-demethylation [unpublished data], and midazolam-4-hydroxylation [unpublished data]) were tested. All activity data were correlated with Spearman correlation coefficients of greater than or equal to 0.8 to each other and with greater than or equal to 0.7 with CYP3A4 protein levels.

RESULTS

Patients background

In total, DNA from 292 renal transplant recipients with available DNA samples, clinical data, and PK data were genotyped for five TSPYL SNVs The baseline characteristics of the 292 patients with complete data are presented in Table 1. Men and women were comparable with the exception of body surface area ($p < 0.05$).

Genetic analyses

Five TSPYL variants were selected. Rs3828743 was selected as it was reported to increase CYP3A4 expression in vitro.[16] Another four eligible SNVs rs10223646, rs6909133, rs1204807, and rs1204811, showed association with the severity of depression or the response to selective serotonin reuptake inhibitor treatment.[23]

The call rates of rS10223646 (66%) and rs1204811 (80%) where below the quality control threshold of greater than or equal to 95% and were removed from further analyses. The allele frequencies of the three remaining TSPYL variants are presented in Table 2. Genotyping results were in Hardy–Weinberg equilibrium and the allele frequencies in the current study are consistent with data reported on gnomAD (https://gnomad.broadinstitute.org/). For 37 samples, genotyping failed for two or more SNVs and these subjects were excluded from the analysis, resulting in a total of 255 subjects ready for modeling.

Univariate analysis

In patients ($n = 244$) in whom both genotyping data of TSPYL variants and *CYP3A4*22* were available, we assessed the effect of TSPYL SNVs rs3828743, rs6909133, and rs1204807 on CYP3A4 activity by comparing the apparent clearance (CL/F) of cyclosporine between different TSPYL genotype groups of each single variant (Figure 1 and Figure S1). Notably, homozygous carriers of rs3828743 showed an ~14% lower CL/F compared to wildtype and heterozygous subjects (median: 30.13 vs.

TABLE 1 Clinical characteristics of patients according to gender.

	Women, $n = 105$	Men, $n = 187$	p value
Age [mean, IQR]	50 [42–59]	51 [42–61]	0.269
BMI [mean, IQR]	25.4 [22.2–29.0]	25.3 [22.8–27.6]	0.864
Body surface area	1.80[1.67–1.90]	2.00 [1.86–2.11]	<0.05
CYP3A4 genotype			
*1/*1	93	165	0.902
*1/*22	11	21	
Unknown	1	1	
CYP3A5 genotype			
*1/*1	2	0	0.112
*1/*3	17	3	
*3/*3	85	149	
Unknown	1	8	

Abbreviations: BMI, body mass index; IQR, interquartile range.

TABLE 2 Allele and genotype frequencies of three TSPYL variants.

	Minor allele frequencies		Genotype frequencies			n, Call rate (%)
	Allele 1	Allele2	Allele 1/Allelle1	Allele 1/Allelle2	Allele 2/Allelle2	$N = 258$
rs3828743	A (26%)	G (74%)	15 (5.8%)	103 (39.9%)	137 (53.1%)	255 98.8%
rs6909133	A (57%)	G (43%)	82 (31.8%)	129 (50%)	43 (16.7%)	254 (98.4%)
rs1204807	A (64%)	C (36%)	106 (41.1%)	114 (44.2%)	35 (13.6%)	255 (98.8%)

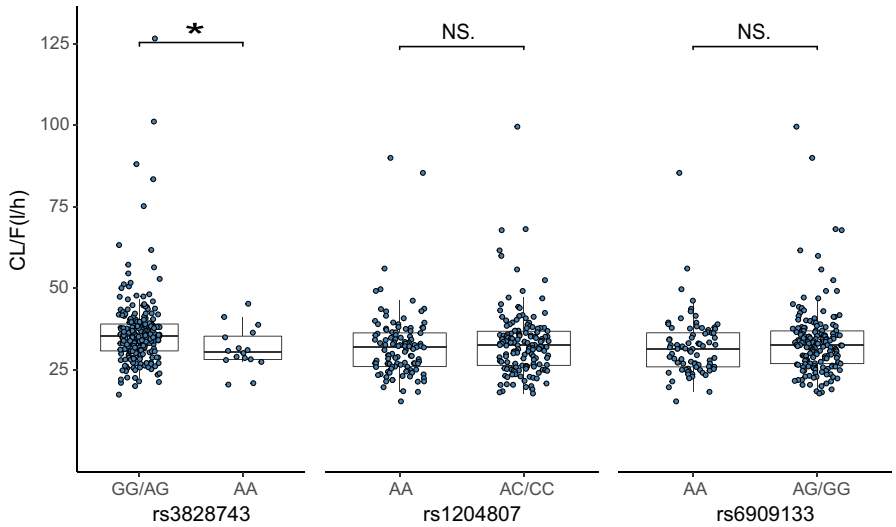

FIGURE 1 The effect of TSPYL SNPs on cyclosporine clearance (L/h). The clearance of cyclosporine based on population PK model was compared between different genotype groups of each TSPYL SNVs. The cyclosporine clearance was compared between homozygous groups (AA) and wildtype/heterozygous groups (GG/GA) in the rs3828743. The cyclosporine clearance was compared between wildtype and heterozygous/homozygous groups in the rs1204807 and rs6909133. CL/F, total apparent clearance; NS, not significant; PK, pharmacokinetic; SNV, single nucleotide variant.

TABLE 3 The comparison of cyclosporine clearance between rs3828743 genotype groups in base model and multivariate model.

		Base model			Multivariate model		
rs3828743	***n***	**CL/F, median (L/h)**	**Range (L/h)**	***p* value[a]**	**CL/F, median (L/h)**	**Range (L/h)**	***p* value[a]**
AA (homozygous)	15	30.13	(20.45–45.27)		28.72	(19.82–45.59)	
AG (heterozygous)	100	35.76	(17.37–126.70)	**0.0061**	35.46	(16.61–124.44)	**0.005**
GG (wildtype)	128	34.57	(20.00–88.13)	0.071	34.73	(19.27–80.87)	0.063
GG/GA (wt + het)	228	35.25	(17.37–126.70)	**0.022**	35.03	(16.61–124.44)	**0.018**

[a]The comparison is between the homozygous and the remaining groups. p-value < 0.05 was considered statistical.

Abbreviation: CL/F, total apparent clearance.

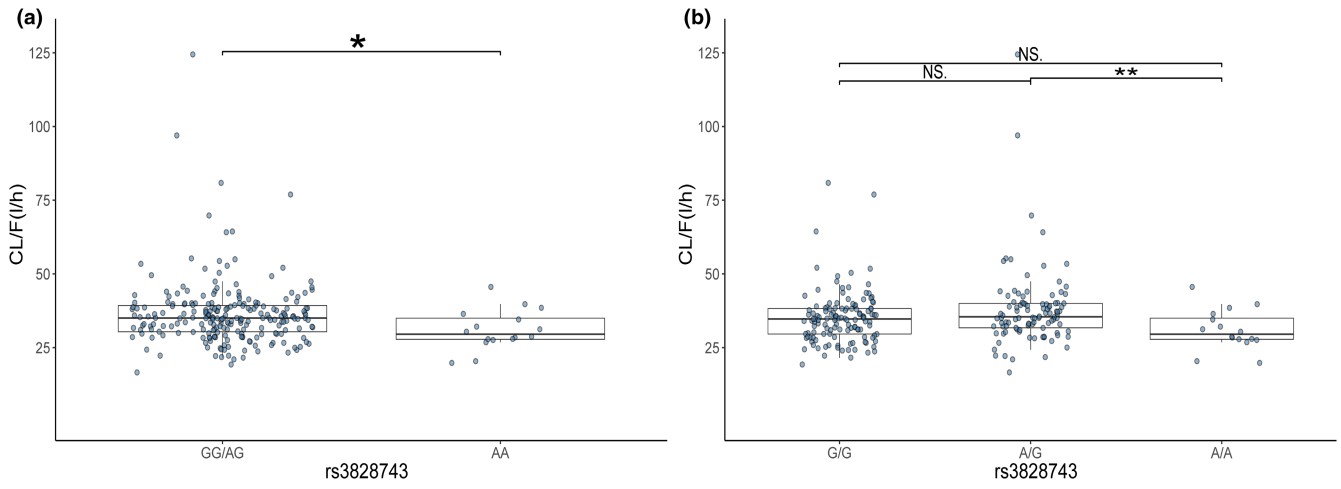

FIGURE 2 The effect of TSPYL_rs3828743 on the clearance of cyclosporine in the multivariate model. (a) The difference of cyclosporine clearance was assessed between rs3828743 homozygous carriers (AA) and wildtype/heterozygous carriers (GG/GA). (b) The difference of cyclosporine clearance was compared among all three genotype groups of rs3828743. CL/F, total apparent clearance; ns, not significant.

35.25, $p = 0.022$; Table 3). Heterozygous carriers showed a slightly increased cyclosporine CL/F compared to the wildtype patients (Figure S1 and Table 3). No significant differences in cyclosporine clearance were observed in variant carriers of rs1204807 and rs6909133 compared to the wildtypes (Figure S1).

Multivariate analysis

In the multivariate PK model, the CYP3A4*22 genotype, steroid usage, body weight, and rs3828743 were independently associated with cyclosporine clearance. Importantly, the effect of rs3828743 was consistent with the univariate model. Rs3828743 homozygous carriers showed a decreased cyclosporine CL/F compared to the combined heterozygous carriers and wildtypes (28.72 vs. 35.03, $p = 0.018$; Figure 2 and Table 3). Furthermore, after adjusting for CYP3A4*22 genotype, steroid usage, and body weight, the effect size of rs3828743 on cyclosporine clearance increased compared to the univariate analysis. In the multivariate model, cyclosporine clearance was 18% lower when comparing homozygous carriers to heterozygous and wildtype patients. However, no differences between heterozygous carriers and wildtype were observed in the multivariate model (Figure 2b).

Gender specificity

Because previously reported results originate from studies evaluating the effect of rs3828743 variant in samples from male subjects only, we performed a subgroup analysis using the multivariate model stratified by gender. Interestingly, the effect of rs3828743 was only observed in men. In men, homozygous variant carriers had an ~21% decreased clearance compared to heterozygous carriers and wildtypes (28.57 vs. 35.97, $p = 0.0052$; Figure S2 and Table 4). By contrast, no effect of rs3828743 genotypes was observed in women with the exception of a significantly increased clearance ($p = 0.026$) in the heterozygous carriers compared to the wildtype women (Figure S3).

Validation with data from a liver cohort

Because the results from our clinical data indicate a decreased CYP3A4 activity in homozygous carriers of rs3828743, whereas the previously published study reported an increased CYP3A4 expression, we conducted additional analyses in the liver bank. Among 150 liver samples, the expression of CYP3A4 mRNA and CYP3A4 protein was comparable in rs3828743 carriers and noncarriers.

TABLE 4 The effect of rs3828743 on cyclosporine clearance stratified for gender for the base and multivariate models.

	Base model				Multivariate model			
rs3828743	CL/F in men, median (range, l/h)	p value[a]	CL/F in womene, median (range, l/h)	p value[a]	CL/F in men, median (range, l/h)	p value[a]	CL/F in women, median (range, l/h)	p value[a]
AA (homozygous)	29.56 (20.45–45.27)		30.81 (27.41–41.21)		28.57 (19.81–45.49)		30.39 (26.82–39.75)	
AG (heterozygous)	35.79 (21.00–126.70)	**0.0077**	35.73 (17.37–101.20)	0.34	36.77 (21.02–124.44)	**0.0052**	34.15 (16.61–96.97)	0.39
GG (wildtype)	35.70 (22.49–61.78)	**0.0073**	30.58 (20.00–88.13)	0.5	35.68 (22.28–64.40)	**0.0079**	29.70 (19.27–80.87)	0.60
GG/GA (wt + het)	35.74 (21.00–126.70)	**0.025**	32.64 (17.37–101.20)	0.95	35.97 (21.02–124.44)	**0.0052**	32.36 (16.61–96.97)	0.92

[a]The comparison is between the homozygous and the remaining groups. *p*- value < 0.05 was considered statistically significant.

Abbreviation: CL/F, total apparent clearance.

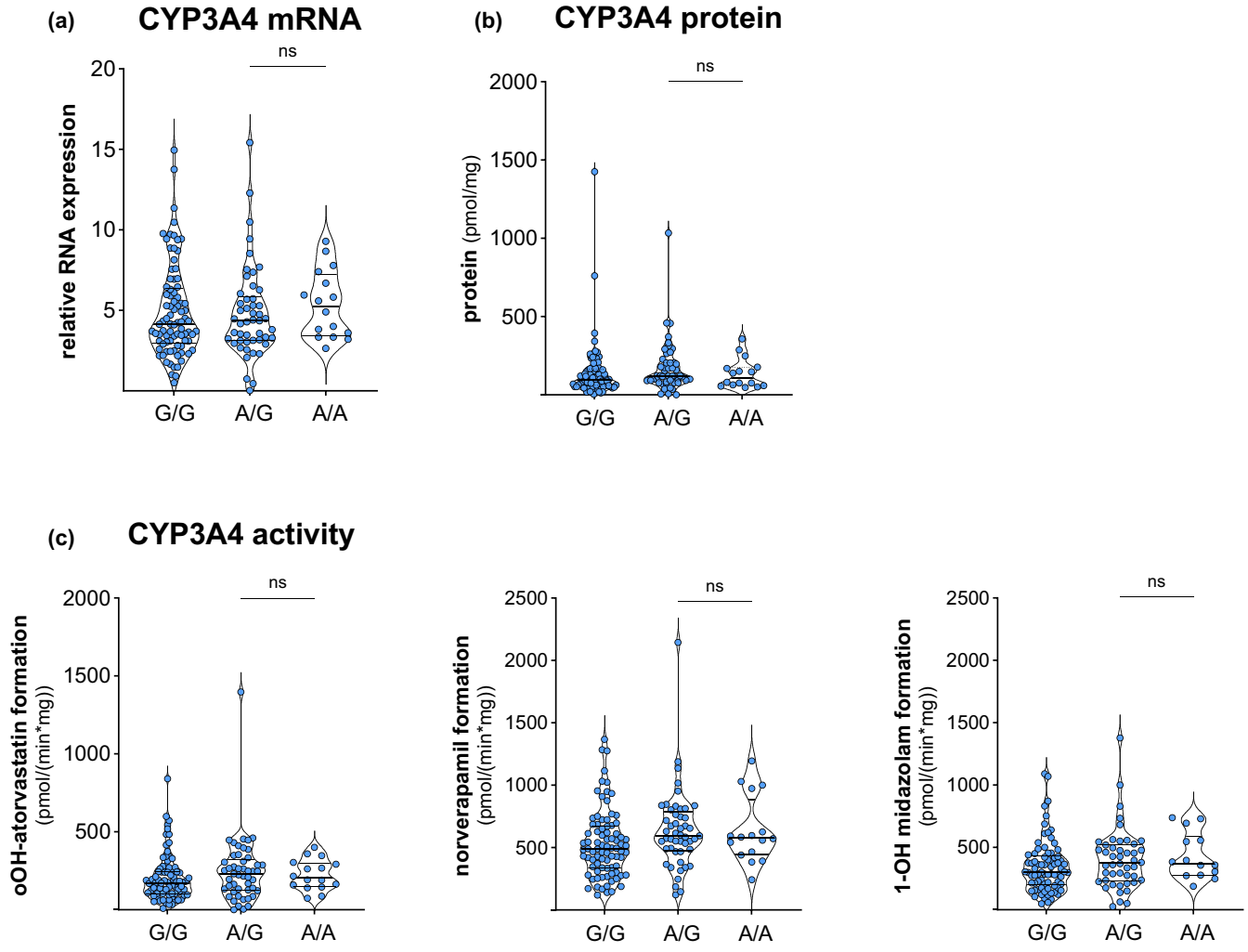

FIGURE 3 The CYP3A4 expression level and CYP3A4 enzyme activity in human liver ($n = 150$) stratified by TSPYL_rs3828743 genotype. (a) CYP3A4 mRNA expression relative to RPLP0. (b) CYP3A4 protein expression in human liver microsomes. (c) CYP3A4 activities determined in human liver microsomes given as pmol metabolite formed per minute and mg microsomal protein. Violin plots show medians and interquartile ranges. ns, not significant.

In addition, no difference was observed in CYP3A4 enzyme activity for any of the three tested substrates (Figure 3).

DISCUSSION

In this study, we evaluated whether genetic variants in *TSPYL* are associated with CYP3A4 metabolism using cyclosporine clearance in kidney transplant recipients as a proxy. The results from our clinical data indicate that the common *TSPYL1* missense mutation rs3828743 is associated with a decreased CYP3A4 enzyme activity. Interestingly, these results are conflicting with previously published data on the effect of rs3828743 on CYP3A4 activity in vitro which indicated an increased CYP3A4 activity.

Qin et al. showed that the mRNA expression level of CYP3A4 can be regulated via knockdown and overexpression of *TSPYLs*. In their in vitro study, rs3828743 was associated with enhanced CYP3A4 protein expression and increased CYP3A4 activity compared to other TSPYLs variants.[16] Notably, these results were obtained with prostate cancer cell lines and the function of several TSPYL1 variants, including rs3828743, was identified in a TSPYL1 overexpressed system. Cyclosporine is eliminated mainly by the CYP3A enzymes in the liver and small intestine, but not in the prostate. The expression level of CYP3A4 differs substantially between the liver and prostate with a median transcripts per million (TPM) of 335.3 in liver compared to 0.14 in prostate (the GTEx Portal on October 19, 2022, https://gtexportal.org/home/gene/CYP3A4). The expression level of TSPYL1 also shows tissue specificity and the liver has a substantially lower expression compared to the prostate (TPM 29.3 vs. 64.5, respectively). Therefore,

it is conceivable that the inconsistency of the effect of rs3828743 in the current study and previously reported in vitro data might be partially explained by the tissue-specific expression level of the CYP3A4 and TSPYL1. In addition, transcriptional regulation is complex as more transcriptional factors might be involved. A recent study identified two other transcriptional factors, REST and ZBTB7A, regulating the expression of TSPYL1, TSPYL2, and TSPYL4 and causing corresponding CYP3A4 expression changes in vitro.[24] Notably, their regulatory effects varied according to the presence of different genetic variants on TSPYL1 and TSPYL4.[19] This study warrants that the TSPYL4 is a non-negligible transcriptional factor involved in the CYP3A4 expression regulation, especially when the function of TSPYL1 is impaired by genetic variants. But neither the study of Qin et al. nor our study considered the contribution of TSPYL4 and its SNVs.

CYP3A4 enzyme activity can be substantially altered by comedication. For example, concomitant use of steroids has been suggested to increase CYP3A4 expression through PXR, RXRa, mRNA, and proteins.[25,26] However, in the MECANO trial, all patients received prednisolone for which conflicting effects on the PKs of cyclosporine have been reported.[27] Still, we cannot exclude that prednisolone administration might have interfered with the effect of rs3828743 on the CYP3A4 activity.

A limitation of our study is that we had to use cyclosporine clearance as a proxy for CYP3A4 activity rather than CYP3A4 expression or activity data. The use of this endophenotype might have resulted in the observed conflicting direction of effect for rs3828743. In an attempt to elucidate the conflicting results of the effect of rs3828743 on CYP3A4 activity in vitro and in vivo, we investigated the effect of this variant on CYP3A4 mRNA and protein expression and enzyme activity with data from a liver bank.[19,20] No association with CYP3A4 expression on mRNA or protein level was observed. Accordingly, no association with CYP3A4 enzyme activity assessed with three different substrates was found. Taken together, these results indicate that it is not likely that *TSPYL* rs3828743 significantly affects CYP3A4 activity. In our study, CYP3A5*3 did not show significant association with cyclosporine clearance which is consistent with other studies that reported that the effect of CYP3A5*3 is either insignificant or very small.[28–30] In addition, a limitation of our study is that ~90% of the patients in our study are of European descent.

In conclusion, the totality of evidence is not in support of a significant role for *TSPYL* SNV rs3828743 in explaining variability in CYP3A4 activity.

AUTHOR CONTRIBUTIONS

Q.Z., M.vL., J.J.S., T.vG., D.J.A.R.M., K.K., and M.S. wrote the manuscript. J.J.S., Q.Z., T.vG., M.vL., and D.J.A.R.M. designed the research. Q.Z., D.J.A.R.M., J.S.S., F.J.B., J.W.dF., K.K., and M.S. performed the research. Q.Z., D.J.A.R.M., M.vL., J.J.S., T.vG., K.K., and M.S. analyzed the data.

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CONFLICT OF INTEREST STATEMENT

The authors declared no competing interests for this work.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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