

Dual Roles for the TSPYL Family in Mediating Serotonin Transport and the Metabolism of Selective Serotonin Reuptake Inhibitors in Patients with Major Depressive Disorder

Sisi Qin¹, Andy R. Eugene¹, Duan Liu¹, Lingxin Zhang¹, Drew Neavin¹, Joanna M. Biernacka², Jia Yu¹, Richard M. Weinshilboum^{1,*} and Liewei Wang^{1,*}

We previously reported that testis-specific Y-encoded-like protein (TSPYLs) are transcription regulators for CYP3A4, CYP2C9, and CYP2C19. Here, we observed dual roles for TSPYLs in mediating serotonin transport and the metabolism of selective serotonin reuptake inhibitors (SSRIs) in patients with major depressive disorder (MDD). The widely prescribed SSRIs, citalopram, and escitalopram are metabolized mainly by CYP2C19. The *TSPYL1* rs3828743 single nucleotide polymorphism (SNP), which decreases its suppression of CYP2C19 expression, was associated with rapid escitalopram metabolism and worse treatment response in the Mayo PGRN-AMPS clinical trial. We also found that TSPYLs can regulate expression of the serotonin transporter protein, SLC6A4, and, in turn, serotonin transport into cells. The SNPs in tight linkage disequilibrium with the *TSPYL1* rs10223646 SNP were significantly correlated with baseline severity of depression in patients with MDD in the Sequenced Treatment Alternatives to Relieve Depression and International SSRI Pharmacogenomics Consortium clinical trials. Our findings suggest that genetic variation in *TSPYL* genes may be novel indicators for baseline severity of depression and SSRI poor response.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

✓ Genetic variation in *SLC6A4* responsible for serotonin transport contributes to susceptibility for major depressive disorder (MDD). Genetic variation in *CYP2C19*, the metabolizing enzyme that catalyzes selective serotonin reuptake inhibitor (SSRI) biotransformation can influence SSRI drug concentrations.

WHAT QUESTION DID THIS STUDY ADDRESS?

✓ This study revealed the function of testis-specific Y-encoded-like protein (*TSPYL*) genes and genetic variation in these genes in the regulation of serotonin transport and metabolism of SSRIs, citalopram (CT) and escitalopram (S-CT).

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

✓ The *TSPYL* family members not only contribute to the regulation of the expression of CYP2C19, affecting plasma SSRI concentrations in patients with MDD, but also regulate the expression of SLC6A4, a transporter that is involved in MDD pathophysiology. Taken together, they contribute to response to S-CT and CT, two commonly used SSRIs in the treatment of depression.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

✓ The genetic variation of *TSPYL* genes may be used as novel biomarkers for the prediction of MDD baseline severity of depression, and response to CT and S-CT, two commonly prescribed SSRIs.

Major depressive disorder (MDD) is the most common psychiatric disorder, and the serotonin transporter (SERT) encoded by the *SLC6A4* gene is thought to play a crucial role in the pathophysiology of MDD.¹ As the most commonly prescribed antidepressants, selective serotonin reuptake inhibitors (SSRIs), such as citalopram (CT) and escitalopram (S-CT), bind to SERT and block serotonin reuptake by neurons, allowing more serotonin to be available for neuron to neuron communication.²

CT is a racemic mixture of S-enantiomer and R-enantiomer, whereas S-CT is only the S-enantiomer.³ Most or all of the therapeutic effect of CT is thought to be mediated by the effect of S-CT, and the equivalent dose of S-CT is half that of CT. The human hepatic cytochrome P450 (CYP) enzyme, CYP2C19, plays a major role in the initial biotransformation step for S-CT to form S-monodesmethylocitalopram (S-DCT),^{4,5} and single nucleotide polymorphisms (SNPs) that impact CYP2C19 protein level

¹Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, Minnesota, USA; ²Department of Health Sciences, Mayo Clinic, Rochester, Minnesota, USA. *Correspondence: Liewei Wang and Richard M. Weinshilboum (Wang.Liewei@mayo.edu and Weinshilboum.Richard@mayo.edu)

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can alter S-CT metabolism. Specifically, the rs1074145 SNP near the *CYP2C19* gene, which is in tight linkage disequilibrium (LD) with the loss of function allele *CYP2C19**2 showed a genome-wide significant association ($P = 4.1 \times 10^{-9}$) with plasma concentrations of S-CT in 435 patients with MDD at 4 and 8 weeks post-CT or S-CT treatment initiation in the Mayo Clinic PGRN-AMPS SSRI clinical trial.⁸

Recently, we demonstrated that three members of the testis-specific Y-encoded-like protein (TSPYL) gene family, TSPYL1, TSPYL2, and TSPYL4, can regulate the expression of many CYP genes, including *CYP17A1*, *CYP3A4*, *CYP2C9*, and *CYP2C19*.⁹ Moreover, a common *TSPYL1* SNP, rs3828743 (G/A) (Pro62Ser), abolishes TSPYL1's suppression of the expression of *CYP2C19* and *CYP3A4*. *CYP3A4* is the most abundant CYP isoform and metabolizes > 50% of all drugs used in the clinic,¹⁰ including, for example, abiraterone¹¹ a *CYP17A1* inhibitor. The variant SNP genotype (A) of *TSPYL1* was significantly associated with worse response and progression-free survival in a prospective clinical trial of 87 patients with metastatic castration-resistant prostate cancer treated with abiraterone acetate/prednisone—presumably as a result of accelerated metabolism of the drug.⁹

Because S-CT is mainly metabolized by *CYP2C19* to form S-DCT, based on our previous findings that the expression of *CYP2C19* is regulated by TSPYL1, TSPYL2, and TSPYL4, and the fact that the common *TSPYL1* SNP rs3828743 abolishes TSPYL1's suppression of *CYP2C19* expression, we examined the association between the *TSPYL1* rs3828743 SNP and plasma drug concentrations in patients with MDD treated with CT or S-CT in the Mayo Clinic PGRN-AMPS SSRI trial.⁸ Moreover, we also observed that TSPYL1, TSPYL2, and TSPYL4 regulate the expression of the SERT gene *SLC6A4*, affecting the transport of serotonin, and that additional *cis*-expression quantitative trait locus (eQTL) SNPs for the *TSPYL1* and *TSPYL4* genes are significantly associated with baseline severity of depressive symptoms in patients with MDD treated with CT or S-CT in the Mayo Clinic PGRN-AMPS trial, the International SSRI Pharmacogenomics Consortium (ISPC), and the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial, one of the largest studies ever conducted to evaluate drug treatment effectiveness in patients with MDD.^{12,13}

METHODS

Cell culture

The HepG2 human hepatoma cells and Caco2 human colorectal adenocarcinoma cells were obtained from American Type Culture Collection (ATCC, Manassas, VA). HepG2 cells were cultured in Eagle's Minimum Essential Medium (ATCC) supplemented with 10% fetal bovine serum (Atlanta Biologicals, Flowery Branch, GA). Caco2 cells were grown in Eagle's Minimum Essential Medium supplemented with 20% fetal bovine serum.

siRNAs and cDNA constructs transfections

Two specific siRNAs that targeted to *TSPYL1*, *TSPYL2*, and *TSPYL4* (Dharmacon, Lafayette, CO), as well as the nontargeting siRNA controls, were transfected into HepG2 and Caco2 cells using the Lipofectamine RNAiMAX Reagent (Thermo Fisher Scientific, Waltham, MA). The *TSPYL1*, *TSPYL2*, and *TSPYL4* cDNA plasmid (OriGene,

Rockville, MD) and empty vectors were transfected into cells by using the Lipofectamin 3000 (Thermo Fisher Scientific) for overexpression study. Total RNAs were extracted 48 hours after transfection for RNA quantification.

mRNA quantification

Total RNA was purified using the Quick-RNA Miniprep Plus Kit (Zymo Research, Irvine, CA). For each reaction, 200 ng of total RNA was used for amplification of the target gene. mRNA levels for *TSPYL1*, *TSPYL2*, *TSPYL4*, and *SLC6A4* were quantified by quantitative real-time polymerase chain reaction using the PrimeTime (IDT, Coralville, IA) pre-designed quantitative polymerase chain reaction primers and the Power SYBR Green RNA-to- C_T 1-Step Kit (Life Technologies, Grand Island, NY). Gene expression analyses were performed using the $\Delta\Delta C_t$ method, and *ACTB* was used as the internal reference gene. Three independent experiments were performed.

Serotonin/citalopram treatment and high-performance liquid chromatography assay for serotonin

Caco2 cells were "starved" in phenol red-free growth media with 5% charcoal stripped serum (Thermo Fisher Scientific) for 48 hours. Cells were then transfected with *TSPYL1/2/4* siRNAs or cDNA plasmids. Twenty-four hours later, equal numbers of transfected cells were seeded into plates for 6 hours until cells attached to the wells, hormone-free media with 10 μ M serotonin (Sigma-Aldrich, St. Louis, MO) alone or combined with 10 μ M citalopram (Sigma-Aldrich) were then replaced with the phenol red-free growth media containing 5% charcoal stripped serum. After 20 minutes, media were removed and the cells were trypsinized, collected, and counted.

The 1×10^6 Caco2 cells were collected in 1 M ice-cold perchloric acid and neutralized by adding ice-cold 2 M potassium hydroxide. The 50 μ l injections was used to analyze samples for serotonin on a Shimadzu high-performance liquid chromatography system (Shimadzu, Kyoto, Japan), equipped with a CBM-20A controller, LC-20AD pump, and with UltiMate 3000 ECD-3000RS Electrochemical Detector (Thermo Scientific, San Jose, CA). Chromatographic separation was achieved on a Shimadzu C18 reversed-phase column 150 \times 4.6 mm, 3 μ m particle size (Shimadzu) coupled with a Phenomenex Security Guard C18 guard column 4 \times 3 mm (Phenomenex, Torrance, CA). The mobile phase was degassed as well as vacuum filtered through 0.22 μ m nylon membranes. The system equilibrated with mobile phase MDTM (Thermo Scientific), through which mobile phase was at a rate of 1.0 mL/min. The oven was held at 40°C and the analyzing time was 10 minutes. The samples were detected by electrode with a two-channel coulometric cell (6011RS; Thermo Scientific) and cell potential was set at E1 = -175 mV (100 nA), E2 = +475 mV (1 μ A). Data were acquired and processed with the Chromeleon software version 7.2.8 (Thermo Scientific). Peaks from the samples were then identified and quantified by comparison with the serotonin (Sigma) standard.

Details for study subjects and statistical analyses are in **Supplemental Materials and Methods**.

Consent and ethics

The study protocol of PGRN-AMPS was reviewed and approved by the Mayo Clinic Institutional Review Board and all patients provided written informed consent. All participants in ISPC and STAR*D study included in these analyses had approval to participate in the consortium from their local ethical review board with written informed consent.

RESULTS

The *TSPYL1* rs3828743 SNP was significantly associated with SSRI metabolism in patients with MDD

In our previous study, we had found that *TSPYL1*, *TSPYL2*, and *TSPYL4* positively regulate the expression of *CYP17A1*,

while they negatively regulate the expression of *CYP3A4*, *CYP2C9*, and *CYP2C19* in human HepG2 and HepaRG hepatic cell lines. Furthermore, the *TSPYL1* rs3828743 SNP (P62S) abolishes *TSPYL1* suppression of *CYP2C19* and *CYP3A4* expression and is significantly associated with clinical response to therapy with abiraterone, a drug which is metabolized by *CYP3A4* in patients with prostate cancer.⁹ Because *CYP2C19* is the major enzyme metabolizing CT/S-CT to form DCT/S-DCT, we hypothesized that the rs3828743 SNP might affect the metabolism of CT/S-CT. To study a possible association between the rs3828743 SNP and plasma drug concentrations, we took advantage of the Mayo Clinic PGRN-AMPS SSRI clinical trial, which included assays of plasma concentrations of CT/S-CT and their respective metabolites measured in blood samples drawn from 435 patients with MDD after 4 and 8 weeks of SSRI therapy. Demographic and clinical characteristics as well as plasma drug and drug metabolite concentrations for the patients enrolled in this study were described in a previous report.⁸

Because the S-CT for the parent drug and metabolites are thought to play the major role in their clinical effect,³ we focused our statistical analyses on S-CTs. The log dose-normalized concentrations of S-CT and its metabolites were not affected by *TSPYL1* rs3828743 SNP genotypes after 4 weeks of therapy (data not shown), whereas after 8 weeks of therapy, the concentrations of parent drug (S-CT) in patients with the *TSPYL1* rs3828743 homozygous wild-type (WT) SNP genotype were higher than in those with the heterozygous genotype ($P = 0.0497$), the ratio of metabolite to parent drug (S-DCT/S-CT) was significantly lower in homozygous WT SNP genotype patients compared with those with the heterozygous genotype ($P = 0.0289$), with no obvious difference between genotypes in the metabolites for S-DCT (Figure 1a). This is consistent with the effect of SNP function on *CYP2C19* expression, as the SNP increased the expression of *CYP2C19*, which may result in rapid metabolism of S-CT.

To take account of the significant effects of the *CYP2C19* alleles that are known to affect *CYP2C19* function and S-CT metabolism,

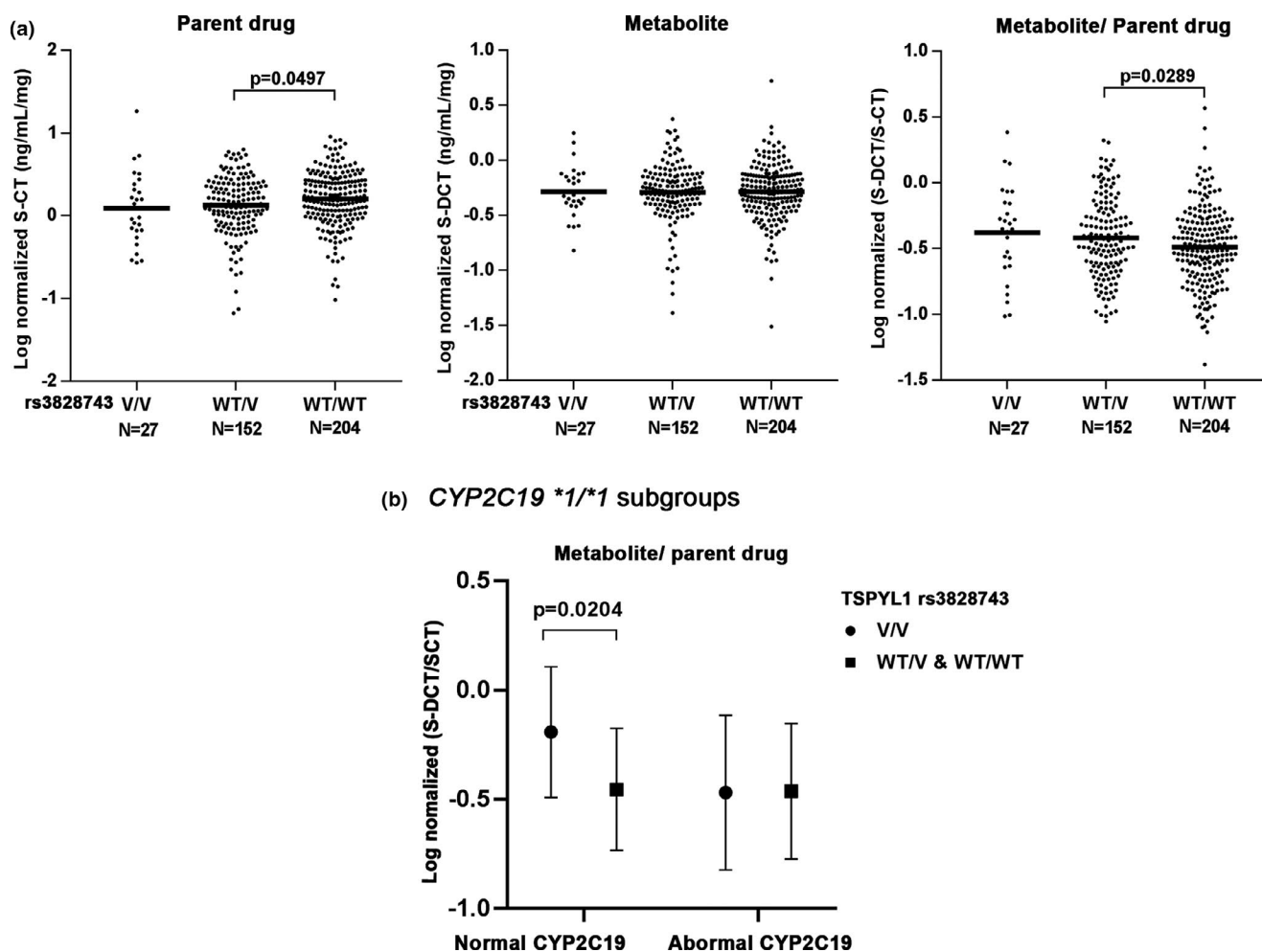


Figure 1 Week 8 plasma S-CT, S-DCT, and the ratio of S-DCT/S-CT for patients with MDD enrolled in PGRN-AMPS compared based on *TSPYL1* rs3828743 single nucleotide polymorphism genotypes. (a) S-CT and its metabolite S-DCT concentrations were normalized to dose, and log normalized S-CT concentrations, S-DCT concentrations and their ratio were compared among rs3828743 genotypes using Welch *t*-tests. (b) Data were subgrouped based on *CYP2C19* *1/*1 genotypes, the ratio of S-DCT and S-CT were compared among rs3828743 genotypes using Welch *t*-tests. MDD, major depressive disorder; S-CT, escitalopram; S-DCT, S-monodesmethylcitalopram; *TSPYL1*, testis-specific Y-encoded-like protein.

Table 1 Regression model for log nom(S-DCT/S-CT) at week 8

Term	Coefficient	SE	P value
Intercept	-0.57805	0.04102	< 0.0001
rs3828743[AA]	0.0793451	0.040245	0.0494
rs3828743[AG]	-0.007903	0.026185	0.7630
rs1074145[AA]	-0.209906	0.068603	0.0024
rs1074145[AG]	-0.00758	0.03943	0.8477
*1/*1 coding[A]	-0.003366	0.023593	0.8866
rs3828743[AA]* *1/*1	0.0920755	0.040245	0.0227
rs3828743[AG]* *1/*1	-0.045578	0.026185	0.0826

R-squared 0.1379; adjusted R-squared 0.1218; number of observations 383. *TSPYL1* SNP rs3828743 [AA] is homozygous variant and [AG] is heterozygous genotype. *CYP2C19* SNP rs1074145 [AA] is homozygous variant and [AG] is heterozygous genotype. *CYP2C19* *1/*1 indicates normal *CYP2C19*, which is not containing rs1074145, *3, or *17 allele.

Bold indicate the predictors with *P* value < 0.05 in the regression model.

we built a regression model using *CYP2C19**1, rs1074145 (in tight LD with *2) *3, *17 together with *TSPYL1* rs3828743 to test their interaction effect on S-CT metabolism (Table 1). The *CYP2C19* rs1074145 SNP was reported to be a genomewide significant SNP associated with plasma S-CT concentrations ($P = 4.1 \times 10^{-9}$)⁸ and it is in tight LD ($r^2 = 0.93$) with the rs4244285 SNP, the most common *CYP2C19* loss-of-function allele, *2. *CYP2C19**3 (SNP rs4986893) is associated with decreased activity, whereas the *17 rs12248560 SNP results in increased *CYP2C19* activity. As shown in Table 1, the S-CT metabolism (ratio of S-DCT and S-CT) is associated with *CYP2C19* rs1074145, *TSPYL1* rs3828743, and the interaction between rs3828743 and *CYP2C19* genotypes. The effect of *TSPYL1* SNP rs3828743 on S-CT metabolism was different between *CYP2C19* subgroups categorized into normal and abnormal functions based on genotypes for known functional SNPs in *CYP2C19*. The regression model (Table 1) supported the conclusion that the *TSPYL1* rs3828743 homozygous variant was associated with rapid S-CT metabolism (high S-DCT/S-CT ratio; Table 1, rs3828743 homozygous variant coefficient > 0, and Figure 1a, right panel), especially within the *CYP2C19* normal function (*1/*1) subgroup (Table 1; coefficient of interaction between rs3828743 homozygous variant and *1/*1 > 0, and Figure 1b).

These results suggested that *TSPYL* affected the initial bio-transformation of S-CT to S-DCT by regulating expression of the metabolizing enzyme *CYP2C19*. The *TSPYL1* rs3828743 variant SNP genotype may imply a rapid S-CT metabolism subtype within *CYP2C19* genotypes, which had previously been thought to possess “normal” catalytic function.

***TSPYL1* SNP rs3828743 was associated with S-CT response in patients with MDD**

To further investigate whether the *TSPYL1* rs3828743 SNP might affect the clinical outcome for CT or S-CT treatment in the PGRN-AMPS study, the same patient cohort for whom plasma drug concentrations were determined, changes of both Hamilton Depression Rating Scale (HAMD) and Quick Inventory of

Depressive Symptomatology Score (QIDS-C) scores, two end points to measure clinical response at week 4 or week 8 post-CT or S-CT treatment, were evaluated based on *TSPYL1* rs3828743 SNP genotypes and additional patient characteristics, including baseline depression score, sex, marital status, level of education, family history, age group, age of onset, and the length of current depressive episode.

When percentage change at week 4 and week 8 from baseline in HAMD score was used as a readout for S-CT response, none of the variables were associated with S-CT drug response. However, in patients who had no response to SSRI treatment at week 4 and were subjected to dose escalation after week 4, only the rs3828743 SNP variant genotype was associated with worse S-CT drug response ($P = 0.0012$). When percentage change at week 4 and week 8 in QIDS-C score from baseline was used as a readout for S-CT response, both rs3828743 and baseline QIDS-C score were associated with drug response (Table S1). Compared with *TSPYL1* rs3828743 WT genotype, the rs3828743 variant that was associated with extensive S-CT metabolizer status was significantly associated with worse drug response (lower percentage change; Figure 2a). Within patients subjected to dose escalation after week 4, the rs3828743 SNP and baseline QIDS-C were also associated with S-CT drug response (Table S2). The percentage change of QIDS-C was significantly lower in patients with the *TSPYL1* rs3828743 variant SNP genotypes compared with the WT genotypes (Figure 2b). These results suggested that the association of the *TSPYL1* rs3828743 variant SNP genotype with a poor response to S-CT treatment may be due, in part, to extensive metabolism of S-CT.

The *TSPYL* family regulates *SLC6A4* expression and mediates serotonin transport

Although previous studies of the PGRN-AMPS trial identified SNPs in or near the *CYP2C19* gene that were associated with plasma drug concentrations with genomewide significance,⁸ an association between plasma drug levels and clinical outcomes was not observed. Because the *TSPYL1* SNP rs3828743 regulation of *CYP2C19* expression influenced both plasma drug levels and drug response (Figures 1 and 2), we wondered whether the *TSPYL* family might also regulate genes in addition to *CYP2C19*, which might be involved in this disease or its treatment outcomes. To test that possibility, we performed RNA-seq in HepaRG cells with *TSPYL1*, *TSPYL2*, and *TSPYL4* knock-down and noticed significantly decreased levels of expression of the serotonin transporter gene, *SLC6A4*. To verify whether *TSPYLs* might regulate the expression of *SLC6A4*, we knocked down or overexpressed *TSPYL1*, *TSPYL2*, and *TSPYL4* in HepG2 cells, which have high levels of *SLC6A4* expression. We observed dramatically decreased mRNA expression of *SLC6A4* after knocking down *TSPYL1*, *TSPYL2*, and *TSPYL4* using two different siRNAs (Figures S1 and S2), but significantly increased levels of *SLC6A4* expression after overexpression of those *TSPYLs* (Figure S1).

Because *SLC6A4* encodes the SERT protein, which transports serotonin from the synaptic cleft into presynaptic neurons, we also determined whether *TSPYL1*, *TSPYL2*, and *TSPYL4* could alter serotonin transport by regulating the expression of *SLC6A4*. As a

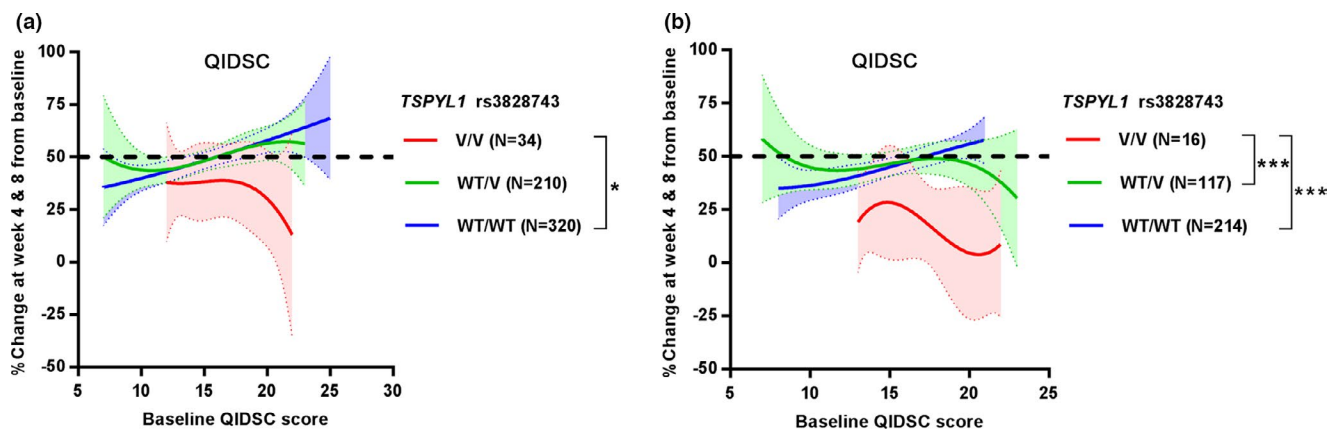


Figure 2 Percentage changes of Quick Inventory of Depressive Symptomatology Score (QIDS-C) at weeks 4 and 8 from baseline compared among testis-specific Y-encoded-like protein (*TSPYL1*) rs3828743 single nucleotide polymorphism (SNP) genotypes. **(a)** Data included plasma samples of patients treated with citalopram (CT) and escitalopram (S-CT) at week 4 and week 8. **(b)** Data only included patients that have higher S-CT concentrations at week 8 compared with week 4. Comparisons were performed using Mann–Whitney test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. X axis indicates baseline QIDS-C score.

first step, the expression of *TSPYL1*, *TSPYL2*, and *TSPYL4* was altered in the human colorectal adenocarcinoma cell line Caco-2, which has a high level of *SLC6A4* expression and has been widely used as a model for transporter functional studies. The positive regulation of *SLC6A4* function by *TSPYLs* was confirmed in Caco-2 cells (**Figure 3a,c**). Next, serotonin alone or combined with CT was added to the cell culture media. Cell lysates were collected 20 minutes later and intracellular serotonin concentrations were measured by high performance liquid chromatography. We observed that knocking down of *TSPYL1*, *TSPYL2*, and *TSPYL4* dramatically decreased serotonin levels in the Caco-2 cells exposed to serotonin (**Figure 3b**), whereas overexpression of *TSPYL1*, *TSPYL2*, and *TSPYL4* significantly increased intracellular serotonin levels (**Figure 3d**). Moreover, addition of CT to block the activity of SERT reduced the serotonin transport into the cells and erased the differences in serotonin levels caused by *TSPYL1*, *TSPYL2*, and *TSPYL4* knockdown or overexpression (**Figure 3b,d**). These results demonstrated that *TSPYL1*, *TSPYL2*, and *TSPYL4* can mediate serotonin transport through the regulation of *SLC6A4* expression.

***TSPYL1* SNPs were significantly associated with the baseline severity of depression in patients with MDD**

Because there is some evidence that lower serotonin levels may result in depression,¹⁸ we hypothesized that the expression of *TSPYL1*, *TSPYL2*, and *TSPYL4* might also be associated with the baseline severity of depression in patients with MDD as a result of influencing serotonin levels in the brain. To test this hypothesis, SNPs within 50 kb of the *TSPYL1*, *TSPYL2*, and *TSPYL4* genes were identified from genomewide association studies (GWAS) that have been performed for the Mayo Clinic PGRN-AMPS, ISPC, and STAR*D studies with treatment outcomes (response and remission) after SSRI therapy as phenotypes.^{15–17,19} Specifically, $\geq 50\%$ reduction in QIDS-C score from baseline to the last visit was defined as “response” and a QIDS-C score of ≤ 5 at the last visit was defined as “remission” in the STAR*D study,

$\geq 50\%$ reduction in HAMD from baseline to the last visit was applied as “response” in the ISPC, whereas both QIDS-C and HAMD were used in the Mayo Clinic PGRN-AMPS. QIDS and HAMD are related questionnaires but there was no one single questionnaire that was available across all three studies. The basic information for PGRN-AMPS, ISPC, and STAR*D trials are described in **Table S3**.

We found *TSPYL1/4* SNPs that mapped to 12 LD blocks (SNPs in the same LD blocks in strong LD, $r^2 > 0.8$) were significantly correlated with baseline severity of depression in these three clinical trials (**Table 2**). In the PGRN-AMPS study, two SNPs, rs62423852 and rs9374600 (**Table 2**; LD block #11) near the *TSPYL1/TSPYL4* genes (the *TSPYL1* and *TSPYL4* genes are close to each other), were associated with baseline severity of depression. Specifically, the rs62423852 SNP was correlated with both baseline HAMD and QIDS-C scores ($P = 0.03149$ and $P = 0.0159$, respectively), whereas the rs9374600 SNP was only correlated with baseline HAMD score ($P = 0.04545$). In the ISPC study, which includes patients from multiple sites, some *TSPYL1/4* SNPs, such as rs9320558 (in tight LD with rs10223646, **Table 2**; LD block #1) were associated with baseline HAMD score ($P = 0.001685$). In the STAR*D trial, the largest National Institute of Mental Health (NIMH) supported national trial, a panel of *TSPYL1/TSPYL4* SNPs was found to be associated with the baseline QIDS-C score. Among these SNPs, rs10223646 and rs6909133 (**Table 2**; LD block #1), SNPs, which are in tight LD ($r^2 = 0.9967$), were significantly associated with QIDS-C score at baseline in white subjects (**Table 3**; $P = 5.48E-06$ and $P = 6.00E-06$, respectively). In addition, rs1204807 and rs1204811 (**Table 2**; LD block #5), which were in tight LD ($r^2 = 1.0$), were significantly associated with QIDS-C score at baseline in all races (**Table 3**; $P = 0.0009828$ and $P = 0.0009909$, respectively).

In summary, the three SNPs, rs10223646, rs6909133, and rs9320558, all of which are in strong LD ($r^2 > 0.8$; **Table 2**; LD block #1), were replicated, with the lowest P values in both ISPC and STAR*D clinical trials within white subjects, whereas the rs62423852 SNP, which was most significant in our PGRN-AMPS

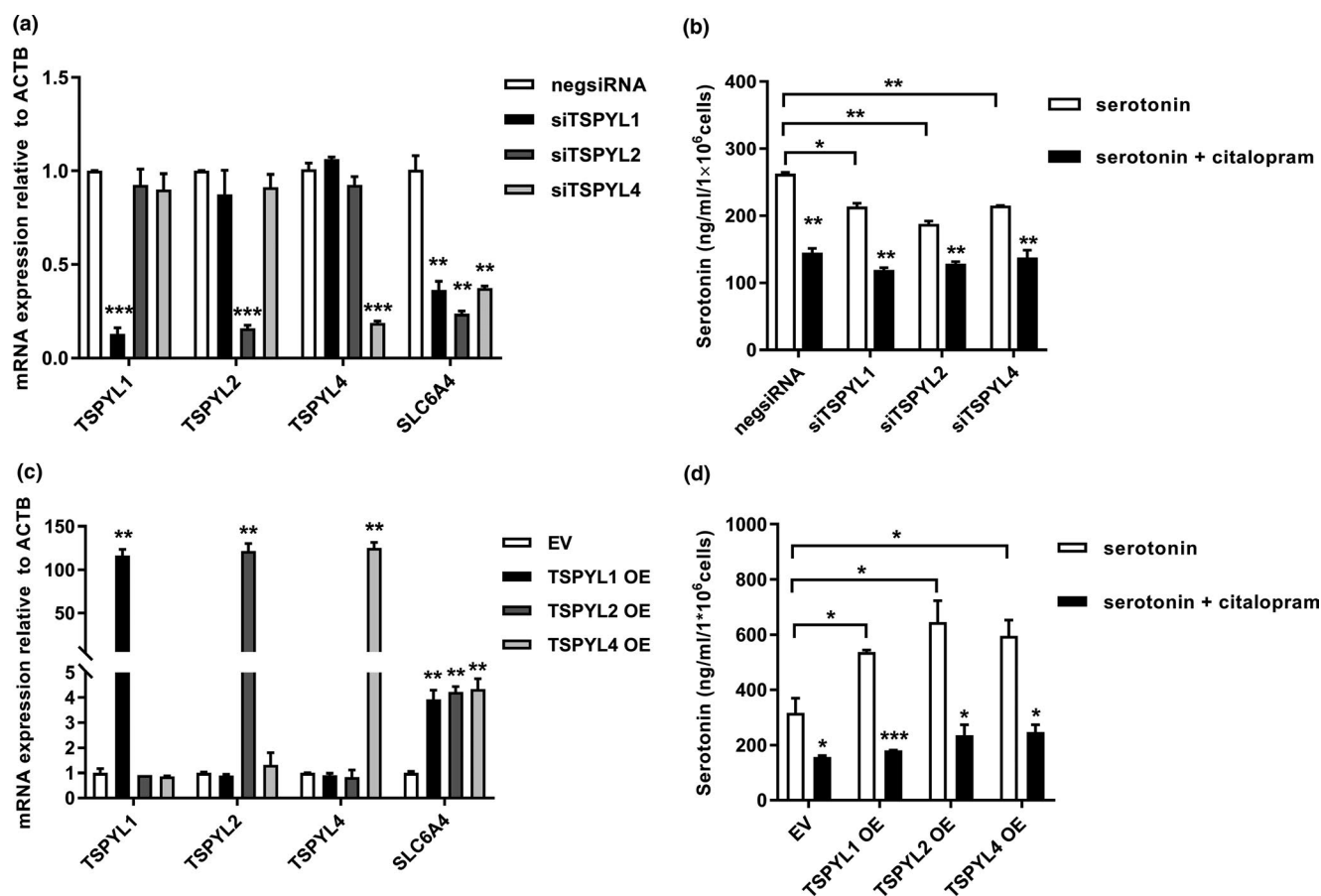


Figure 3 Testis-specific Y-encoded-like proteins (TSPYLs) regulate SLC6A4 expression and serotonin transport in Caco2 cells. **(a)** SLC6A4 expression after knockdown of TSPYLs with TSPYL siRNAs. **(b)** Intracellular concentrations of serotonin after knockdown of TSPYLs in Caco2 cells after 20-minute incubation of 10 μ M serotonin alone or together with 10 μ M citalopram. **(c)** SLC6A4 expression after overexpression of TSPYLs. **(d)** Intracellular concentrations of serotonin after overexpression of TSPYLs in Caco2 cells with 20-minute incubation of 10 μ M serotonin alone or in addition to 10 μ M citalopram. The mRNA expression levels relative to ACTB are shown as means of three independent experiments (\pm SEM), expression levels of TSPYLs and SLC6A4 after knockdown or overexpression of TSPYLs were compared with cells transfected with negative siRNA or empty vector by the use of two-tailed Student's *t*-test. Serotonin concentrations were assayed by high-performance liquid chromatography and compared with negative siRNA or empty vector control by two-tailed Student's *t*-test, **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

study (Table 2; LD block #11) could be replicated in the ISPC but not the STAR*D study. Notably, rs9374600 and rs6927341, in tight LD with SNP rs3828743 (Table 2; LD block #12) that was correlated with plasma drug level and response (Figures 1 and 2), were correlated with baseline severity of depression in all three studies, although overexpression of the rs3828743 SNP variant (Pro62Ser) protein did not affect the regulation by TSPYL1 of SLC6A4 protein (data not shown).

Most importantly, in the Genotype-Tissue Expression (GTEx) Project, which represents a comprehensive public resource for studying tissue-specific gene expression and regulation,²¹ SNPs rs10223646, rs6909133, rs9320558, rs1204807, and rs1204811 that belong to two different LD blocks (Table 2; LD blocks #1 and #5) were *cis*-eQTL SNPs for the *TSPYL1* gene but not *SLC6A4* in brain tissues, especially in anterior cingulate cortex, cerebellum, hypothalamus, and basal ganglia where SERT is expressed (Figure 4). These results suggest that *TSPYL* genetic variation may contribute to the severity of depression in patients with MDD as a result of the modulation of serotonin levels in the brain.

DISCUSSION

Our previous study demonstrated that the *TSPYL* family regulates the expression of several important drug metabolizing enzymes, including *CYP2C9*, *CYP2C19*, and *CYP3A4*, and that the *TSPYL1* nsSNP rs3828743, which abolishes the negative regulation of *CYP2C19* and *CYP3A4* by *TSPYL1* is correlated with poor drug response to the *CYP3A4* metabolized drug abiraterone in patients with prostate cancer.⁹ Moving beyond these previous findings, the present study supports dual functions of members of the *TSPYL* family in antidepressant therapy and baseline severity of depression in patients with MDD. On the one hand, we found that the *TSPYL1* rs3828743 variant SNP genotype, which was associated with higher *CYP2C19* expression, resulted in rapid S-CT metabolism, especially within subtypes possessing the normal WT *CYP2C19* protein (Figure 1 and Table 1). On the other hand, members of the *TSPYL* family also regulated the expression of *SLC6A4*, encoding SERT, and modulated serotonin transport in hepatic and colorectal cells that highly express SERT (Figures S1, S2 and Figure 3). Moreover, the *TSPYL1* SNPs rs10223646 and

rs6909133 that were *cis*-eQTLs for *TSPYL1* in the brain were associated with baseline severity of depression in white patients with MDD with $P = 5.48E-06$ and $P = 6.00E-06$, respectively (Table 3). Collectively, *TSPYL* family members regulated both serotonin transport and the metabolism of two SSRIs, CT and S-CT, that block the serotonin transporter.

Of interest, our RNA-seq studies conducted with human hepatic HepaRG cells showed that, other than *SLC6A4*, the *TSPYL* family did not affect the expression of any other solute carrier superfamily members. This pattern differs from *TSPYL* regulation of the CYP superfamily, although most of the solute carrier family members have low levels of expression in HepaRG cells.

In previous GWAS studies, many genes were identified as candidates affecting susceptibility for the development of MDD but, due in part to heterogeneity of the MDD phenotype, the replication of these findings has proven difficult, with only SNPs in *APOE*, *DRD4*, *GNB3*, *MTHFR*, *SLC6A3*, and *SLC6A4* being widely replicated in different studies.^{22,23} Here, we have identified *TSPYL* SNPs that may contribute to baseline severity of depression in the ISPC study as well as the STAR*D study, and the top SNPs identified from these two studies were in tight LD (Table 2; LD block #1). Obviously, due to lack of healthy controls in these GWAS, the effect of *TSPYL* SNPs on depression requires further validation.

As we pointed out, the top *TSPYL1* SNPs rs10223646/rs6909133 and rs1204807/1204811 were associated with the baseline severity of depression in the STAR*D trial but were not involved in the alteration of SSRI metabolism (Table 3). That might be due to the fact that these SNPs are *cis*-eQTLs for *TSPYL1* in brain tissues but they are not eQTLs for *TSPYL1* in the liver (Figure 4) where the SSRI metabolizing enzyme CYP2C19 is expressed. Moreover, the direction of the eQTLs in brain tissues could also explain the influence of these SNPs on the baseline depression. Specifically, SNPs rs10223646/rs6909133 and rs1204807/1204811 were significantly associated with higher *TSPYL1* expression in the brain (Figure 4; when $P < 0.05$, normalized effect size [NES] > 0). Based on the positive regulation of *SLC6A4* by *TSPYLs* (Figure 3), the variant SNP genotypes might imply higher *SLC6A4* expression, more serotonin transported into cells, and lower serotonin levels in synaptic clefts, which, in turn, might result in more severe baseline depression symptoms (BETA > 0; Table 3).

The *TSPYL1* rs3828743 SNP was found to be associated with rapid S-CT metabolism at week 8, but not at week 4 (Figure 1). This association that was found at 8 weeks but not 4 weeks might be due to dose adjustments after week 4. In the PGRN-AMPS study, more than half of the patients had dose escalations after 4 weeks and three patients had dose reductions. These dose adjustments might have affected the differences in results between 8 weeks and 4 weeks. Moreover, the *TSPYL1* SNP rs3828743 not only affected the plasma concentrations of CT, S-CT, and their metabolites, but also affected clinical response to SSRI therapy (Figure 2). Although our previous study using PGRN-AMPS data did not find correlations between plasma levels of SSRIs (CT and S-CT) and clinical outcomes,^{8,17} meta-analysis, including patients in PGRN-AMPS, GENDEP, STAR*D, and GenPod studies, showed correlations between *CYP2C19* metabolic phenotypes

Table 2 *TSPYL1/4* SNPs correlates with baseline severity of depression across different clinical trials

LD block ^a	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12
Representative SNP	rs10223646	rs9481617	rs910391	rs10456903	rs1204807	rs62423797	rs111762856	rs142686690	rs7742336	rs1204790	rs62423852	rs3828743 ^b
PGRN-AMPS (HAMD)											0.03149	
PGRN-AMPS (QIDS-C)											0.0159	0.04646
ISPC (HAMD)	0.001685	0.003581						0.005427	0.0407		0.02491	0.01086
STAR*D CA (QIDS-C)	5.48E-06	0.001156	0.004205	0.009243	0.01108	0.03982	0.04533	0.00507	0.0109	0.03746		0.0253
STAR*D all (QIDS-C)	0.00158		0.01258	0.005516	0.000983	0.00158	0.03398					0.04289

Blank means no significant correlation between the SNPs and baseline severity of depression within the indicated LD block of *TSPYL1/4* genes. P value in **bold** indicates the lowest P value across different LD blocks in each of the study.

HAMD, Hamilton Depression Rating Scale; ISPC, International SSRI Pharmacogenomics Consortium; LD, linkage disequilibrium; QIDS-C, Quick Inventory of Depressive Symptomatology Score; SNPs, single nucleotide polymorphisms; STAR*D, Sequenced Treatment Alternatives to Relieve Depression; *TSPYL1*, testis-specific Y-encoded-like protein.

^aThe *TSPYL1/4* SNPs in the same LD block are in strong LD ($r^2 > 0.8$) and have higher chance to be co-inherited. ^bSNP rs3828743 was also associated with plasma drug level and SSRI response (Figures 1 and 2).

Table 3 Top TSPYL SNPs correlate with baseline severity of depression in patients with MDD in STAR*D GWAS

Gene	Chr	Position	SNP	MAF	BETA.linear.3ev	SE	P.linear.3ev	genotype	MA	CA
TSPYL1/4	6	1.17E + 08	rs10223646 ^a	0.3378	0.5707	0.1410	5.48E-06	I	T	C
TSPYL1/4	6	1.17E + 08	rs6909133 ^a	0.3383	0.5670	0.1408	6.00E-06	I	G	A
TSPYL1/4	6	1.17E + 08	rs1204807 ^b	0.3408	0.4055	0.1229	0.0009828	I	C	A
TSPYL1/4	6	116552103	rs1204811 ^b	0.3402	0.4055	0.1229	0.0009909	I	T	C

GWAS, genomewide association studies; MAF, minor allele frequency; MDD, major depressive disorder; SNP, single nucleotide polymorphism; STAR*D, Sequenced Treatment Alternatives to Relieve Depression; TSPYL, testis-specific Y-encoded-like protein.

^aTop TSPYL SNPs correlate with baseline Quick Inventory of Depressive Symptomatology Score (QIDS-C) scores in patients with MDD in STAR*D GWAS (white patients). ^bTop TSPYL SNPs correlate with baseline QIDS-C scores in patients with MDD in STAR*D GWAS (all races).

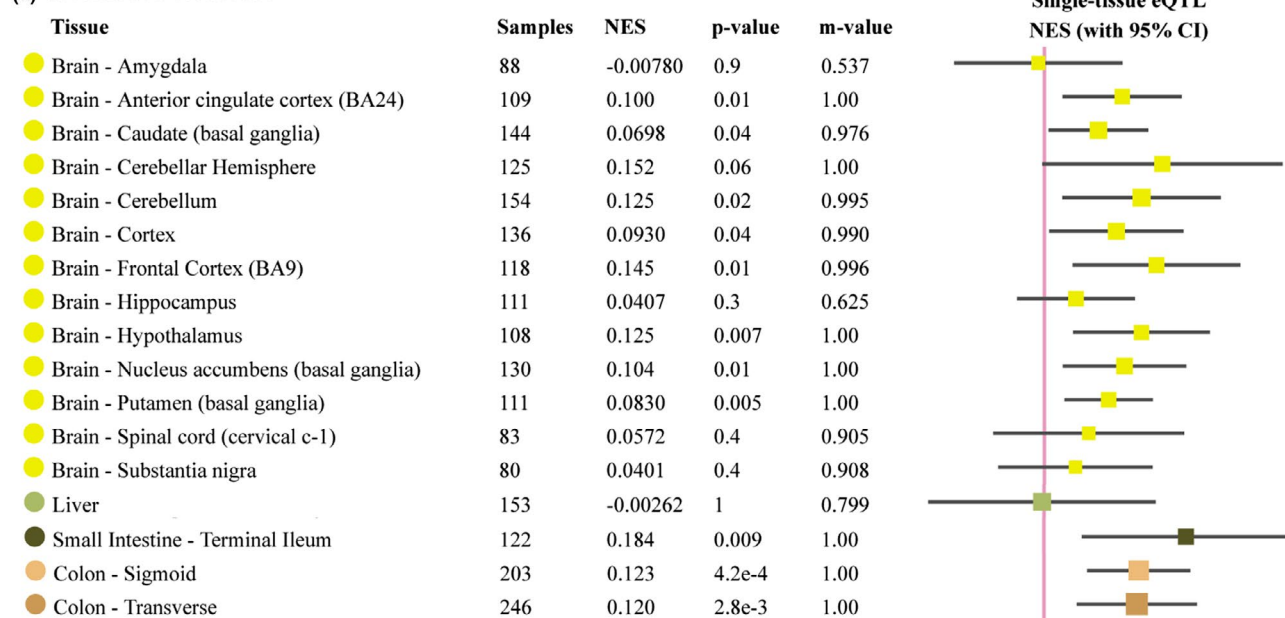
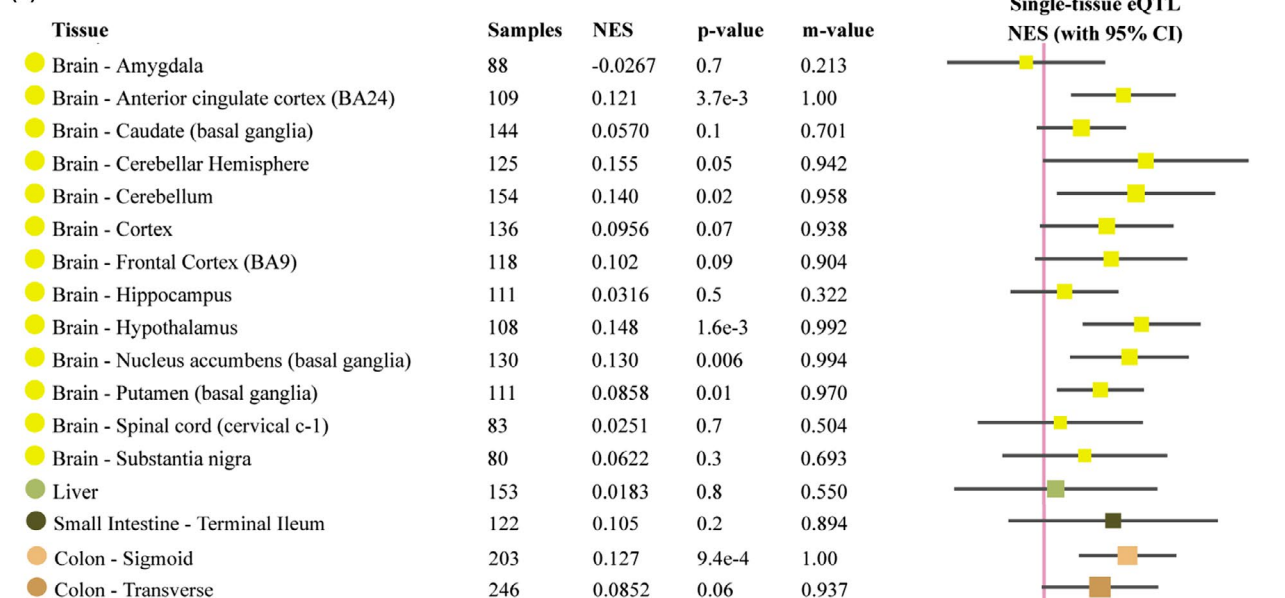
(a) rs10223646/ rs6909133**(b) rs1204807/ rs1204811**

Figure 4 Multi-tissue testis-specific Y-encoded-like protein (*TSPYL1*) expression quantitative trait locus (eQTLs) plots for top *TSPYL1/4* single nucleotide polymorphisms (SNPs) correlated with severity of depression in patients with major depressive disorder (MDD). Data for brain, liver, small intestine and colon tissues were obtained from the GTEx Portal. **(a)** Top SNPs correlated with severity of depression at baseline in white patients with MDD. **(b)** Top SNPs correlated with severity of depression at baseline in all patients with MDD. CI, confidence interval; NES, normalized effect size.

and CT/S-CT efficacy and side effects.²⁴ Thus, the effect of the *TSPYL1* rs3828743 SNP on SSRI response may be mediated through its effect on the metabolism of SSRIs. Because *TSPYL* family members all have high expression in brain tissues (GTEx), and because they regulate the expression of proteins and pathways other than *CYPs* or *SLC6A4* based on our RNA-seq (data not shown), it is likely that additional mechanisms by which *TSPYLs* regulate SSRI response might also be involved.

The fact that patients with the worst baseline severity (QIDS-C score) are likely to have better S-CT response (higher percentage change in QIDS-C; **Tables S1 and S2**) suggests that baseline symptom severity can be included as one predictor for outcome.^{25,26} In future clinical trials, this relationship can be further pursued. SNP genotypes and baseline severity may eventually help us predict SSRI response and select alternative therapies for those who may not respond to SSRIs at early treatment stages, rather than waiting, leading toward truly individualized antidepressant therapy.

In summary, we have demonstrated dual roles for *TSPYL* family members in the pathophysiology of MDD and in SSRI metabolism. Our findings suggest that genetic variation in or near *TSPYL* genes may be novel biomarkers for prediction of baseline depression severity and response to CT and S-CT, two very commonly prescribed SSRIs.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

Figures S1-S2.

Table S1.

Table S2.

Table S3.

Supplemental Materials and Methods.

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CONFLICT OF INTEREST

Drs. Wang and Weinshilboum are cofounders of and stockholders in OneOme, LLC. All other authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

S.Q., L.W., and R.W. wrote the manuscript. S.Q., D.L., L.W., and R.W. designed the research. S.Q., L.Z., and J.Y. performed the research. S.Q., A.E., D.N., and J.B. analyzed the data.

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