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. Weinshilboum¹,* and Liewei Wang¹,*

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-monodesmethylcitalopram (S-DCT),⁴ 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)

Received June 14, 2019; accepted September 30, 2019. doi:10.1002/cpt.1692
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 genomewide significant association (P = 4.1 × 10−8) 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 isofrom 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 worsened 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-Ct 1-Step Kit (Life Technologies, Grand Island, NY). Gene expression analyses were performed using the ΔΔCt 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 μM serotonin (Sigma-Aldrich, St. Louis, MO) alone or combined with 10 μM 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 μl 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 × 4.6 mm, 3 μm particle size (Shimadzu) coupled with a Phenomenex Security Guard C18 guard column 4 × 3 mm (Phenomenex, Torrance, CA). The mobile phase was degassed as well as vacuum filtered through 0.22 μm 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 μA). 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. 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. Because the S-CT for the parent drug and metabolites are thought to play the major role in their clinical effect, 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,
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[AA]         | -0.003366   | 0.023593| 0.8866  |
| rs3828743[AA]*           | 0.0920755   | 0.040245| 0.0227  |
| *1/*1                    | -0.045578   | 0.026185| 0.8082  |

R-squared 0.1379; adjusted R-squared 0.1218; number of observations 383. TSPYL1 SNP rs3828743 [AA] is homozgyous 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.

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, 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 knockdown 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

we built a regression model using CYP2C19*1, rs1074145 (in tight LD with *2) *3, *1/*1 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 × 10−9) and it is in tight LD (r² = 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 *1/*1 versus *1/*3 allele.

SNP rs3828743 [AA] is homozygous variant and [AG] is heterozygous genotype. TSPYL1 rs3828743 [AA] is homozygous variant and [AG] is heterozygous genotype. TSPYL1 *1/*1 indicates normal TSPYL1, which is not containing rs1074145, *3, or *17 allele. Bold indicate the predictors with P value < 0.05 in the regression model.
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, ≥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, where 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.

In summary, the three SNPs, rs10223646, rs6909133, and rs9320558, all of which are in strong LD (r² > 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
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 rsSNP 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 \( P = 5.48 \times 10^{-6} \) and \( P = 6.00 \times 10^{-6} \), 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 is different 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, SLC6A4, and SLC6A4 being widely replicated in different studies.22,23 Here, we have identified TSPYL SNPs that may contribute to baseline severity in 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 and depression symptoms (BETA > 0; Table 3).

| LD block | SNP | TSPYL1 (AMPS) (HAMD) | TSPYL1 (AMPS) (QIDS-C) | TSPYL1 (ISPC) (HAMD) | TSPYL1 (ISPC) (QIDS-C) | TSPYL1 (STAR*D) all (QIDS-C) | TSPYL1 (STAR*D) all (QIDS-C) |
|----------|-----|---------------------|-----------------------|---------------------|-----------------------|-----------------------------|-----------------------------|
| 1        | rs10223646 | m9481617 | 0.00491 0.00458 | 0.00546 0.00547 | 0.00338 0.00343 | 0.00274 0.00278 | 0.00274 0.00278 |
| 2        | rs6909133  | m930391  | 0.00116 0.00125 | 0.00108 0.00118 | 0.00109 0.00120 | 0.00158 0.00159 | 0.00158 0.00159 |
| 3        | rs1204807  | m10456903 | 0.00102 0.00104 | 0.00105 0.00106 | 0.00105 0.00106 | 0.00105 0.00106 | 0.00105 0.00106 |
| 4        | rs1204811  | m910391  | 0.00102 0.00104 | 0.00105 0.00106 | 0.00105 0.00106 | 0.00105 0.00106 | 0.00105 0.00106 |
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).

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 at baseline 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. 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. 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.

FUNDING
This work was supported by National Institutes of Health Grants U19 GM13886 (The Pharmacogenomics Research Network), R01 GM28157, R01 GM125633, Minnesota Partnership for Biotechnology and Medical Genomics Grant #14.37, Department of Defense, and Private and Philanthropy funding sources: (i) Mayo Clinic Centre for Individualized Medicine; (ii) A. T. Suharya and Ghan D. H, Gail and Joseph Gassner; (iii) The International SSRI Pharmacogenomics Consortium (ISPC): a genome-wide association study of antidepressant treatment response. Transl. Psychiatry 5, e553 (2015).

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., D.L., and J.Y. performed the research. S.Q., A.E., D.N., and J.B. analyzed the data.

2019 The Authors. Clinical Pharmacology & Therapeutics published by Wiley Periodicals, Inc. on behalf of American Society for Clinical Pharmacology and Therapeutics.

This is an open access article under the terms of the Creative Commons Attribution NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

1. Cowen, P.J. & Browning, M. What has serotonin to do with depression? World Psychiatry 14, 158–160 (2015).

2. Morrissette, D.A. & Stahl, S.M. Modulating the serotonin system in the treatment of major depressive disorder. CNS Spectrums 19, 54–68 (2014).

3. Hyttel, J., Bogeso, K.P., Perregaard, J. & Sanchez, C. The pharmacological effect of citalopram residues in the (S)-(+) -enantiomer. J. Neural. Transm. Gen. Sect. 88, 157–160 (1992).

4. Rochat, B., Amey, M., Gillet, M., Meyer, U.A. & Baumann, P. Identification of three cytochrome P450 isozymes involved in N-demethylation of citalopram enantiomers in human liver microsomes. Pharmacogenetics 7, 1–10 (1997).

5. Kobayashi, K. et al. Identification of cytochrome p450 isoforms involved in citalopram N-demethylation by human liver microsomes. J. Pharmacol. Exp. Ther. 280, 927–933 (1997).

6. Von Moltke, L.L., Greenblatt, D.J., Giancarlo, G.M., Granda, B.W., Harmatz, J.S. & Shader, R.I. Escitalopram (S-citalopram) and its metabolites in vitro: cytochromes mediating biotransformation, inhibitory effects, and comparison to R-citalopram. Drug Metab. Dispos. 29, 1102–1109 (2001).

7. von Moltke, L.L. et al. Citalopram and desmethylcitalopram in vitro: human cytochromes mediating transformation, and cytochrome inhibitory effects. Biol. Psychiatry 46, 839–849 (1999).

8. Ji, Y. et al. Citalopram and escitalopram plasma drug and metabolite concentrations: genome-wide associations. Br. J. Clin. Pharmacol. 78, 373–383 (2014).

9. Qin, S. et al. TSPYL family regulates CYP17A1 and CYP3A4 expression: potential mechanism contributing to abiraterone response in metastatic castration-resistant prostate cancer. Clin. Pharmacol. Ther. 104, 201–210 (2018).

10. Li, A.P., Kaminski, D.L. & Rasmussen, A. Substrates of human hepatic cytochrome P450 3A4. Toxicology 104, 1–8 (1995).

11. Yin, L. & Hu, Q. CYP17 inhibitors—abiraterone, C17,20-lyase inhibitors and multi-targeting agents. Nat. Rev. Urol. 11, 32–42 (2014).

12. Mrazek, D.A. et al. CYP2C19 variation and citalopram response. Pharmacogenet. Genomics 21, 1–9 (2011).

13. Trivedi, M.H. et al. Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. Am. J. Psychiatry 163, 28–40 (2006).

14. Rush, A.J. et al. Sequenced treatment alternatives to relieve depression (STAR*D): rationale and design. Control Clin. Trials 25, 119–142 (2004).

15. Fava, M. et al. Background and rationale for the sequenced treatment alternatives to relieve depression (STAR*D) study. Psychiatr. Clin. North Am. 26, 457–494 (2003).

16. Biernacka, J.M. et al. The International SSRI Pharmacogenomics Consortium (ISPC): a genome-wide association study of antidepressant treatment response. Transl. Psychiatry 5, e553 (2015).

17. Ji, Y. et al. Pharmacogenomics of selective serotonin reuptake inhibitor treatment for major depressive disorder: genome-wide associations and functional genomics. Pharmacogenomics J. 13, 456–463 (2013).

18. aan het Rot, M., Mathew, S.J. & Charney, D.S. Neurobiological mechanisms in major depressive disorder. CMAJ 180, 305–313 (2009).

19. Biernacka, J.M. et al. The International SSRI Pharmacogenomics Consortium (ISPC): a genome-wide association study of antidepressant treatment response. Transl. Psychiatry 5, e553 (2015).

20. Mrazek, D.A. et al. Treatment outcomes of depression: the pharmacogenomic research network antidepressant medication pharmacogenomic study. J. Clin. Psychopharmacol. 34, 313–7 (2014).

21. GTEXPortal. Resource overview <https://gtexportal.org/home/> (2014).

22. Dunn, E.C. et al. Genetic determinants of depression: recent findings and future directions. Harv. Rev. Psychiatry 23, 1–18 (2015).

23. Lopez-Leon, S. et al. Meta-analyses of genetic studies on major depressive disorder. Mol. Psychiatry 13, 772–785 (2008).

24. Fabbri, C. et al. Effect of cytochrome CYP2C19 metabolizing activity on antidepressant response and side effects: meta-analysis of data from genome-wide association studies. Eur. Neuropsychopharmacol. 28, 945–954 (2018).

25. Henkel, V. et al. Relationship between baseline severity of depression and antidepressant treatment outcome. Pharmacopsychiatry 44, 27–32 (2011).

26. Kilts, C.D., Wade, A.G., Andersen, H.F. & Schlaepfer, T.E. Baseline severity of depression predicts antidepressant drug response relative to escitalopram. Expert. Opin. Pharmacother. 10, 927–936 (2009).