Genetic polymorphisms of cytochrome P450 enzymes influence metabolism of the antidepressant escitalopram and treatment response

Aims: The antidepressant escitalopram (*S*-CIT) is metabolized by the cytochrome-P450 (CYP) enzymes CYP 2D6, 2C19 and 3A4. This study evaluated the impact of *CYP2D6*, *2C19* and *3A4* genetic polymorphisms on plasma concentrations of *S*-CIT and patient treatment response. **Materials & methods:** A total of 100 patients diagnosed with major depressive disorder were recruited to the study and their depression symptoms were assessed using the Hamilton Depression Rating Scale. The genetic polymorphisms **4*, **5* and **10* on *CYP2D6*, **2*, **3* and **17* on *CYP2C19*, and **18* on *CYP3A4* were selected based on their function and respective allele frequencies in Asian populations. Polymorphisms were analyzed using the SNPstream® genotyping system, PCR and direct sequencing methods. The steady-state serum concentrations of *S*-CIT and its metabolites *S*-desmethylcitalopram and *S*-didesmethylcitalopram were analyzed by HPLC. According to semiquantitative gene dose (SGD) and gene dose (GD) models for allele combinations of these polymorphisms, *CYP2D6* was clustered into intermediate (0.5, 1 and 1.5 SGD) and extensive (2 SGD) metabolizers, while *CYP2C19* was clustered into poor (0 GD) and extensive (1 and 2 GDs) metabolizers. **Results:** The group of patients with intermediate CYP2D6 metabolism (0.5 SGD) had a significantly higher frequency of remitters from major depressive disorder during the 8-week treatment ($p = 0.0001$). Furthermore, CYP2C19 poor metabolizers had significantly higher *S*-CIT serum levels than did extensive metabolizers at weeks 2, 4 and 8 ($p < 0.05$). The allele frequencies in *CYP3A4*18* and *CYP2C19*17* were too low to permit further subgroup analyses. **Conclusion:** Our results suggest that the genetic polymorphisms in *CYP2C19* may be influencing *S*-CIT serum concentrations, and that specific *CYP2D6* polymorphisms may be predicting patient treatment outcomes based on gene dosage analyses.

KEYWORDS: CYP2C19 ■ CYP2D6 ■ CYP3A4 ■ escitalopram ■ major depression ■ therapeutic drug monitoring

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Major depressive disorder (MDD) is a severe psychiatric illness characterized by successive recurring episodes, which often require a series of antidepressant treatments [1]. Escitalopram (*S*-CIT) is a selective serotonin reuptake inhibitor that effectively treats major depressive disorder caused by a range of anxiety disorders [2]. *S*-CIT is metabolized by the liver cytochrome P450 (CYP) enzymes CYP2D6, 2C19 and 3A4 [3]. The first step of the metabolic process involves *N*-demethylation by CYP3A4 and 2C19, forming *S*-desmethylcitalopram (*S*-DCIT) [4]. This metabolite is further demethylated, mainly with CYP2D6, to become *S*-didemethylcitalopram (*S*-DDCIT) [5]. Like *S*-CIT, *S*-DCIT is an active metabolite with antidepressant effects, whereas *S*-DDCIT is an inactive metabolite [4]. Patients administered *S*-CIT have various plasma concentrations of *S*-CIT and its metabolites *S*-DCIT and *S*-DDCIT [6], which may contribute to different individual clinical outcomes.

Expression of genes encoding metabolic enzymes that contain genetic polymorphisms may not be altered by the treatment drug, making them possible markers predictive of enzymatic activities related to the metabolism of *S*-CIT [7] and possibly treatment response [8]. The prevalence of genetic polymorphisms varies amongst different ethnic groups [9]. The *CYP2D6* gene has more than 80 identified variations among the *CYP* genetic superfamily [101]. Specific gene variants can alter the catalytic activity of CYP2D6, which can result in clinically relevant interindividual differences in therapeutic efficacy or adverse drug reactions [10]. The polymorphic allelic types that may possibly alter CYP enzymatic activities include *CYP2D6*5*, caused by the deletion of the entire *CYP2D6* gene, which is present in 2–7% of Caucasians and 3–7% of ethnic Chinese populations [11–15]. *CYP2D6*4*, which is caused by a defective splicing site, was reported in approximately 21% of Caucasians and 1% of Asians [12]. and *CYP2D6*10*, which results in protein instability and occurs in 50–70% of ethnic Chinese populations [16]. Furthermore, *CYP2D6*36*, which is caused by a six amino acid difference

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in exon 9 (converted to *CYP2D7*), occurs in 2.63% of Asians, but has not been reported in Caucasians [17].

CYP2C19 has two SNPs, rs4244285 (*CYP2C19*2*) and rs4986893 (*CYP2C19*3*), which cause poor metabolism in approximately 20% of the ethnic Chinese [18] and 22.5% of the Japanese population [19]. These two defective alleles may account for 100% of Chinese patients characterized as poor metabolizers [7]. A novel SNP that causes *CYP2C19*17* mutation accounts for reported rapid antidepressant metabolism [20,21]. The *CYP2C19*17* allele was also selected in this study.

It has been estimated that *CYP3A4* metabolizes approximately 50% of all clinical therapeutic drugs [22–23]. The *CYP3A4*18* allele type has a T–C transition in exon 10 and may be associated with increased *CYP3A4* enzyme activity [24]. Identification of changes to drug metabolism and enzymatic activities caused by these genetic polymorphisms is crucial for predicting treatment drug serum levels *in vivo* and patient responses to treatment. The present study evaluated whether genetic polymorphisms in *CYP2D6*, *2C19* and *3A4* could serve as biomarkers predictive of *S*-CIT serum levels or treatment responses.

Materials & methods

Subjects

This study was approved by the institutional review board of the National Health Research Institutes and participating hospitals. Written informed consent was obtained from all patients participating in the study. This study was also registered as a clinical trial with the US NIH [102] and assigned the study identifier NCT00384020. We recruited 100 patients with MDD from the outpatient clinics of five hospitals in or around Taipei, Taiwan; the hospitals were Chang Gung Memorial Hospital, Mackay Memorial Hospital, Far Eastern Memorial Hospital, TMU-Wan Fang Hospital and Songde branch of the Taipei City Hospital (Figure 1). All patients were aged at least 18 years of age and of Han Chinese ethnicity, as defined by both of their parents and at least three of their grandparents being of Han ethnicity. Clinical interviews were performed by trained research nurses. Diagnosis of MDD was made according to the diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV using the Structured Clinical Interview for DSM-IV-TR Axis-I Disorders [25]. For study enrollment, patients needed to have a moderate depressive episode, defined as at least 14 points on the 21-item Hamilton Depression (HAM-D) assessment tool [26,27], at first visit to be used as a baseline. Participants could not have been previously refractory or intolerant to *S*-CIT treatment and must have completed a 7-day washout period from any previous antidepressant treatment. Patients with primary or comorbid diagnosis of schizophrenia, schizoaffective disorder, bipolar disorder, alcohol or substance dependence, dementia or other significant medical conditions were excluded from the study. Patients received *S*-CIT at a fixed dose of 10 mg/day for the first 4 weeks, followed by dosages of 10–30 mg/day according to clinical response for another 4 weeks. Other psychotropic drugs were prohibited during the study, with exceptions for hypnotics (zolpidem 10 mg taken per night as needed, but not exceeding four nights per week) and anxiolytics (lorazepam 1–2 mg per day) for severe anxiety. Blood samples (collected 12–20 h after the most recent dose) were taken at weeks 2, 4 and 8 and sent to a central laboratory for analyses of *S*-CIT and its metabolites, and for DNA genotyping. The sampling time, drug dosage and patient compliance with study medication were documented according to the protocol.

Depression symptoms rating scale

Medication efficacy was assessed using the 21-item HAM-D [28] and Hamilton Rating Scale for Anxiety (HAM-A) [26]. The subgroup analyses of HAM-D were further clustered into Core, Sleep, Activity, Psychic anxiety, Somatic anxiety and Delusion [29]. The severity of depression was also rated using the Beck Depression Inventory (BDI) and Clinical Global Impression (CGI) for severity of illness. Patients were evaluated at baseline and later at weeks 2, 4, 6 and 8 of continuous treatment. All raters for the HAM-D and HAM-A received the same training module as investigators. The inter-rater reliability coefficient for all raters was consistent with an α-value of 0.9. Remitters from MDD were defined as HAM-D scores less than 10 [27]. Side effects were assessed using the Treatment Emergent Signs and Symptoms (TESS) scale and the Arizona Sexual Experiences Scale [30], which has a five-item rating scale quantifying sex drive, arousal, vaginal lubrication/penile erection, ability to reach orgasm and satisfaction from orgasm.

Serum S-CIT & metabolites

We measured serum concentrations of *S*-CIT as well as its metabolites, *S*-DCIT and *S*-DDCIT, using HPLC [31]. The HPLC system used in this

study consisted of a Waters (Milford, MA, USA) 2795 Alliance solvent pump and autosampler, a Waters 2475 multi-λ fluorescence detector (245 nm excitation, 295 nm emission), and an Hewlwtt Packard (CA, USA) computer recorder installed with the Waters Empower™ software. The analytical column was a reverse-phase C8 column (Sphere-Image; 5 µm, 100 × 3 mm) with a C18 guard column (Phenomenex [CA, USA]; 4 × 3 mm). We set the column oven at 35°C. The mobile phase was composed of 35% acetonitrile, 65% potassium phosphate buffer (10 mM), and triethylamine (1 ml/l of mobile phase), with a final pH of 3.2.

Patient sera samples were obtained from whole blood centrifuged at 3000 rpm (1710 × g) for 15 min. Serum levels of *S*-CIT, *S*-DCIT, *S*-DDCIT and 100 ng supplement imipramine (internal standard) were extracted with a C18-E 100 mg/ml capacity STRATA® (Phenomenex) column. After conditioning the column with a Waters vacuum manifold with 1 ml of methanol, 1 ml of water and 1.5 ml of 45 mM potassium phosphate buffer (pH 4.5), 800 µl of a serum sample and imipramine were added onto the column on the manifold. Samples were allowed to equilibrate for 2–3 min (stopcock closed) and then drained with suction force. The column was then washed with 1 ml of water, 1 ml of a 50% methanol–water solution, and vacuum dried for 1 min. The retained *S*-CIT, *S*-DCIT, *S*-DDCIT and internal standard were eluted using 1 ml of methanol/ammonium acetate (1 g/100 ml) and collected in a 12 × 75 mm test tube. The collected eluent was evaporated in a water bath at 55°C under a stream of air for 17 min. The remaining residue was dissolved in 100 µl of mobile phase and 50 µl of each sample was subjected to chromatography.

Escitalopram was a gift from the Research and Education Institute for Texas Health Resources (TX, USA) and showed the same retention time as citalopram obtained from Sigma (MO, USA). *S*-DCIT and *S*-DDCIT were purchased from Toronto Research Chemicals Inc. (ON, Canada). Using 1.953 ng each of *S*-CIT, *S*-DCIT and *S*-DDCIT as standards for reproducibility analyses, the intraday and interday coefficients of variation were 0.59 and 2.25% for *S*-CIT, 0.41 and 1.83% for *S*-DCIT, and 0.4 and 1.54% for *S*-DDCIT, respectively. The recovery rates for *S*-CIT, *S*-DCIT and *S*-DDCIT were 107.5 ± 2.1%, 104.2 ± 3.7%, and 106.1 ± 3.3%, respectively. The lower detection limit was 0.2 ng/ml for all three compounds.

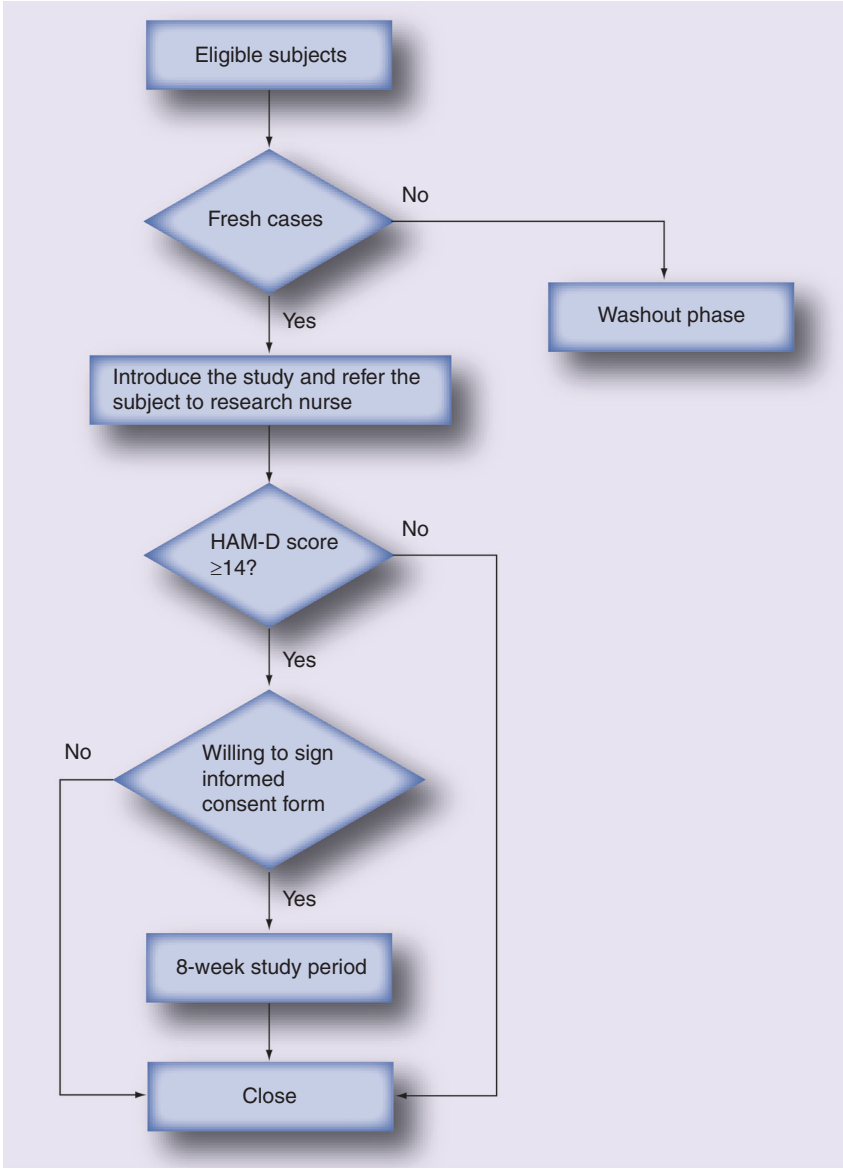


Figure 1. Study procedures followed at five hospitals in northern Taiwan to recruit patients with major depression.
HAM-D: Hamilton Depression.

Genomic DNA purification & SNP genotyping

Study patient genomic DNA was extracted from 8 ml of whole-blood lymphocyte pellets using the Puregene kit (Gentra Systems, MN, USA). The genomic DNA was used for genotyping allelic polymorphisms in *CYP2D6* (**4*, **5* and **10*), *CYP2C19* (**2*, **3* and **17*) and *CYP3A4*18* genes.

*CYP2D6*4* (rs3892097) was genotyped using an allele-specific PCR method to produce a PCR product of 358 bp [32]. *CYP2D6*10* (rs1065852) was genotyped using the C_11484460_40 validated ABI TaqMan® SNP genotyping assay (Applied Biosystems, CA, USA). *CYP2D6*5* was genotyped using copy number variation produced with the Hs00010001_cn ABI TaqMan

Table 1. Description and frequency of major human *CYP2D6*, *CYP2C19* and *CYP3A4* gene polymorphisms.

Allele	Allele description	Effect	Predicted activity	Allele frequency (%)†
CYP2D6				
**1*	Wild-type, C allele at rs1065852 and G allele at rs3892097	None	Normal	37.8
**4*	1846G>A, T allele at rs1065852 and A allele at rs3892097	Splice-site mutation	Inactive	1.02
**5*	Whole gene deletion	No enzyme	None	6.63
**10*	100C>T, T allele at rs1065852 and G allele at rs3892097	Unstable enzyme	Decrease	53.6
CYP2C19				
**1*	Wild-type, G allele at rs4244285 and G allele at rs4986893	None	Normal	65.5
**2*	681G>A, A allele at rs4244285 and G allele at rs4986893	Splice-site mutation	Inactive	32.0
**3*	636G>A, G allele at rs4244285 and A allele at rs4986893	W212X	Inactive	2.5
**17*	-806C>T, T allele at rs12248560	Promoter region	Increase	0.5
CYP3A4				
**1*	Wild-type, T allele at rs28371759	None	Normal	98.4
**18*	878T>C, C allele at rs28371759	Unstable enzyme	Increase	1.6

†*Allele frequency (%) in the present study.*

Copy Number assay (Applied Biosystems) with the TaqMan RNAse P control kit as internal standard (Applied Biosystems) [33]. All TaqMan assays were performed according to the manufacturer's protocol. *CYP2D6*5* genotyping was verified by using long PCR amplification [34].

The SNPs of rs4244285 (*CYP2C19*2*), rs4986893 (*CYP2C19*3*) and *CYP3A4*18* were genotyped using the GenomeLab™ SNPstream® genotyping platform (Beckman Coulter Inc. CA, USA) and its accompanying SNPstream software suite according to the manufacturer's protocol. The PCR reactions were carried out in a total volume of 5 µl with 2.5 ng of template DNA in the cycling conditions (MJ-225 thermal cycler) of 94°C for 1 min, followed by 39 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min. The amplified DNA fragments were incubated with a clean-up cocktail of exonuclease I and shrimp alkaline phosphatase in order to degrade any unincorporated PCR primers and deoxynucleotide triphosphates. The tagged extension reaction was carried out using 'cleaned' PCR products as a template and a mixture of 12 site-specific SNP primers.

The tagged extension primers were extended with single tetramethylrhodamine- or bodipy-fluorescein-labeled nucleotide, and then spatially resolved by hybridization to complementary oligonucleotides arrayed on 384-well microplates (GenomeLab SNPstream Genotyping System). The Tag array plates were imaged using a two-laser, two-color, charged couple device-based imager (GenomeLab SNPstream array imager). The 12 individual SNPs were identified by their position and the fluorescent color in each well according to the position of the tagged oligonucleotides. The error rate based on blind replicates was 0.1–1% for the SNPs examined in the present study.

*CYP2C19*2* and **17* were verified with probe C_25986767_70 and C_469857_10 of ABI TaqMan SNP genotyping assay according to the manufacturer's protocol. Each allele type was independently verified for at least two subjects using direct sequencing.

Statistical analyses

All statistical analyses were performed by Statistical Analysis System Package 9.13 (SAS Institute Inc., NC, USA) to calculate descriptive statistics of *S*-CIT treatment dose and the specific levels of its serum metabolites. The Hardy–Weinberg equilibrium was computed on single SNP by an exact test [35]. The predicted functional activities of *CYP2D6* and *2C19* were analyzed using allelic modeling, where the semi-quantitative gene dose (SGD) model was applied for *CYP2D6* [36] and the gene dose (GD) model was applied for *CYP2C19*. Using one-way analysis of variance (ANOVA), we examined possible associations between predicted enzymatic activity

and quantified severity of depressive symptoms for the *CYP2C19* and *2D6* allele types in each separate week. The interactions between the two genetic dose models were analyzed using two-way ANOVA. The generalized estimating equations model was also applied [37] to adjust the covariates within the association analyses.

The associations between predicted enzymatic activity and time to remission were assessed with the log-rank (Mantel–Cox) test and analysis of 'survival' (time to nonremission rate) curves using GraphPad Prism 5 software (GraphPad Inc., CA, USA). Power analyses of remission rates and gene dose models were estimated by comparing two survival curves during 8 weeks of *S*-CIT treatment using StatMate 2.0 (GraphPad Inc.). The sample size justified from the HAM-D remission rate had a power above 99%. Group differences were considered significant when p-values were less than 0.05.

Results

A total of 100 patients with MDD who were an average age of 42 years were recruited for this study. Study patients were 82% female, 39% were naive to the study drug, 60% had previously used antidepressants, 67% had comorbidities, and 38% of patients reported having experienced recurrent episode and first episode of depression. Liver function as assessed using measurements of glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, γ-glutamyl transferase, serum alkaline phosphatase. Direct bilirubin, total bilirubin and thyroid function as indicated by thyroid stimulating hormone and thyroxine (T4) levels were all within normal ranges, with the exception of six subjects who had higher values for glutamate oxaloacetate transaminase.

Patient body weight and sexual function were not altered by 8 weeks of *S*-CIT treatment. All depressive symptoms showed improvement following treatment, with a significant decrease in scores for the severity of depression as rated using the BDI, HAM-D, HAM-A and the CGI scales ($p < 0.0001$ for all rating scales). The mean values for HAM-D and HAM-A were 22.14 and 25.89, respectively, at baseline, 14.37 and 18.17 at week 2, 12.76 and 15.35 at week 4 and 10.09 and 12.58 at week 8. The actual rates of remitters were 22% at week 2, 40% at week 4 and 65% at week 8. The patient dropout rate was 30%. Side effects evaluated using TESS were increased at week 2, and then significantly decreased ($p = 0.0151$) after the 8-week treatment. The average compliance rate for each week was approximately 65% (Figure 1).

Serum concentrations of S-CIT & its metabolites

Escitalopram treatment dose and serum concentrations of *S*-CIT and its active metabolite *S*-DCIT increased significantly over the 8-week treatment period ($p \leq 0.0001$, 0.0113 and 0.0035, respectively). However, the *S*-DDCIT, *S*-DCIT:*S*-CIT ratio, *S*-DDCIT:*S*-CIT ratio, and *S*-DDCIT:*S*-DCIT ratio were not altered from baseline after 8 weeks of *S*-CIT treatment.

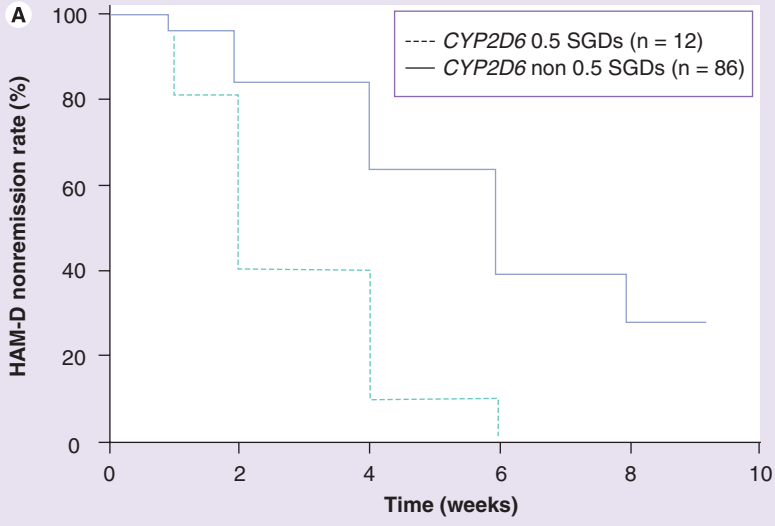
Genetic polymorphisms in *S*-CIT-metabolizing CYP enzymes

Genetic polymorphisms in *CYP2D6, 2C19* and *3A4* are presented in Table 1, along with individual allelic frequencies and the allelic alterations, which may cause a reduction or increase in enzymatic activity. All SNPs achieved Hardy–Weinberg equilibrium, with the exception of rs4244285 (*CYP2C19*2*; $p = 0.029$).

Table 2. Definition of *CYP2D6* polymorphisms using semiquantitative gene dose model.

SGD value	n	Genotype		Allele combination	Predicted phenotype
		rs1065852	*rs3892097*		
0	1	Del/del	Del/del	**5/*5*	PM
0.5	11	T/del	G/del	**10/*5*	IM
	1	T/T	G/A	**4/*10*	IM
1	26	T/T	G/G	**10/*10*	IM
	2	C/del	G/ del	Wt/**5*	IM
	1	C/T	G/A	Wt/**4*	IM
1.5	41	C/T	G/G	Wt/**10*	IM
2	16	C/C	G/G	Wt/wt	EM
3	1	C/C	G/G	**1x2/*1*	UM

*Del: *5 heterozygous deletion; EM: Extensive metabolizer; IM: Intermediate metabolizer; PM: Poor metabolizer; SGD: Semiquantitative gene dose; UM: Ultra-rapid metabolizer; wt: Wild-type.*



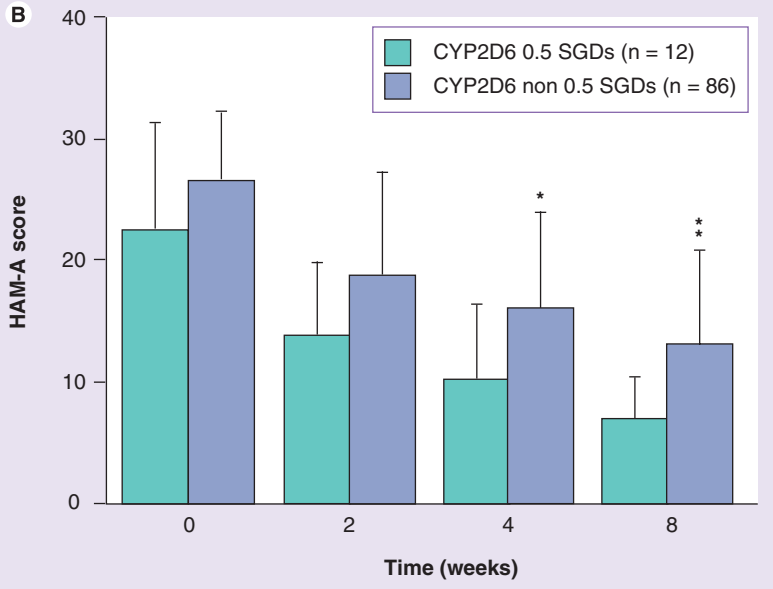


Figure 2. *CYP2D6* genetic modeling of escitalopram treatment remission response. (A) HAM-D score nonremission rates calculated by log-rank regression analyses. **(B)** Changes in HAM-A score were compared between *CYP2D6* 0.5 SGD versus non-0.5 SGD in patients with major depressive disorder. The HAM-A data are presented as mean ± standard deviation.
*p-value < 0.05. **p-value < 0.005.
HAM-A: Hamilton Rating Scale for Anxiety; HAM-D: Hamilton Depression; SGD: Semiquantitative gene dose.

*CYP2C19*17* and *CYP3A4*18* had allele frequencies of only 0.5 and 1.6%, respectively, which limited their utility in subgrouping for further analyses.

CYP2D6 & treatment response

The SGD model subgrouped different allelic combinations on *CYP2D6* genetic polymorphisms into intermediate and extensive metabolizers (Table 2). The poor metabolizer included the 0 SGD (allelic combination of **5/*5*). The intermediate metabolizers included the 0.5 SGD (allelic combinations of **10/*5* and **4/*10*), the one SGD (allelic combinations of **10/*10*, **1/*5* and **1/*4*), and the 1.5 SGD (allelic combinations of **1/*10*). The extensive metabolizers included the two SGD (allelic combination of **1/*1*), while the ultra-rapid metabolizers included the three SGD (allelic combination of **1xN/*1*). The poor and the ultra-rapid metabolizers were not included in further analyses owing to there being only one subject in each group.

The *S*-CIT treatment dose and serum concentrations of *S*-CIT, and its metabolites *S*-DCIT and *S*-DDCIT, were not different amongst the allelic combinations of *CYP2D6*. The 0.5 SGD of intermediate metabolizers had higher numbers of fast remitters compared with the non-0.5 SGD metabolizers in the HAM-D rating scale for depression (log rank test, $p < 0.0001$) during an 8-week *S*-CIT treatment course. Similar results were found for the HAM-A rating scale ($p = 0.0277$ at week 4 and $p = 0.0013$ at week 8) for anxiety (Figure 2). *Post-hoc* power analyses for the HAM-D nonremission rates of two groups at week 2, 4, and 8 were 60, 99 and 100%, respectively. Remitters were defined as having a HAM-D score below 10 and a HAM-A score below 17. The serum levels of *S*-CIT, *S*-DCIT and the ratios of *S*-DCIT:*S*-CIT were not significantly different between the 0.5 SGD and non-0.5 SGD groups during the 8-week treatment course.

CYP2C19 & serum concentrations of *S*-CIT

The GD model subgrouped the allelic combinations of *CYP2C19* into poor and extensive metabolizers (Table 3). The poor metabolizers included the 0 GD (allelic combinations of **2/*2*, **2/*3* and **3/*3*), and the extensive metabolizers included the one GD (allelic combinations of **1/*2* and **1/*3*) and the two GD (allelic combinations of **1/*1*).

The three subgroups of functional allelic frequencies were not statistically different in reference to subject's age, body weight and treatment doses. The poor metabolizer had significantly higher *S*-CIT serum concentrations than the extensive metabolizers of both one GD and two GDs at weeks 2, 4 and 8 (Figure 2). The metabolic ratio of *S*-DCIT:*S*-CIT had genetic dose relations with the GD of *CYP2C19* during *S*-CIT treatment at week 2 ($p < 0.0001$), 4 ($p < 0.0001$) and 8 ($p = 0.0402$). However, the treatment response

estimated by HAM-D for depression and HAM-A for anxiety showed no differences between the poor and extensive metabolizers in *CYP2C19*.

Discussion

Three liver metabolic enzymes CYP2D6, 2C19 and 3A4 are responsible for the metabolism of *S*-CIT. No reports have simultaneously discussed the metabolic effect of these enzymes in patients under treatment with *S*-CIT in relation to their genetic variants in a Taiwanese population. In this preliminary report of an ongoing study, we collected 100 patients with MDD from five hospitals in northern Taiwan over the course of 3 years. The genetic variants showed that all the allele type frequencies were within the reported ranges of the Asian population [11,12,16,18,24].

Classification of the different *CYP2D6* genetic polymorphisms in a statistical model could possibly influence the outcome of predicted correlations between patients' drug metabolic activity and treatment responses [22,38]. Original differences in *CYP2D6* metabolic rates within the patient population were evaluated by phenotyping with debrisoquine [39]. Classification of patients as intermediate metabolizers was found later to be equal to the genotyping defined as 0.5, 1 and 1.5 in the SGD model. Genotyping of the SGD model was found to work better with nortriptyline, which more precisely predicted patient steady-state serum concentrations [40]. This model has subsequently been successfully applied in patients treated with the antidepressant imipramine, in relation to treatment dose [36], and also to the metabolic ratios of venlafaxine, fluoxetine and risperidone [41].

Using this model, we found that the patients classified as intermediate metabolizers at 0.5 SGD of *CYP2D6* responded faster than the non-0.5 SGD patients to treatment with the antidepressant *S*-CIT (Figure 2). However, serum concentrations of *S*-CIT, *S*-DCIT or the *S*-DCIT:*S*-CIT ratio in the patients at 0.5 SGD of *CYP2D6* were not shown to be significantly higher than the non-0.5 SGD groups over the 8-week treatment course. This result suggests that *CYP2D6* may be involved in the *S*-CIT treatment response pathway in patients with MDD.

In contrast with the SGD for *CYP2D6*, the *CYP2C19* group had fewer functional genetic polymorphisms (**2*, **3* and **17*) in Asian populations. When GD was applied to analyses of patients in the *CYP2C19* polymorphism group, we found that the poor metabolizers had *S*-CIT serum levels that were significantly higher than levels in the extensive metabolizers (Figure 3). This result suggests that the GD model may be a better tool in predicting *S*-CIT serum levels than *CYP2C19* phenotyping of classification [39]. Use of phenotyping classification may miss the relationships between the metabolic ratios of *S*-DCIT:*S*-CIT and the GDs of *CYP2C19* at weeks 2, 4 and 8. However, the GD model in *CYP2C19* correlates only with *S*-CIT serum concentrations and not treatment response as rated using the HAM-D and HAM-A scales, or even the patient remission ratios. This suggests that the serum *S*-CIT levels alone may not fully explain the drug treatment response.

In *CYP2D6* and *CYP2C19* gene–gene polymorphism interaction analyses using a multivariate generalized estimating equations statistical model adjusted for age, gender, time of treatment and treatment dose, we found that gene interaction was significantly associated with patient serum levels of *S*-CIT, *S*-DCIT, and the *S*-DCIT:*S*-CIT ratio (data not shown). The main contributor to this interaction was *CYP2C19*. The *CYP2D6* GD of the 0.5 and non-0.5 subgroups were mainly associated with HAM-D score ($p = 0.0027$) and HAM-A score ($p = 0.0025$) (data not shown). Using two-way ANOVA, it was found that the interaction between these two genetic models was mainly associated with the serum concentrations of *S*-CIT at week 4 ($p < 0.0001$) and week 8 ($p < 0.0001$) (data not shown). Although this result is tentative owing to the low patient numbers in some of the subgroups, the results of this study are similar to those of previously reported genetic modeling analyses [36]. Further confirmation of these findings is required.

Table 3. Definition of *CYP2C19* using a gene-dosage model.

Gene dose	n	Genotype		Allele combination	Predicted phenotype
		rs4244285	*rs4986893*		
0	15	AA	GG	**2/*2*	PM
	1	GA	GA	**2/*3*	PM
1	33	GA	GG	**1/*2*	EM
	4	GG	GA	**1/*3*	EM
2	47	GG	GG	**1/*1*	EM

EM: Extensive metabolizer; PM: Poor metabolizer.

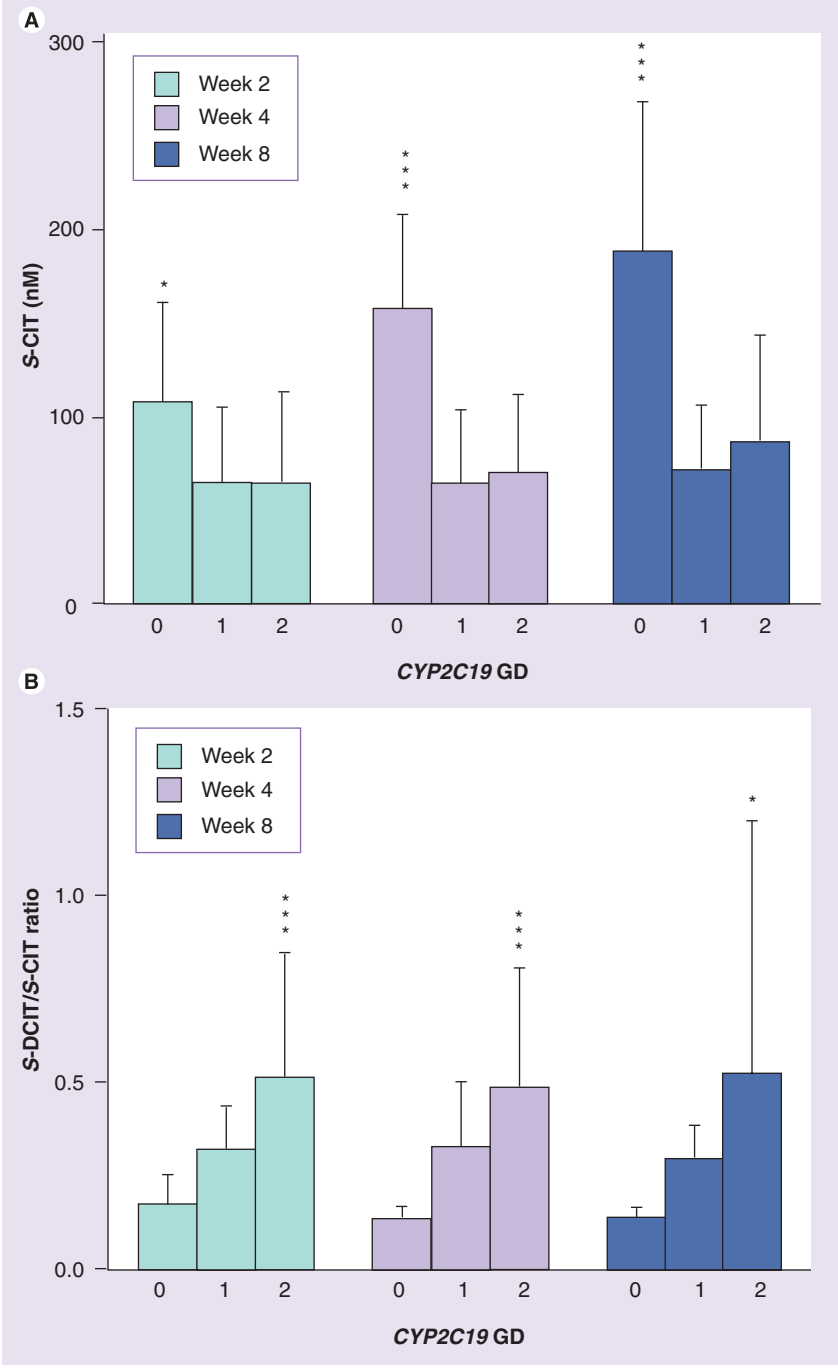


Figure 3. *CYP2C19* genetic modeling and serum concentrations of *S*-CIT or its metabolic ratio. (A) Average steady-state serum *S*-CIT concentration and **(B)** average metabolic ratio of *S*-DCIT:*S*-CIT in poor and extensive metabolizers defined by *CYP2C19* gene dosage at weeks 2, 4 and 8. Data are presented as mean ± standard deviation. Asterisks represent a significant difference between gene dose 0 and gene dose 1 or 2 **(A)**, or between gene dose 2 and gene dose 0 or 1 **(B)**. *p-value < 0.05. ***p-value < 0.001.
GD: Gene dose; *S*-CIT: Escitalopram; *S*-DCIT: *S*-desmethylcitalopram.

Conclusion

In summary, this study found that the statistical models used in defining the *CYP* enzyme gene allelic combinations suggestive of predicting patient antidepressant serum levels and treatment response. Results indicate that the *CYP2D6* genetic polymorphisms analyzed using the SGD model with a cutoff at 0.5 might provide a better predictor of fast *S*-CIT treatment response in patients with MDD as related to the severity of their depressive symptoms, as rated with the HAM-D scale, and the severity of their anxiety symptoms, as rated with the HAM-A scale. Furthermore, *CYP2C19* genetic polymorphisms analyzed by the GD model may serve as better predictors of patient *S*-CIT serum levels and the *S*-DCIT:*S*-CIT ratio. As the main objective of the study covered multiple polymorphisms in more than one gene, these proposed models warrant further validation in other patient cohorts.

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Ethical conduct of research

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

Executive summary

- The antidepressant escitalopram is metabolized by *CYP2D6*, *2C19* and *3A4*.
- The genetic polymorphisms of **4*, **5* and **10* in *CYP2D6*, and **2* and **3* on *CYP2C19* have allele frequencies high enough to explore their predictive effect on CYP enzymatic activities in the Taiwanese population.
- The combination of genetic variants of metabolic CYP enzymes may affect the predictions of escitalopram (*S*-CIT) serum concentrations and treatment responses.
- The *CYP2D6* genetic polymorphisms analyzed using the semiquantitative gene dose model with a cutoff at 0.5 might be a better predictor of fast *S*-CIT treatment responses than other semiquantitative gene dose cutoffs.
- The *CYP2C19* genetic polymorphisms analyzed using the gene dose model may provide a model for the prediction of patient *S*-CIT serum levels and the *S*-desmethylcitalopram:*S*-CIT ratio.
- These proposed models will need to be validated in other patient cohorts or applied to multiple CYP enzyme metabolic analyses.

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