ABCB1 gene polymorphisms are associated with the severity of major depressive disorder and its response to escitalopram treatment

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Objective **ATP-binding cassette, sub-family B (MDR/TAP), member 1 (ABCB1) is a drug transporter protein expressed on the epithelial cells of the intestine and the endothelial cells of the blood–brain barrier. Intestinal ABCB1 actively transports drugs from the cell membrane and prevents them from entering the blood stream whereas the blood–brain barrier ABCB1 prevents drugs from entering the central nervous system. In this study, we tested whether genetic polymorphisms within the *ABCB1* gene are associated with the severity of depression and the effectiveness of the antidepressant, escitalopram (*S*-CIT), in treating major depressive disorder (MDD).**

Methods **Twenty single nucleotide polymorphisms in the *ABCB1* gene were selected and genotyped in 100 MDD patients who had undergone *S*-CIT treatment continuously for 8 weeks. The serum concentrations of *S*-CIT and its metabolites (*S*-desmethylcitalopram and *S*-didesmethylcitalopram) were then measured at weeks 2, 4, and 8.**

Results **The *ABCB1* genotypes of rs1922242 ($P=0.0028$) and rs1202184 ($P=0.0021$) showed significant association with the severity of depressive symptoms as assessed by the Hamilton Rating Scale for Depression adjusted with Hamilton Rating Scale for Anxiety. The haplotype block, rs1882478-rs2235048-rs2235047-rs1045642-rs6949448 (from intron 27 to intron 26), of *ABCB1* was found strongly associated with the remission rate (global $P=0.003$, d.f.$=69$) in which haplotype T-T-T-C-C was associated with a slower remission rate on *S*-CIT treatment ($P=0.001$). The haplotypes may not be indicators of the severity of depression or anxiety.**

Conclusion **Our findings suggest that single nucleotide polymorphisms in the *ABCB1* gene may be indicators of the severity of depression and of the likely *S*-CIT treatment remission response in MDD.** ***Pharmacogenetics and Genomics*** **00:000–000 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Major depressive disorder (MDD) is a severe psychiatric illness that tends to recur [1] and current practice often involves continuous treatment. Escitalopram (*S*-citalopram, or *S*-CIT) is a selective serotonin reuptake inhibitor that is commonly prescribed for MDD and anxiety disorder [2] and has been reported to have favorable efficacy and acceptability, which are comparable with the antidepressant, sertraline [3]. *S*-CIT has also been reported to be both safe and well tolerated over both short and long-term treatments [2].

The treatment efficacy of *S*-CIT relies on the drug transporter activity of the ATP-binding cassette, sub-family B (MDR/TAP), member 1 (ABCB1) at the blood–brain barrier to regulate the drug concentrations close to the target site of the serotonin nerve terminals [4,5]. The ABCB1 protein, also known as multidrug resistance 1 or P-glycoprotein 1, is a drug transporter and an ATP-consuming enzyme found in the cell membranes of the intestinal epithelium and the blood–brain barrier endothelium and regulates the drug concentrations in both the plasma and the brain [5]. Although no direct evidence

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was shown between the blood levels of *S*-CIT and its treatment response, indirect evidence was supported by the effective serum concentration of *S*-CIT correlates with the serotonin transporter occupancy [6], citalopram concentration in the brain is increased in *abcb1* knockout mice [7], and the plasma citalopram concentration is also related to treatment responses in patients with obsessive–compulsive disorder [8]. In *Abcb1* knockout mice, *S*-CIT has been shown to be a substrate of *ABCB1* [7]. An association study of single nucleotide polymorphisms (SNPs) within *ABCB1* and antidepressant treatment responses further suggested that these genetic variants are predictors of the treatment response [7]. Concurrent studies have reported similar findings with other antidepressants that are also *ABCB1* substrates [9,10]. However, conflicting results have been obtained in a reported Sequenced Treatment Alternatives to Relieve Depression trial [11]. To examine the complexity surrounding the involvement of *ABCB1* genetic polymorphisms on the severity of MDD and also their association with the *S*-CIT treatment response, we analyzed SNPs within exon regions, tagSNPs and potentially functional SNPs.

Earlier susceptibility genetic studies of MDD and bipolar disorder have shown some of the underlying mechanisms, including the serotonin [12] and inflammatory [13,14] pathways. These findings have indicated that MDD is a complex genetic disorder. The involvement of the pharmacokinetic gene, *ABCB1*, in MDD was first reported in a Japanese population with a significant association found with mood disorders, including depression [15]. However, this P-glycoprotein, which is encoded by the *ABCB1* gene, has not yet been studied in a Taiwanese MDD cohort. We therefore examined the role of the *ABCB1* gene in the *S*-CIT antidepressant treatment response in Taiwanese MDD patients.

Materials and methods

Patients

We recruited 100 Taiwanese patients with MDD from the outpatient clinics of five hospitals in the Taipei area. All patients were at least 18 years of age and were determined to be of Han Chinese ethnicity based on the documented background of their parents and at least three of their grandparents. Clinical interviews were conducted by trained research nurses. Diagnosis was made according to the criteria of the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* using the Structured Clinical Interview for *DSM-IV*-TR Axis-I Disorders [16]. Inclusion criteria for enrollment in this study included a moderate depressive episode at first visit of at least 14 points (regarded as the baseline) on the 21-item Hamilton Rating Scale for Depression (HAM-D) [7,17]. The patients must not have shown an earlier refractory response or intolerance to *S*-CIT treatment, and needed to have completed a 7-day washout period for any earlier antidepressant treatments. Patients with a primary or comorbid diagnosis of schizophrenia, schizoaffective disorder, bipolar disorder, alcohol or substance dependence, dementia, or other significant medical conditions were excluded from this study.

Patients received *S*-CIT at a fixed dose of 10 mg for the first 4 weeks, followed by dosages of 10–30 mg/day based on their clinical response over an 8-week treatment. No other psychotropic drugs were allowed during this period except for hypnotics (zolpidem at 10 mg per night was permitted as needed, but not exceeding four nights per week), or anxiolytics (lorazepam 1–2 mg per day) to treat severe anxiety. Serum and blood samples were collected at 12–20 h after the last dose on weeks 2, 4, and 8 and sent to the laboratory for analyses of *S*-CIT and its metabolites and for DNA genotyping. Sampling time, dosage, and patient compliance were also documented.

This study was approved by the institutional review board of the National Health Research Institutes and participating hospitals, with written informed consent obtained from all patients. This study was also registered for clinical trial at National Institutes of Health (*http://www.clinicaltrial.gov/ct/show/NCT00384020*).

Depression symptoms rating scale

Medication efficacy was assessed using the 21-item HAM-D [18], the Hamilton Rating Scale for Anxiety (HAM-A) [17], and the Clinical Global Impression scale for determining the severity of illness and improvement after treatment. Subgroup analyses of the HAM-D scores enabled further clustering of the MDD cases into remitters and nonremitters. We evaluated the patients at baseline (at the time of enrollment) and at weeks 2, 4, 6, and 8 of the continuous treatment period. All raters for the HAM-D and HAM-A underwent the same investigator training module. The interrater reliability coefficient was therefore consistent with an α value of 0.9. Remitters from MDD were defined by a HAM-D score of less than 10 [7]. Side effects were assessed using the Treatment Emergent Symptom Scale (TESS) and the Arizona Sexual Experiences Scale [19], which has a 5-item rating scale that quantifies sex drive, arousal, vaginal lubrication/penile erection, ability to reach orgasm, and satisfaction from orgasm.

Serum escitalopram and metabolite analysis

The serum concentrations of *S*-CIT and its metabolites, *S*-desmethylcitalopram (*S*-DCIT) and *S*-didesmethylcitalopram (*S*-DDCIT), were measured by high-performance liquid chromatography [20] using a Waters 2795 Alliance solvent pump and an autosampler, a Waters 2475 multi-λ fluorescence detector (with an excitation wavelength of 245 nm and emission wavelength of 295 nm), and an HP computer recorder installed with the Waters Empower software (Milford, Massachusetts, USA). The analytical column used was a reverse-phase C8 column

(Sphere-Image, 5 μm, 100 × 3 mm) with a C18 guard column (Phenomenex, 4 × 3 mm). We set the column oven temperature at 35°C. The mobile phase comprised 35% acetonitrile, 65% potassium phosphate buffer (10 mmol/l), and triethylamine (1 ml/l of mobile phase), with a final pH of 3.2.

Patients' sera were obtained by whole-blood centrifugation at 3000 rpm (1710 × *g*) for 15 min. From these serum samples, we extracted *S*-CIT, *S*-DCIT, *S*-DDCIT, and 100 ng of an imipramine supplement (internal standard) using a C18-E 100 mg/ml capacity STRATA (Phenomenex, Torrance, California, USA) column. In brief, the column was conditioned with a Waters vacuum manifold with 1 ml of methanol, 1 ml of water, and 1.5 ml of 45 mmol/l potassium phosphate buffer (pH 4.5), and loaded with 800 μl of serum sample and imipramine. The samples were equilibrated for 2–3 min (stopcock closed) and 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 into 12 × 75 mm test tubes. The collected eluent was then evaporated in a water bath at 55°C under a stream of air for 17 min. We dissolved the remaining residue in a 100 μl volume of mobile phase, and chromatographed 50 μl of this sample.

The *S*-CIT, which was a gift from the Research and Education Institute for Texas Health Resources (Dallas, Texas, USA), showed the same retention time as citalopram (Sigma, St Louis, Missouri, USA). *S*-DCIT and *S*-DDCIT were purchased from Toronto Research Chemicals Incorporation (North York, Ontario, Canada). Using 1.95 ng each of *S*-CIT, *S*-DCIT, and *S*-DDCIT standards in a reproducibility analysis, the intra-day and inter-day coefficients of variation were measured at 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 lowest detection limit was 0.2 ng/ml for all the three compounds.

ABCB1 SNP selection and genotyping

Genomic DNA was extracted from 8 ml of whole-blood lymphocyte pellets from each patient using Puregene kit (Gentra Systems, Minneapolis, Minnesota, USA). We chose the *ABCB1* SNPs from three main sources: (i) literature reports [15] of polymorphisms relating to MDD in ethnic groups from Asia; (ii) tagSNPs within the *ABCB1*gene with a minor allelic frequency greater than 0.1 and an r^2 cutoff at 0.8 from HapMap (*http://www.hapmap.org/index.html.en*) with about 6% tagging interval; and (iii) functional SNPs predicted by FastSNP (*http://fastsnp.ibms.sinica.edu.tw/pages/PrioritizeResult.jsp?taskid = TK2781&Submit = ENST00000265724*).

Genotyping was performed by a 12-plex PCR reaction in a 384-well plate, using the GenomeLab SNPstream genotyping platform and its accompanying SNPstream software suite (Beckman Coulter Inc., Fullerton, California, USA). We designed three primers using *www.autoprimer.com* for each SNP detection. We used forward and reverse PCR primers to amplify a short stretch of DNA (approximately 90–150 bp) covering the SNP area of interest, and a tagged SNP primer for single base primer extension to identify the SNP. The 5′ portion of the tagged primer was complementary to one of 12 unique single-stranded DNA oligonucleotides, which were spotted at a specific location within each well of a 384-well SNPware Tag array (Beckman Coulter Inc.). The 3′ portion of the tagged primer was complementary and precisely adjacent to the SNP, enabling detection of the polymorphism through the incorporation of a fluorescent-labeled terminating nucleotide.

Multiplex primer SNP extension reactions were performed in a total volume of 7 μl, containing 3.76 μl SNP extension dilution buffer, 0.06 μl SNP primer mix (each 5 μmol/l), 0.2 μl C/T (or C/G, T/C etc) extension mix, 2.96 μl water, and 0.02 μl SNPware DNA polymerase. The cycling conditions were 96°C for 3 min followed by 45 cycles of 94°C for 20 s, and 40°C for 11 s. The tagged extension primers were extended with single TAMRA-labeled nucleotides or bodipy-fluorescein-labeled nucleotides and then spatially resolved by hybridization to the complementary oligonucleotides arrayed on the 384-well SNPware Tag array. 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 fluorescent color in each well according to the position of the tagged oligonucleotides. The error rate on the basis of blind replicates was 0.1–1% for the SNPs examined in this study. The call rate for each SNP was 100%.

Statistics

The average serum concentrations of *S*-CIT and its metabolites are presented as the mean ± standard error of the mean. A Hardy–Weinberg equilibrium (HWE) was computed using a recently reported exact test method for each SNP [21]. The kinetic change trends in the depression severity rating, treatment dose, serum *S*-CIT and metabolite concentration, and the TESS score of the side effects, were calculated using the generalized estimating equations (GEE) approach adjusted for the time effect of treatment weeks using Statistical Analysis System 9.13 software (SAS Institute Inc. Cary, North Carolina, USA) [22]. Correction for multiple testing was made to adjust the *P* value of each test using a permutation algorithm implemented by the SAS MULTTEST procedure. The procedure adjusted the *P* values from a family of hypothesis tests to control the family-wise error rate. The permutation adjustments

resample the data without replacement to approximate the distribution of the minimum *P* value of all tests. The MULTTEST procedure was applied for the associations between SNPs and the remission rates, the severity of MDD, the symptoms of insomnia and depression. The odds ratios (remission ratios) and 95% confidence interval in each case were used to assess the strength of the relationship in the allelic models. Power analysis was done to justify the sample size using the GLMPOWER procedure of the SAS software. The last observation carried forward was used to add up the missing data because of the dropouts.

Haplotype blocks of 20 SNPs were constructed using Haploview version 4.1 [23]. The haplotype blocks were determined using Gabriel's method [24]. Within each haplotype block, PROC MIXED procedure was used in the SAS software to carry out the association of overall haplotype and remission, HAM-D, and HAM-A adjusted for age, sex, and dose. The Hochberg method [25] implemented in PROC MULTTEST procedure was used to adjust for the multiple testing resulting from analyses of multiple phenotypes. The level of statistical significance was a *P* value of less than 0.05.

Results

We recruited 100 MDD patients (81 female and 19 male) using an open-label study design. The average age of this population at the time of study was 42 years. Their liver and thyroid functions were within normal ranges except in six cases that showed higher glutamate oxaloacetate transaminase values.

Body weight and sexual function were unaltered in the patients after an 8-week *S*-CIT treatment. In addition, their depression and anxiety symptoms showed improvement by HAM-D and HAM-A measurements, respectively, and the severity of illness rated using the Clinical Global Impression scale showed significantly decreased scores (GEE $P < 0.0001$ in all rating scales). The 65% *S*-CIT treatment response rate observed was similar to that reported earlier (51% for female cases at week 8) [26].

Serum concentrations of *S*-CIT and its metabolites

The average serum concentrations of *S*-CIT and its active metabolite, *S*-DCIT, were 72 ± 5.5 and 21 ± 1.0 nmol/l at week 2, 78.4 ± 6.3 and 22.6 ± 1.2 nmol/l at week 4, and 95.2 ± 7.9 and 27.7 ± 2.2 nmol/l at week 8. The *S*-CIT treatment dose, and the serum concentrations of *S*-CIT and its active metabolite, *S*-DCIT, all significantly increased over the 8 weeks of treatment (GEE $P < 0.0001$, < 0.011, and < 0.0035, respectively; data not shown). The *S*-DDCIT, *S*-DCIT/*S*-CIT, *S*-DDCIT/*S*-CIT, and *S*-DDCIT/*S*-DCIT ratios were unaltered after the 8-week *S*-CIT treatment.

ABCB1 SNPs and depressive symptoms

Table 1 lists 20 validated SNPs across the *ABCB1* gene region. These SNPs were found to be in the HWE with minor allele frequencies above 0.04. As the HAM-D and HAM-A values strongly correlate with each other (Pearson's correlation coefficient = 0.52, $P < 0.0001$), the SNP genotypes of rs2235046 (intron 17), rs1128503 (exon 13), and rs1202184 (intron 5) were thus significantly associated with both the severity of MDD rated by the HAM-D adjusted with HAM-A and also the severity of anxiety rated with HAM-A adjusted with HAM-D, even after multiple corrections at baseline. In addition, the rs1922242 SNP showed an association with the severity of MDD only after adjustment (permutation, $P = 0.0028$).

SNPs and *S*-CIT treatment responses

The remission status of MDD and its association with *ABCB1* SNPs at week 8 are listed in Table 2. Patients with HAM-D scores of less than 10 were counted as remitters, and all others as nonremitters. Three SNPs, rs1882478 ($P = 0.037$), rs1045642 ($P = 0.045$) and rs10256836 ($P = 0.021$), showed significant associations with the remission rates for the allele types after correction with a multiple test for permutation. The rs10256836 SNP has more nonremitters in the minor C allele than remitters, which differs from the situation observed for rs1882478 and rs1045642, in which there were more remitters for the minor allele than nonremitters.

Haplotype association with treatment response

Haplotype blocks containing two groups of five SNP markers were constructed using Haploview method [24]. This method creates a block when the informative (i.e. noninconclusive) comparisons have strong linkage disequilibrium and span no more than 30 kb. Figure 1 shows a couple of five SNP haplotype blocks in the *ABCB1* genetic region. Block 1 covers the SNPs, rs1882478-rs2235048-rs2235047-rs1045642-rs6949448, in introns 27–26, and block 2 contains the SNPs, rs1922242-rs2235046-rs1128503-rs2235018-rs10256836, in introns 17–5. Table 3 lists the haplotype blocks and their associations with MDD remission, the severity of MDD rated with HAM-D adjusted with HAM-A, and the severity of anxiety symptoms rated with HAM-A adjusted with HAM-D. Block 1 showed significant association with remission (multiple testing and adjusted age, sex and dose of global $P = 0.003$). The T-T-T-C-C combination showed a negative *t* score association with remission ($t = -4.92$; multiple testing and adjusted age, sex and dose of $P = 0.001$). The T-T-T-C-C combination cases showed a slower rate of remission compared with patients without this combination during 8 weeks of *S*-CIT treatment, in which all the dropout patients were treated as nonremitters. The block 2 haplotype also showed significant association with MDD remission in the T-G-C-A-C combination ($t = -3.3$; multiple testing and

Table 1 Validated *ABCB1* gene single nucleotide polymorphisms and their associations with major depressive disorder

					HAM-D[e]	HAM-A[f]
SNP_ID[a]	Chromosome 7[b]	Allele[c]	Role	MAF[d]	*N*	*P*[g]	*P*[g]
rs1882478	86974954	T/C	Intron 27	0.47 (0.49)	100	0.062	0.28
rs2235048	86976447	T/C	Intron (boundary) 27	0.42 (0.4)	100	0.087	0.37
rs2235047	86976468	T/G	Intron (boundary) 27	0.39 (0.47)	100	0.74	0.66
rs1045642 **(C3435T)**	86976581	C/T	Coding exon 27	0.42 (0.4)	95	0.057	0.27
rs6949448	86979750	C/T	Intron 26	0.42 (0.44)	81	0.18	0.28
rs10234411	87002828	A/T	Intron 21	0.49 (0.43)	99	0.26	0.56
rs3789246	87005963	G/A	Intron 20	0.15 (0.19)	100	0.52	0.15
rs1922242	87011603	A/T	Intron (boundary) 17	0.28 (0.29)	98	**0.0028**	0.38
rs2235046	87012002	A/G	Intron (boundary) 17	0.38 (0.32)	100	**0.011**	**0.041**
rs1128503 **(C1236T)**	87017537	T/C	Coding exon 13	0.41 (0.31)	100	**0.015**	**0.020**
rs2235018	87037301	A/G	Intron 6	0.17 (0.19)	98	0.97	0.30
rs2235016	87037348	T/G	Intron (boundary) 6	0.04 (0.1)	99	0.37	0.81
rs10256836	87038709	G/C	Intron 5	0.14 (0.11)	100	0.61	0.95
rs1989831	87043415	T/A	Intron 5	0.06 (0.14)	100	0.39	0.80
rs1202184	87051837	A/G	Intron 5	0.33 (0.26)	100	**0.0021**	**0.0013**
rs3789243	87058822	C/T	Intron 4	0.28 (0.28)	100	0.11	0.58
rs2188524	87068371	A/G	Intron (boundary) 1	0.07 (0.12)	100	0.34	0.81
rs28381796	87069847	T/C	Intron 1	0.05 (0.065)	100	0.36	0.80
rs4148732	87071985	A/G	Intron 1	0.12 (0.1)	100	0.088	0.44
rs1978095	87089577	T/C	Intron 1	0.20 (0.20)	100	0.33	0.34

Bold text: $P<0.05$.

ABCB1, ATP-binding cassette, sub-family B (MDR/TAP), member 1; HAM-A, Hamilton Depression Rating Scale for anxiety; HAM-D, Hamilton Depression Rating Scale for Depression; MAF, minor allele frequency; *N*, subject number; *P*, permutation bootstrap for 10 000 *P* values; SNP, single nucleotide polymorphism.

[a]According to the dbSNP database (parentheses indicate that the SNP is within an exon locus).

[b]Based upon NCBI Human Genome Build 36.

[c]The second allele is the minor allele.

[d]Minor allele frequency (parenthesis: minor allele frequency in HapMap Chinese population, except rs28381796 which is from an Asian population).

[e]Hamilton Depression Rating Scale to quantify the severity of depression symptoms adjusted with HAM-A.

[f]Hamilton Anxiety Rating Scale to quantify the severity of anxiety symptoms adjusted with HAM-D.

[g]Permutation bootstrap for 10000 *P* values for the SNP genotype and adjusted with HAM-D or HAM-A association analyses.

Table 2 Genotyped single nucleotide polymorphisms of the *ABCB1* gene and their associations with *S*-CIT treatment response at week 8

		Remitters		Non-remitters		Remission association in allele type		
SNP ID	Allele[a]	*N*	MAF	*N*	MAF	*P*	Odds ratio	95% CI
rs1882478	T/C	48	0.56	26	0.31	**0.037**	0.35	0.17–0.71
rs2235048	T/C	48	0.49	26	0.27	0.11	0.38	0.18–0.80
rs2235047	T/G	48	0.36	26	0.35	1.00	0.92	0.46–1.87
rs1045642	C/T	44	0.51	25	0.26	**0.045**	0.34	0.16–0.72
rs6949448	C/T	37	0.51	20	0.26	0.12	0.34	0.14–0.79
rs10234411	A/T	47	0.47	26	0.52	1.00	1.23	0.62–2.42
rs3789246	G/A	48	0.16	26	0.10	0.98	0.57	0.20–1.68
rs1922242	A/T	47	0.26	25	0.38	0.77	1.79	0.86–3.73
rs2235046	A/G	48	0.32	26	0.54	0.11	2.45	1.22–4.89
rs1128503	T/C	48	0.31	26	0.54	0.08	2.57	1.28–5.14
rs2235018	A/G	47	0.14	26	0.15	1.00	1.13	0.44–2.94
rs2235016	T/G	48	0.02	26	0.02	1.00	0.92	0.08 ~10.41
rs10256836	G/C	48	0.10	26	0.31	**0.021**	3.82	1.58–9.22
rs1989831	T/A	48	0.03	26	0.02	1.00	0.61	0.06–6.00
rs1202184	A/G	48	0.29	26	0.42	0.74	1.78	0.88–3.60
rs3789243	C/T	48	0.23	26	0.37	0.59	1.94	0.93–4.05
rs2188524	A/G	48	0.06	26	0.02	0.96	0.29	0.03–2.51
rs28381796	T/C	48	0.02	26	0.04	1.00	1.88	0.26–13.75
rs4148732	A/G	48	0.08	26	0.21	0.27	2.95	1.10–7.89
rs1978095	T/C	48	0.18	26	0.17	1.00	0.97	0.40–2.37

Bold text, $P<0.05$.

ABCB1, ATP-binding cassette, sub-family B (MDR/TAP), member 1; CI, confidence interval; MAF, minor allele frequency; *N*, subject number; *P*, permutation bootstrap for 10 000 *P* values; S-CIT, escitalopram; SNP, single nucleotide polymorphism.

[a]The second allele is the minor allele.

adjusted age, sex and dose of $P = 0.047$). None of the haplotypes showed significant associations with the severity of depression or anxiety after multiple testing and adjustment for age, sex and dose of S-CIT.

Genotype and side effects

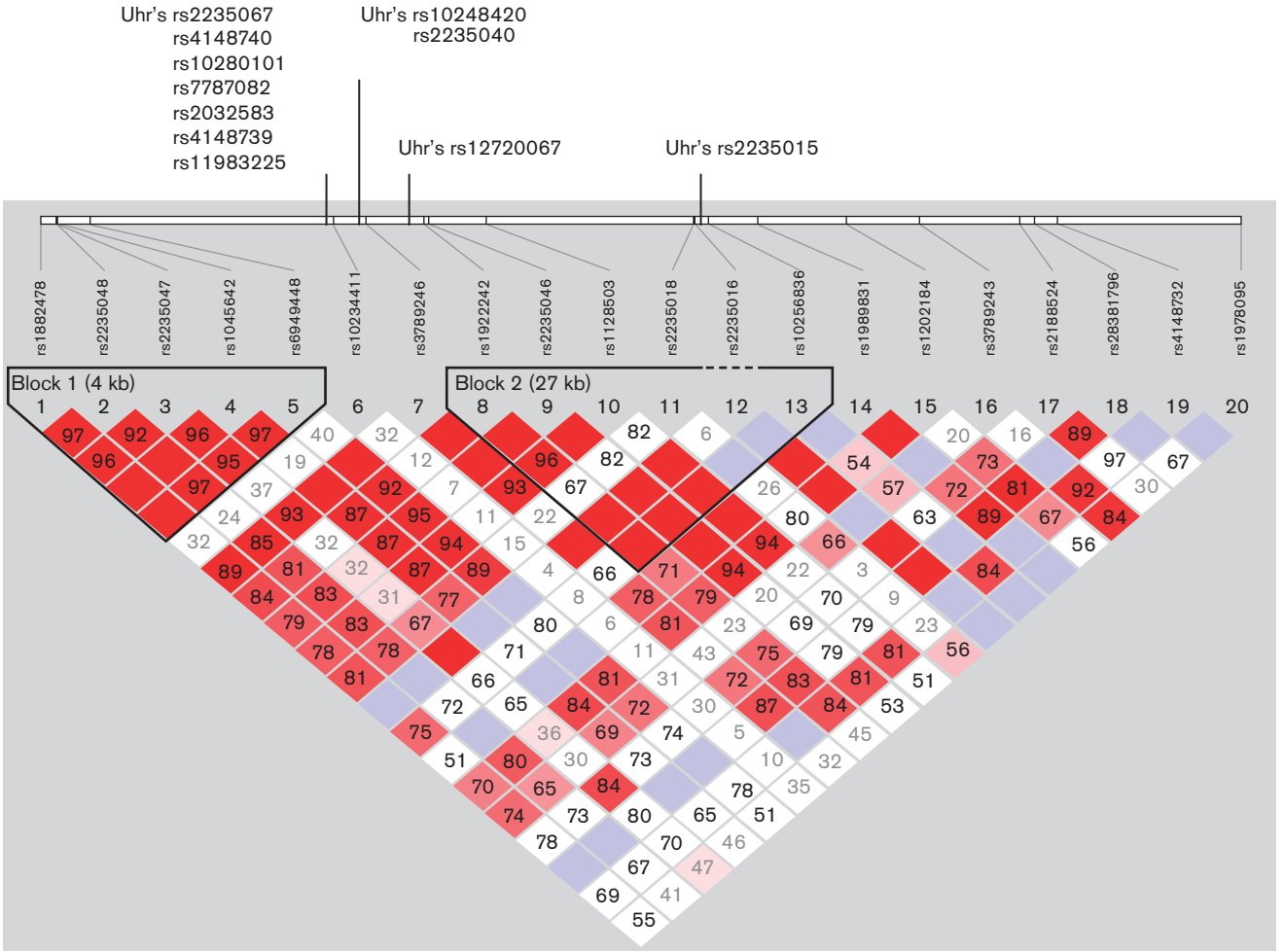
The treatment emergent symptoms scale values rated with TESS were increased at weeks 1 and 2, and then significantly decreased by the eighth week of *S*-CIT treatment (GEE $P = 0.0151$). In week 1, the rs1882478 SNP showed a significant association with symptoms of insomnia (permutation $P = 0.0051$; power = 0.73). The T allele carriers had more severe insomnia symptoms than the C allele carriers ($P = 0.002$). During week 2, the rs1922242 SNP showed significant association with symptoms of depression (permutation $P = 0.035$; power = 0.7), in which the T allele carrier had stronger symptoms than the A allele carriers ($P = 0.0054$).

Discussion

In this study, we genotyped 20 known SNPs within the *ABCB1* gene region in 100 MDD patients. The allele frequencies were found to be comparable with those reported by HapMap for a Chinese population and were in the HWE (Table 1). These results suggest that our current randomized sample size and the data obtained from it are a good reflection of the general Chinese population.

The *ABCB1* genetic region that associates with the severity of depression may be mainly located between

Fig. 1



Haploview linkage disequilibrium (*D*′) for the two haplotype blocks within the 20 ATP-binding cassette, sub-family B (MDR/TAP), member 1 single nucleotide polymorphism (SNP) markers (numbered by primer ID according to the physical length of each SNP). The number in each square is D′ × 100 between two SNPs. *D*′ = 1 in both block 1 (rs1882478 and rs6949448) and 2 (rs1922242 and rs10256836). The log of the odds values were 30.38 in block 1 and 10.31 in block 2. The rs2235016 SNP (dashed-line) was not included in block 2. The significant SNPs reported in Uhr's earlier study were compared with those analyzed in this study.

introns 17 and 5. As shown in Table 1, the single locus rs1922242 (intron 17, $P = 0.0028$), rs2235046 (intron 17, $P = 0.011$), rs1128503 (exon 13, $P = 0.015$) and rs1202184 (intron 5, $P = 0.0021$) between introns 17 and 5 showed a significant association with the severity of depression as rated with HAM-D after adjustment with HAM-A as a covariate at baseline. All of these SNPs further showed a significant association with the severity of anxiety as rated using HAM-A after adjustment with HAM-D as a covariate with the exception of rs1922242. The fact that the HAM-A and HAM-D measurements also showed a strong correlation with each other indicates that anxiety may be a feature of depressive symptoms [27]. These results suggest that variations in the *ABCB1* gene may predispose the carriers to differing levels of depression. The haplotype did not show significant association with the severity of depression or anxiety (Table 3), which suggests that the haplotype may not be indicators of the predisposition of depression.

ABCB1 genetic variations were further found to associate with the *S*-CIT treatment response. In our remission rate association analyses of a single locus, three SNP allele types were found to have significant associations with the *S*-CIT treatment responses in our MDD patient cohort (Table 2). These SNPs included rs1882478 (intron 27), rs1045642 (exon 27) and rs10256836 (intron 5). The minor C allele of the rs1882478 SNP was associated with more remitters than nonremitters and similar observations were made for other SNPs, which were found to be significantly associated with the remission rate after *S*-CIT treatment. For example, exon 27 of rs1045642 (also known as C3435T) harbors the minor T allele and appeared at a higher frequency among remitters. The

Table 3 Haplotype frequencies (HF) of the *ABCB1* gene and their association with remission, the depression score rating by HAM-D and the anxiety score rating by HAM-A in major depressive patients

Haplotypes	Frequency	Remission (t)	Remission *P* value	HAM-D (t)	HAM-D *P* value	HAM-A (t)	HAM-A *P* value
Block 1 (rs1882478-rs2235048-rs2235047-rs1045642-rs6949448) (intron 27-intron 27-intron 27-exon 27-intron 26)							
Global *P* value			**<0.0001 (0.003)** (d.f.=69)		**0.03** (0.84) (d.f.=93)		0.45 (d.f.=93)
C-C-T-T-T	0.39	2.46	**0.016 (0.042)**	−1.64	0.11	1.41	0.16
C-T-T-C-C	0.03	0.34	0.73	−0.49	0.62	−0.73	0.47
C-T-T-C-T	0.031	0.23	0.82	0.91	0.36	0.38	0.70
T-T-G-C-C	0.35	0.37	0.72	0.56	0.58	−0.85	0.40
T-T-T-C-C	0.18	−4.92	**<0.0001 (0.001)**	1.25	0.21	−0.45	0.65
Block 2 (rs1922242-rs2235046-rs1128503-rs2235018-rs10256836) (intron 17-intron 17-exon 13-intron 6-intron 5)							
Global *P* value			0.11 (d.f.=69)		0.099 (d.f.=93)		0.14 (d.f.=93)
A-A-T-A-G	0.46	2.53	**0.014** (0.075)	−1.64	0.11	1.63	0.11
A-A-T-G-G	0.15	0.13	0.90	−1.16	0.25	0.3	0.76
A-G-C-A-G	0.088	−1.36	0.18	0.70	0.48	−2.01	**0.048** (0.28)
T-G-C-A-C	0.18	−3.30	**0.002 (0.047)**	1.13	0.26	−0.77	0.44
T-G-C-A-G	0.13	0.79	0.44	1.83	0.07	−0.62	0.54

[illegible]ion (t), haplotype block association t score with escitalopram treatment of remission rates at week 8.
[illegible]1, ATP-binding cassette, sub-family B (MDR/TAP), member 1. d.f., [illegible]inator degrees of freedom.
HAMD (t), haplotype block association t score with Hamilton Depression (HAMD) rating scale of the severity of depression symptoms adjusted with HAM-A at baseline.
HAMA (t), haplotype block association t score with the Hamilton Anxiety (HAMD) rating scale of the severity of anxiety symptoms adjusted with HAM-D at baseline.
Bold text, $P<0.05$.
Parenthesis *P* value: adjusted *P* value for multiple testing after permutation for 10 000 times and for age, sex, and dose.

C3435T polymorphism has been shown in several studies to be an important marker of schizophrenia olanzapine [28], and also of the bromperidol [29] treatment response and depressive side effects of nortriptyline [30]. No interactions were found among rs1882478, rs1045642 and rs10256836. We therefore speculate that the T allele may be associated with less ABCB1 activity than the C allele [31]. Hence, higher concentrations of *S*-CIT may enter the brains of C allele carriers from the blood stream to exert a greater treatment effect. Our remission association loci were different from Uhr's study (Fig. 1). It may be because of ethnicity differences.

The findings from our haplotype block association analyses further suggested that the intron 26–27 region and the T-T-T-C-C combination are important remission indicators for the *S*-CIT treatment response. On the basis of the results of our *ABCB1* haplotype block 1 analysis listed in Table 3, and also our remission rate association analyses, the T-T-T-C-C combination in the rs1882478-rs2235048-rs2235047-rs1045642-rs6949448 block exhibited a significant association with an inverse correlation with the *S*-CIT treatment response ($t=-4.92$, $P=0.001$). Patients with the T-T-T-C-C combination, which is a risk haplotype, exhibited a lower remission rate than other haplotype combinations.

The side effects of *S*-CIT, evaluated using the TESS scale, were found not to have increased proportionally over the 8-week treatment period. Detectable side effects occurred prominently in weeks 1 and 2 and then gradually decreased as the depressive symptoms showed continual improvement up to week 8. Insomnia is a common side effect of S-CIT [32] and the rs1882478 SNP of T allele carriers with stronger depressive symptoms showed an association with more severe insomnia than the C allele carriers during week 1 of *S*-CIT treatment. This SNP may therefore be an indicator of more prominent insomnia side effects. The rs1922242 variation was also found to associate with more severe depression in the A allele carrier compared with the T allele carriers at week 2 of the *S*-CIT therapy. This SNP may thus be an indicator of the severity of depressive symptoms as a side effect of *S*-CIT.

The dropout rate in our current patient cohort was around 23% at treatment week 8, similar to that reported in the earlier study of Uhr [7]. No comorbidities or deaths occurred in this study. Notable limitations of this study include the higher ratio of female participants (four times), although women have only twice the incidence rate of MDD compared with men [33], the small sample size and the lack of a placebo arm. As a placebo may provide a cutoff for better estimation of the remission rate, a future study design should involve a placebo but this could not be adopted in these analyses because of ethical considerations. The major advantage of this study was our ability to monitor *S*-CIT treatment response in major depressive patients over 8 weeks. The sample size justification was calculated from the reduction of HAM-D over 8 weeks of *S*-CIT treatment. It showed 99.4% (CI 54.9–100%) in retrospective power analysis. Similar results were obtained after the last observation carried forward correction considering the dropouts of missing data. This indicated that the depression symptom was

indeed improved at week 8 even though the sample size was small. Our data indicating that the *ABCB1* genotype is associated with the severity of MDD and the response to *S*-CIT in a Taiwanese population are thus significant.

In summary, these findings show that the *ABCB1* genetic locus harbors SNPs that could be indicators of the severity of depressive symptoms. The haplotype combination of T-T-T-C-C in block 1 was found to associate with a slower *S*-CIT treatment remission response. The role of these SNP variants, particularly in relation to how they regulate *ABCB1* expression and its enzymatic activity, thus warrants further investigation.

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