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CYP2D6 P34S Polymorphism and Mirtazapine Responses in Koreans with Major Depression

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Abstract

Drug metabolism is a critical determinant of the therapeutic and adverse effects of many psychotropic drugs. The metabolism depends on the pharmacokinetics of a drug, which includes its absorption, distribution, and elimination. Psychotropic drugs are metabolized mainly by cytochrome P450 (CYP) enzymes; about 20 of these enzymes exist and they are often responsible for the rate-limiting step of drug metabolism. CYP2D6 is the best-characterized P450 enzyme that exhibits polymorphism in humans. This study determined the relationship between the *CYP2D6*10* (*P34S*) polymorphism and the response to mirtazapine in 153 Koreans with major depressive disorder (MDD). The genotype frequencies were compared using logistic regression analysis, and between-genotype differences in the decrease in the 21-item Hamilton Depression (HAMD21) score over the 12-week treatment period were analyzed using a linear regression analysis. The proportion of remitters was lower in patients with MDD possessing the S allele than in P allele carriers after 2 weeks of mirtazapine treatment. Similarly, the reductions in the HAMD21 and Clinical Global Impression (CGI) scores in S allele carriers were smaller than those in patients with the P allele after 2 weeks of mirtazapine treatment. In the analysis of depression symptoms, the sleep and delusion scores had smaller reductions in S allele carriers. Based on the Liverpool University Neuroleptic Side Effect Rating Scale (LUNSERS), the psychic adverse effects of mirtazapine were associated with CYP2D6 P34S, while weight gain was not. These results suggest that CYP2D6 P34S affects the outcome of mirtazapine treatment in patients with MDD, and that this polymorphism may be a good genetic marker for predicting the clinical outcome of mirtazapine treatment.

Keywords: CYP2D6, Major depressive disorder, Mirtazapine, Treatment response

The hepatic cytochrome P450 system is responsible for the first phase in the metabolism and elimination of numerous endogenous and exogenous molecules, and ingested chemicals. P450 enzymes convert these substances into electrophilic intermediates, which are then conjugated by phase II enzymes to give hydrophilic derivatives that can be excreted[1].

Drug metabolism and transport genes such as *CYP2D6* and *CYP2C19* are obvious pharmacogenetic candidates given their known interaction with drugs like selective serotonin reuptake inhibitors (SSRIs) and their metabolites *in vivo*[2]. Moreover, several of these pharmacokinetic genes have common variants that have been shown to impair enzyme function[3]. For example, Yin *et al.* found that homozygous carriers of the nonfunctional allele of *CYP2C19* show a 42% decrease in clearance of the SSRI citalopram compared to homozygous carriers of the wild-type allele[4]. Gräsmader *et al.* showed that the plasma concentrations of several antidepressants were significantly influenced by the *CYP2D6* and *CYP2C19* genotype, although the clinical response was not associated with the plasma concentrations of these drugs[5].

Despite these known *in vivo* relationships between antidepressant medications and pharmacogenetic genes, few epidemiological studies have investigated the relationship between the antidepressant response and pharmacokinetic gene variants. A retrospective study of 28 patients who experienced adverse events and 16 patients who were non-responsive to a variety of antidepressants showed an association with the *CYP2D6* genotype[6]. A prospective study of 246 elder-

Table 1. Demographic characteristics in the MDD intention-to-treat (ITT) group.

	*CYP2D6*10 P34S*			*P*
	PP	*PS*	*SS*	
N	45	70	38	0.304*
Age (yr, Mean ± SE)	51.02 ± 2.07	51.34 ± 1.81	46.03 ± 2.36	0.168†
Onset age (yr, Mean ± SE)	49.76 ± 2.13	49.06 ± 1.84	44 ± 2.47	0.165†
SEX (M, %)	10 (22.2%)	17 (24.3%)	11 (28.9%)	0.771**
Suicide attempt (Y, %)	3 (6.7%)	3 (4.3%)	2 (5.3%)	0.855**
Family history of depression (Y, %)	5 (11.1%)	10 (14.3%)	5 (13.2%)	0.885**
Family history of other psychotic disease (Y, %)	4 (8.9%)	7 (10%)	1 (2.6%)	0.378**
Baseline HAMD21 score	21.20 ± 0.54	21.99 ± 0.55	21.32 ± 0.55	0.528†
core	8.96 ± 0.29 (45)	9.09 ± 0.3 (70)	8.89 ± 0.32 (38)	0.887†
sleep	2.87 ± 0.28 (45)	3.4 ± 0.26 (70)	3.39 ± 0.34 (38)	0.241†
activity	2.69 ± 0.15 (45)	2.7 ± 0.13 (70)	2.76 ± 0.18 (38)	0.914†
psychic anxiety	3.04 ± 0.22 (45)	3.19 ± 0.14 (70)	3.08 ± 0.18 (38)	0.75†
somatic anxiety	3.53 ± 0.18 (45)	3.89 ± 0.16 (70)	3.45 ± 0.23 (38)	0.169†
delusion	2.71 ± 0.23 (45)	2.87 ± 0.16 (70)	2.58 ± 0.22 (38)	0.484†
Baseline CGI	4.49 ± 0.11	4.54 ± 0.09	4.34 ± 0.1	0.498†
Baseline weight (kg)	59.66 ± 1.47	60.93 ± 1.24	61.82 ± 1.81	0.653†

**P* value for Hardy-Weinberg equilibrium

Genotype comparisons were made by ANOVA† and chi-square test**

ly subjects taking the SSRI paroxetine found that the *CYP2D6* genotype was not associated with the side-effect burden[7]. Another recent study of 100 subjects with depression taking fluvoxamine found that the *CYP2D6* genotype does not influence the frequency of gastrointestinal side effects, although when the *CYP2D6* genotype was combined with a serotonin 2A receptor polymorphism, such an association was observed[8]. Despite the equivocal results of these studies, some studies have advocated the use of pharmacokinetic enzyme variant information to guide clinical therapy with SSRIs, particularly by adjusting the dose[9].

Mirtazapine, which is a noradrenergic specific serotonergic antidepressant (NaSSA), enhances noradrenergic transmission via the blockade of α2-adrenoceptors[10-12] and serotonergic transmission indirectly via noradrenergic stimulation of α1-adrenoceptors and blockade of α2-heteroreceptors[13,14]. It also has anxiolytic, hypnotic, antiemetic, orexigenic, antihistaminic, and antipruritic effects via antagonism of the 5-HT2A, 5-HT2C, 5-HT3, and H1 neurotransmitter receptors[15-17]. Like most antidepressants, mirtazapine is metabolized by the cytochrome P450 system, especially CYP2D6[18].

Ethnicity is a factor in the occurrence of CYP2D6 variability, which is based on the differences in the allele frequencies of CYP2D6 among populations. For example, about 10% of whites have the nonfunctional CYP2D6*4 allele, while about 50% of Asians possess the CYP2D6*10 allele, which should cause decreased CYP2D6 activity[19].

Although an intuitive appeal exists in ascribing differences in drug tolerance and efficacy to variation in pharmacokinetic genes, no adequately powered studies have consistently reported a significant clinical effect. This study examined the relationship between the *CYP2D6*10* polymorphism (*C188T* or *P34S*) and the mirtazapine treatment response in Korean patients with major depressive disorder (MDD).

Clinical Characteristics of the Study Subjects and Hardy-Weinberg Equilibrium for the CYP2D6*10 Polymorphism

In the 153 initial subjects studied, the distribution of CYP2D6 P34S genotypes was in Hardy-Weinberg equilibrium (Table 1). Table 1 summarizes the patient gender, mean age, age at onset, frequency of suicide attempts, and family history of MDD or other psychotic diseases. None of these parameters differed significantly among the genotypes. The baseline HAMD21 score, each symptom score, and Clinical Global Impression (CGI) score also did not differ among the genotypes. In addition, the body weights of the study subjects were not associated with the CYP2D6 P34S genotypes.

Of the 153 patients chosen to participate in this study, 66 withdrew because of a failure to draw blood, lack of efficacy, personal conflict or other personal decision, loss to treatment, or adverse events during 12 weeks of treatment. The genotype distributions did not differ from those of the initial patients (data not shown), which suggested that the subsequent genetic analysis was not affected by withdrawal.

Table 2. Association analysis of CYP2D6 P34S polymorphism with the remission status by mirtazapine treatment in MDD patients.

Duration (weeks)	Remission status	*CYP2D6 P34S* gentoype, % (N)				Codominant		Dominant		Recessive		*CYP2D6 P34S* allele, % (N)				
		PP	*PS*	*SS*	Total	*P**	OR (±95% CI)	*P**	OR (±95% CI)	P*	OR (±95% CI)	*P*	*S*	Total	*P**	OR (±95% CI)
1	Remission	40.0% (2)	20.0% (1)	40.0% (2)	100% (5)	0.772	0.83 (0.24-2.9)	0.648	1.53 (0.24-9.64)	0.322	0.39 (0.06-2.52)	50.0% (5)	50.0% (5)	100% (10)	0.768	0.83 (0.23-2.94)
	Non-remission	29.3% (43)	46.3% (68)	24.5% (36)	100% (147)							52.4% (154)	47.6% (140)	100% (294)		
2	Remission	40.9% (9)	45.5% (10)	13.6% (3)	100% (22)	0.042	2.01 (1.03-3.96)	0.094	2.34 (0.87-6.33)	0.089	3.17 (0.84-11.99)	63.6% (28)	36.4% (16)	100% (44)	0.030	2.16 (1.08-4.32)
	Non-remission	26.7% (28)	43.8% (46)	29.5% (31)	100% (105)							48.6% (102)	51.4% (108)	100% (210)		
4	Remission	28.6% (8)	53.6% (15)	17.9% (5)	100% (28)	0.527	1.21 (0.67-2.19)	0.99	0.99 (0.38-2.58)	0.279	1.84 (0.61-5.53)	55.4% (31)	44.6% (25)	100% (56)	0.496	1.24 (0.67-2.29)
	Non-remission	30.2% (26)	41.9% (36)	27.9% (24)	100% (86)							51.2% (88)	48.8% (84)	100% (172)		
8	Remission	25.0% (9)	44.4% (16)	30.6% (11)	100% (36)	0.609	0.86 (0.48-1.54)	0.749	0.85 (0.33-2.25)	0.602	0.78 (0.30-2.01)	47.2% (34)	52.8% (38)	100% (72)	0.579	0.84 (0.46-1.54)
	Non-remission	27.9% (17)	45.9% (28)	26.2% (16)	100% (61)							50.8% (62)	49.2% (60)	100% (122)		
12	Remission	26.3% (10)	42.1% (16)	31.6% (12)	100% (38)	0.529	0.83 (0.45-1.50)	0.966	0.98 (0.37-2.60)	0.326	0.61 (0.23-1.63)	47.4% (36)	52.6% (40)	100% (76)	0.522	0.82 (0.45-1.51)
	Non-remission	26.5% (13)	51.0% (25)	22.4% (11)	100% (49)							52.0% (51)	48.0% (47)	100% (98)		

*Obtained by logistic regression controlling age and sex as covariates.

Association between Remission Atatus and the CYP2D6 P34S Polymorphism

We also tested the association between the CYP2D6 P34S polymorphism and the remission status with mirtazapine treatment. As shown in Table 2, a significant association was seen after 2 weeks of treatment in the codominant model ($P=0.042$). The proportion of S allele homozygotes was lower in the patients entering remission than in non-remitters (13.6% vs. 29.5%, respectively), while the proportion of P allele homozygotes was higher in remitters (40.9% vs. 26.7 %, respectively).

Similar results were seen in the allele analysis, in which the remission status after 2 weeks of mirtazapine treatment was significantly associated with CYP2D6 P34S [$P=0.030$, odds ratio=2.16 (1.08−4.32); Table 2]. The frequency of the S allele in remitters was lower than that in patients who did not enter remission (36.4% vs. 51.4%, respectively).

These results suggest that CYP2D6 influences remission status with mirtazapine treatment and that S allele carriers tend not to enter remission.

Association of the CYP2D6 P34S Polymorphism and the Reduction in the HAMD21 Score with Mirtazapine Treatment

Since remission status was associated with CYP2D6 P34S, we also analyzed the association between the polymorphism and HAMD21 score using linear regression. As shown in Figure 1A, a significant association was observed between them ($P=0.032$ in the recessive model). The HAMD21 score in the S allele homozygotic patients (14.24 ± 0.95) was higher than in the patients possessing the PS genotype (12.59 ± 0.69) and in PP homozygotes (11.81 ± 0.79). In the allele analysis, the score of patients possessing an S allele was higher than that of P allele carriers (13.49 ± 0.49 vs. 12.15 ± 0.43, respectively; $P=0.017$). These results support the results of the logistic regression analysis and suggest that the S allele has a protective effect on the antidepressant action of mirtazapine.

Association of the CYP2D6 P34S Polymorphism and the Reduction in the CGI Score with Mirtazapine Treatment

To confirm these results, we also tested the association between the CYP2D6 P34S polymorphism and the percent reduction of the CGI scores in the mirtazapine-treated patients. Similar to the results of the HAMD21 analysis, a significant association was detected between the two after 2 weeks of mirtazapine treatment (Figure 1B). The reduction rate in SS homozygotic patients was $22.63\pm3.72\%$, which was lower than those in patients with the PP ($31.01\pm3.18\%$) or

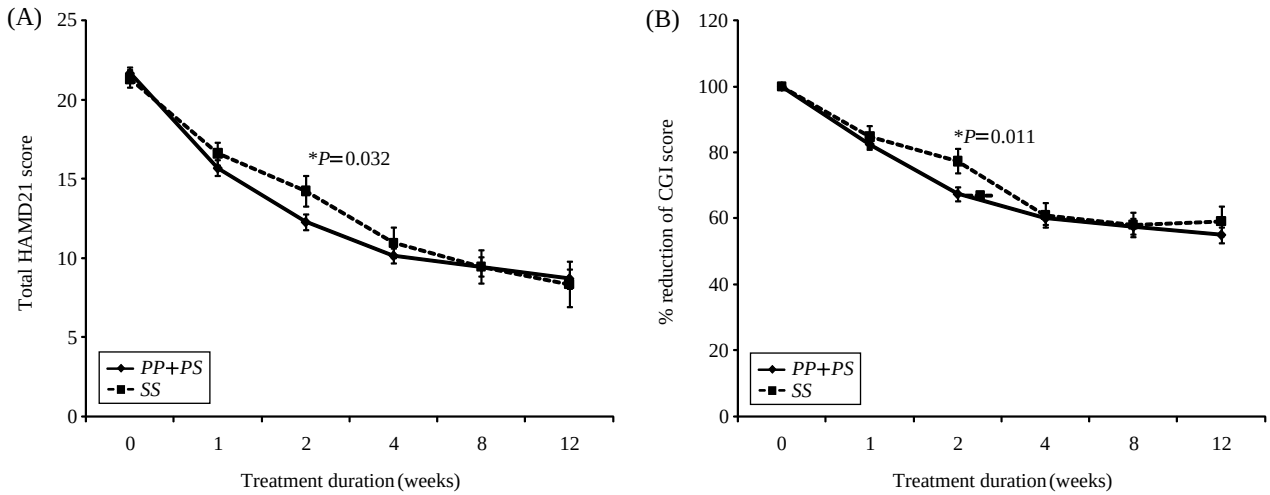

Figure 1. The changes of HAMD21 score (A) and the percent reduction of CGI scores (B) after mirtazapine treatment according to genotypes of CYP2D6 P34S. Data are presented by mean ± SE. **P* values were obtained using linear regression analysis controlling age and sex as covariates.

Table 3. Association analysis of CYP2D6 P34S polymorphism with symptom scores at 2 weeks of mirtazapine treatment in MDD patients.

Symptoms	Symptom score, Mean ± SE (N)			*P* values*		
	PP	PS	SS	Cod	Dom	Rec
core	5.51 ± 0.38 (37)	6 ± 0.34 (56)	6.18 ± 0.38 (34)	0.409	0.191	0.443
sleep	1.19 ± 0.26 (37)	1.29 ± 0.24 (56)	1.82 ± 0.34 (34)	0.100	0.229	0.035
delusion	1.78 ± 0.21 (37)	1.84 ± 0.17 (56)	2.24 ± 0.19 (34)	0.139	0.305	0.049
activity	1.59 ± 0.17 (37)	1.73 ± 0.15 (56)	2.03 ± 0.2 (34)	0.197	0.191	0.100
psychic anxiety	1.92 ± 0.17 (37)	2.11 ± 0.15 (56)	2.21 ± 0.19 (34)	0.481	0.261	0.398
somatic anxiety	2.24 ± 0.18 (37)	2.57 ± 0.19 (56)	2.53 ± 0.25 (34)	0.410	0.181	0.581

**P* values were obtained using linear regression analysis controlling age and sex as covariates.
Cod, codominant model; Dom, dominant model; Rec, recessive model

PS (33.52 ± 2.64%, $P=0.036$ in the codominant and $P=0.011$ in the recessive model) genotype.

Association of CYP2D6 P34S and Changes in Depressive Symptoms with Mirtazapine Treatment

We tested the association between the polymorphism and the changes in each depressive symptom, based on the HAMD21 scores, with mirtazapine treatment (Table 3). We found that the symptom scores representing sleep and delusion at 2 weeks were associated with CYP2D6 P34S. The sleep and delusion scores at 2 weeks were higher in SS homozygotes than in patients with the PP or PS genotype ($P=0.035$ for sleep scores and 0.049 for delusion scores in the recessive model). These results suggest that the antidepressant action of mirtazapine is influenced by the CYP2D6 P34S polymorphism, especially in symptoms related to sleep and delusion.

Association of CYP2D6 P34S and Mirtazapine-induced Weight Gain

We tested the association of CYP2D6 P34S with mirtazapine-induced adverse effects assessed using LUNSERS. The major adverse effects of mirtazapine treatment were psychic side effects (data not shown). The risk of psychic side effects was associated with CYP2D6 P34S at 1, 2, and 4 weeks of mirtazapine treatment (Table 4). The proportion of S allele homozygotes was higher in patients with psychic side effects than in other patients, and the odds ratio was between 2.06 and 6.44. This suggests that patients with the S allele of CYP2D6 P34S are more susceptible to developing psychic adverse effects.

Mirtazapine can induce weight gain as an adverse effect, which may be a reason for reduced compliance. Therefore, we also tested the relationship between CYP2D6 P34S and the percent increase in body weight of patients after mirtazapine treatment; however, we

Table 4. Association analysis of CYP2D6 P34S polymorphism with the presence of mirtazapine-induced psychic side effects in MDD patients.

Duration (weeks)	Psychic side effects	CYP2D6 P34S gentoype, % (N)				Codominant		Dominant		Recessive	
		PP	PS	SS	Total	*P**	OR (±95%CI)	*P**	OR (±95% CI)	*P**	OR (±95% CI)
1	−	36.7% (18)	49% (24)	14.3% (7)	100% (49)	0.012	2.06 (1.17-3.61)	0.14	1.95 (0.8-4.75)	0.007	3.93 (1.47-10.55)
	+	22.9% (11)	37.5% (18)	39.6% (19)	100% (48)						
2	−	40% (26)	41.5% (27)	18.5% (12)	100% (65)	0.001	2.94 (1.54-5.59)	0.005	6.44 (1.78-23.37)	0.010	3.44 (1.34-8.78)
	+	9.4% (3)	46.9% (15)	43.8% (14)	100% (32)						
4	−	35.2% (25)	45.1% (32)	19.7% (14)	100% (71)	0.009	2.38 (1.24-4.56)	0.067	2.99 (0.93-9.64)	0.011	3.49 (1.33-9.18)
	+	15.4% (4)	38.5% (10)	46.2% (12)	100% (26)						
8	−	30.1% (25)	44.6% (37)	25.3% (21)	100% (83)	0.583	1.24 (0.58-2.63)	0.907	1.08 (0.31-3.76)	0.419	1.64 (0.49-5.45)
	+	28.6% (4)	35.7% (5)	35.7% (5)	100% (14)						
Total	−	44.4% (16)	44.4% (16)	11.1% (4)	100% (36)	0.003	2.49 (1.36-4.58)	0.018	2.95 (1.2-7.26)	0.011	4.51 (1.41-14.44)
	+	21.3% (13)	42.6% (26)	36.1% (22)	100% (61)						

*Obtained by logistic regression controlling age and sex as covariates.

could not find a significant association between them (data not shown).

Discussion

Cytochrome P450 CYP2D6 is an extensively characterized polymorphic drug-metabolizing enzyme. The *CYP2D6* gene is highly polymorphic and more than 70 different alleles are known[20,21]. The activity of the enzyme varies markedly among individuals from poor to intermediate to extensive to ultra-rapid metabolism according to the polymorphisms of the *CYP2D6* gene. Association studies provide growing evidence for the clinical importance of the CYP2D6 polymorphism and in investigating whether the *CYP2D6* genotype distribution differs from that of the normal population either in patients developing marked adverse effects or in nonresponders during treatment with CYP2D6 substrates[11,22,23]. However, these important studies present less information on the dose adjustments necessary to individualize pharmacotherapy in a given clinical case[11]. With respect to psychopharmacological drug metabolism, several antidepressants have been characterized as CYP2D6 substrates[24]. Therefore, we investigated the genetic association between CYP2D6*10 and the outcome of mirtazapine treatment in patients with MDD.

Ethnicity is a factor in the occurrence of CYP2D6 variability. For example, the allele frequency of CYP2D6*4 is 20.7% in Caucasians[25], but 0.2% in Japanese[26]. For CYP2D6*10, the frequency is 45% in Koreans[27], but 1.53% in Caucasians[25], and even differs among Asian populations; it is 38.1% in Japanese[26] and 51.3% in Chinese[28]. This variability suggests that the clinical outcome with the same drug can vary among populations. Lee *et al.* reported that the CYP2D6 alleles with frequencies exceeding 5% in Koreans were *1, *2, *4, and *10[27]. Of these, we choose to study *10 based on statistical power and its effects on enzyme activity. In this study, the frequency of *10 was 47.7%, which concurs with a previous report.

Age, history of depression, and the baseline HAMD21 score and CGI did not differ among genotypes, which suggests that CYP2D6*10 is not a risk factor for developing depression. In fact, Bijl *et al.* reported that CYP2D6 was not associated with the susceptibility to depression or anxiety disorder in the elderly, although 5-methoxytryptamine (5-MT), a precursor of serotonin, is a substrate of CYP2D6 and the concentration of serotonin in the brain was lower in poor metabolizers than in normal subjects[29]. Considering their report and our observations, the enzyme activity might not be a contributing factor to the development of depression.

Our study revealed that CYP2D6 P34S was significantly associated with remission status. More recently, Serretti *et al.* classified the drug metabolism of 278 Europeans as phenotypes according to the genotypes of five alleles: CYP2D6*3, *4, *6, *9, and *19[30]. They found no association between the phenotypes and either response or remission status with antidepressant treatment. Although our study focused on a single locus, P34S, and did not measure the enzyme activity, our results contradict their observation. A possible explanation is the difference in the study designs. Serretti *et al.* studied 21 drugs, including SSRIs, tricyclic antidepressants, norepinephrine reuptake inhibitors, serotonin norepinephrine reuptake inhibitors, and NaSSA, and 278 subjects[30]. This caused the stratification of the study subjects, and just 21 subjects were treated with mirtazapine. These subjects were further divided into four phenotypes, poor (PM), intermediate (IM), extensive (EM), and ultrarapid (UM)

metabolizers, which may have lowered the statistical power of the study. In our study, 153 subjects were treated with mirtazapine only, and were classified using the genotypes of a single polymorphism for which the allele frequency was about 0.47. In fact, the power of our analysis exceeded 80%. In addition, our finding of an association between remission status and CYP2D6 P34S was supported by the analysis of the HAMD21 and CGI scores. Therefore, we concluded that the S allele of CYP2D6 P34S is a risk factor for a low response to mirtazapine treatment.

Our result indicated that the association between CYP2D6 P34S and the treatment effect of mirtazapine was restricted to that at 2 weeks. After 4 weeks, no difference was seen in the HAMD21 score and remission status among genotypes. These observations may have been due to the pharmacokinetics of mirtazapine. The blood mirtazapine concentration reaches a steady state 4-6 days after uptake[18], and we can expect this time point to vary according to the enzyme activity; PM may need less than 6 days to reach a steady state and EM may need longer than PM or IM. After 2 weeks, all subjects reach an effective blood concentration regardless of their genotype. Consequently, the antidepressant activity of mirtazapine may not differ among subjects, although the psychic anxiety symptom score was associated with the CYP2D6 P34S at 8 weeks.

Two recent studies demonstrated an increased hospitalization rate in PMs treated with antidepressants and antipsychotics and an increased trend for developing adverse effects in PMs as compared to Ems[6,31]. However, another study failed to detect differences in the *CYP2D6* genotype distribution in psychiatric patients with adverse drug effects[7]. We used LUNSERS to assess the adverse effects of mirtazapine, making ours the first study on antidepressant-induced side effects. We found that the major adverse effects of mirtazapine were psychic side effects, including difficulty staying awake during the day. These side effects may be based on the antihistamine effect of mirtazapine, although further studies on mirtazapine-induced psychic side effects are necessary. Furthermore, we found that this side effect was associated with CYP2D6 P34S, while weight gain, a major side effect of mirtazapine[32], was not; the S allele, which was associated with low efficacy, was also associated with the development of psychic side effects. These observations suggest that PMs produce significantly higher plasma drug concentrations and may be more susceptible to psychic adverse effects, and that CYP2D6 activity may not be a risk factor for weight gain.

This study has several limitations; we evaluated only one SNP of the *CYP2D6* gene. Therefore, whole-gene screening and association studies for other variants of the *CYP2D6* gene may be necessary to identify markers associated with the response to antidepressants in various populations. We also cannot exclude the presence of a population stratification bias[33]. However, because the Korean population is characterized by a relatively high degree of genetic homogeneity[34], we consider such stratification bias unlikely in our sample.

Previous pharmacogenetic studies have found associations between the *CYP2D6*10* polymorphism and the clinical response to SSRIs. To our knowledge, no study has examined the relationship between the clinical outcome of mirtazapine administration and *CYP2D6* polymorphisms; further studies are required.

In summary, we found that CYP2D6 P34S was associated with the remission status and decline in the HAMD21 score after 2 weeks of mirtazapine treatment, especially symptoms related to sleep and delusion, and it was also related to mirtazapine-induced psychic side effects. These results suggest that CYP2D6 P34S is a genetic marker predicting the clinical outcome of mirtazapine treatment.

Materials & Methods

Subjects

Trained psychiatrists examined all of the subjects using the Structured Clinical Interview for DSM-IV Axis I disorders (SCID-I) and the Korean version of the Diagnostic Interview for Genetic Studies (K-DIGS). The severity of depression was assessed using the 21-item Hamilton Depression Rating (HAMD21) scale. Only subjects with a minimum score of 18 on the HAMD21 scale were enrolled.

Subjects with primary or comorbid diagnoses of schizophrenia, schizoaffective disorder, rapid cycling bipolar disorder, dementia, and alcohol or substance dependence based on DSM-IV criteria within the previous 6 months were excluded from the study. Patients with serious or unstable medical illness were also excluded. We also disqualified subjects with a personal or family history of substance abuse/dependence. Patients already on medication were permitted a 2-week washout period. Demographic data, medical history, and laboratory data were documented. All subjects were at least 18 years of age.

During the treatment period in the study, all subjects took mirtazapine (Remeron®; Schering-Plough, Kenilworth, NJ, USA) at a daily dose of 15-60 mg. Psychotropic drugs such as benzodiazepines and mood stabilizers were not permitted. All subjects gave informed consent to participate in the study, and the study protocol was approved by the ethics committee of the

Korea University Medical Center.

Clinical symptoms were evaluated using the HAMD21 scale at baseline and after 1, 2, 4, 8, and 12 weeks of treatment. Responders were those who showed a 50% or more decrease in the HAMD21 score compared to baseline, and remitters were defined by a HAMD21 total score of 7 points or less[35]. To evaluate specific clusters of depressive symptoms, the HAMD21 items were grouped according to the following factors, as described by Serretti *et al.*[36]: core (items 1, 2, 7, 8, 10, and 13), sleep (items 4, 5, and 6), activity (items 7 and 8), psychic anxiety (items 9 and 10), somatic anxiety (items 11, 12, and 13), and delusion (items 2, 15, and 20).

The side-effect profile during treatment was assessed using the modified Liverpool University Neuroleptic Side Effect Rating Scale (LUNSERS). To evaluate specific clusters of adverse effects, LUNSERS items were grouped as extrapyramidal (items 17, 25, 30, 36, and 40), psychic side effects (items 2, 4, 12, 16, 19, 21, 27, and 35), other autonomic (items 13, 14, 18, 23, and 31), allergic reactions (items 1 and 41), anticholinergic (items 6, 9, 28, 32, and 45), hormonal (items 7, 42, 15, 22, and 39), and miscellaneous side effects (items 5, 20, 33, and 37).

DNA Analysis

DNA was extracted from peripheral blood and the polymerase chain reaction (PCR) was performed using the sense primer 5′-CCA TTT GGT AGT GAG GCA GGT AT-3′ and antisense primer 5′-CAC CAT CCA TGT TTG CTT CTG GT-3′. Each amplification mixture contained 50 ng of DNA, 3 μL of 10× PCR buffer, 2.5 μL of 2.5 mM dNTP, 10 pmol of each primer, and 0.5 μL of *Taq* polymerase (5 U/μL; TaKaRa, Kyoto, Japan). Samples were amplified using a thermocycler (Perkin-Elmer, Boston, MA, USA) for an initial 7 min at 94˚C, followed by 35 cycles of 45 s at 94˚C, 45 s at 56˚C, and 45 s at 72˚C. After a final 5-min extension at 72˚C, the reaction was terminated at 4˚C. The amplified DNA was digested with the restriction endonuclease *Hph*I (New England Biolabs, Ipswich, MA, USA), which cleaves at site 188C. The product was electrophoresed on 3% agarose gels stained with ethidium bromide; the 213- and 58-bp fragments correspond to the 188C (34Pro) allele and the 122-, 101-, and 58-bp fragments correspond to the 188T (34Ser) allele.

Statistical Analysis

The Hardy-Weinberg equilibrium for the *CYP2D6*10* polymorphism was tested using the χ^2 test. The genetic association of the single nucleotide polymorphism (SNP) was analyzed using multiple logistic regression for categorical data and linear regression for continuous variables, controlling for age and sex as covariates. A P value ≤ 0.05 was regarded as statistically significant. The power to detect associations given the sample size was analyzed using G · Power[37]. All statistical analyses were performed using SPSS version 10.0 (SPSS Inc., Chicago, IL, USA).

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