plasma, to which cisapride had been added as an internal standard, was extracted with hexane-chloroform, and the extract was subjected to automated column-switching high-performance liquid chromatography using a TSK BSA-C8 precolumn (Tosoh, Tokyo, Japan) for sample clean-up, and a TSK gel ODS-80TS column (Tosoh) for separation.

Statistical Analysis

Kaplan–Meier survival analysis and Cox regression analysis were used to compare the probabilities of the incidence of side effects. Genotype and allele distributions were analyzed by the χ^2-test. The clinical and demographic characteristics, onset weeks, onset doses, onset concentrations, and cumulative numbers of side effects were compared among groups by the unpaired *t*-test or one-way analysis of variance. The level of significance was set at less than 0.05.

RESULTS

The genotype frequencies of the 5-HT2A, 5-HT3A, and 5-HT3B genes are shown in Tables 1, 2 and 3. All of these genetic variations were in Hardy–Weinberg equilibrium. The genotypes of the 5-HT3A C195T polymorphism were not detected in seven patients. The other three genotypes of four patients could not be identified. No significant differences were demonstrated for sex, age, and baseline HAM-D-17 scores among the genotype groups.

Effect of the 5-HT2A Gene A-1438G Polymorphism

The cumulative incidences of fluvoxamine-induced gastrointestinal side effects are presented in Figure 1. Cox regression was used to analyze the effect of the 5-HT2A gene A-1438G polymorphism on the gastrointestinal side effects. The number of G alleles was entered into the analysis as an independent variable, and sex, age, and baseline HAM-D-17 score were added as potential confounders. The Cox regression analysis showed that patients with one G allele had a 2.171-fold higher risk of developing gastrointestinal side effects ($P=0.041$; 95% confidence interval (CI), 1.032–4.566) and patients with two G alleles had a 2.926-fold higher risk of developing gastrointestinal side effects ($P=0.008$; 95% CI, 1.321–6.481) than patients with no G allele. Sex, age, and baseline HAM-D-17 scores showed no significant effects on the risk of developing gastrointestinal side effects. There were no significant differences in the incidence of discontinuation between the three genotype groups ($\chi^2=0.029$, df$=2$, $P=0.986$) (Table 1).

Significant trends were demonstrated for the cumulative number of gastrointestinal side effects between the three genotype groups, although no significant differences were observed for the onset weeks, onset doses, and onset concentrations of fluvoxamine.

On the other hand, survival analyses showed no significant effect of the A-1438G polymorphism on the onset rate of all side effects, including the gastrointestinal side effects. No significant differences were demonstrated for the onset weeks, onset doses, onset concentrations, and cumulative numbers of all side effects between the genotype groups (Table 1).

Table 1 Characteristics of the Demographic Data and Fluvoxamine-Induced Side Effects by Comparison of the A-1438G Genotypes of the 5-HT2A Receptor

	5-HT2A gene A-1438G polymorphism[a]		
	A/A (*N*=28)	A/G (*N*=41)	G/G (*N*=27)
Sex (M/F)	12/16	24/17	11/16
Age	40.8 (17.6)	41.3 (15.4)	38.2 (14.7)
Baseline HAM-D-17 score	21.6 (5.2)	20.2 (5.4)	20.0 (4.9)
Discontinuation	7	11	7
P		0.986	
Gastrointestinal side effects			
Number of patients	10	25	18
Onset week	3.4 (2.9)	3.9 (2.7)	2.1 (2.0)
P		0.073	
Onset dose (mg)	60.0 (42.8)	75.0 (53.5)	45.8 (43.9)
P		0.16	
Onset concentration (ng/ml)	46.5 (70.3)	25.0 (27.8)	24.6 (32.9)
P		0.366	
Cumulative number of side effects	0.7 (1.2)	1.7 (2.0)	2.0 (2.7)
P		0.05	
All side effects			
Number of patients	21	34	22
Onset week	3.2 (2.9)	3.1 (2.8)	1.9 (1.7)
P		0.139	
Onset dose (mg)	56.0 (31.5)	62.5 (47.8)	39.8 (24.0)
P		0.097	
Cumulative number of side effects	2.8 (3.3)	5.3 (6.7)	5.0 (4.6)
P		0.147	

[a]Analysis stratified by the three genotype groups.
5-HT = 5-hydroxytryptamine; HAM-D-17 = 17-item Hamilton Rating Scale for Depression.

Effects of the 5-HT3A and 5-HT3B Gene Polymorphisms

Although Cox regression analysis was performed to investigate the effects of Pro16Ser and C195T of the 5-HT3A gene and Tyr129Ser of the 5-HT3B gene on the gastrointestinal side effects or all side effects, these polymorphisms had no significant effects on the occurrence of fluvoxamine-induced side effects (Tables 2 and 3). No significant differences were demonstrated for the onset weeks and doses of gastrointestinal side effects or all side effects in each genotype group.

Effect of the CYP2D6 Gene Polymorphism

The allele frequencies of the *5 and *10 alleles were 3.6 and 38.1%, respectively. The patients were divided into two genotype groups by the degree of enzyme activity: 75

Table 2 Characteristics of the Demographic Data and Fluvoxamine-Induced Side Effects by Comparison of the 5-HT3A Receptor Gene Polymorphisms

	5-HT3A gene					
	Pro16Ser			C195T		
	Pro/Pro (*N*=76)	Pro/Ser (*N*=18)	Ser/Ser (*N*=2)	C/C (*N*=8)	C/T (*N*=35)	T/T (*N*=50)
Sex (M/F)	38/38	9/9	0/2	2/6	18/17	25/25
Age	41.2 (15.5)	39.1 (16.2)	17.0 (5.7)	29.3 (15.5)	38.5 (13.3)	43.5 (16.6)
Baseline HAM-D-17 score	20.1 (5.2)	21.8 (5.2)	24.5 (0.7)	22.3 (5.7)	19.9 (5.1)	20.6 (5.3)
Gastrointestinal side effects						
Number of patients	44	7	2	5	19	26
All side effects						
Number of patients	63	12	2	6	29	39

5-HT = 5-hydroxytryptamine; HAM-D-17 = 17-item Hamilton Rating Scale for Depression.

Table 3 Characteristics of the Demographic Data and Fluvoxamine-Induced Side Effects by Comparison of the 5-HT3B Receptor Gene Polymorphisms

	5-HT3B gene		
	Tyr129Ser		
	Tyr/Tyr (*N*=54)	Tyr/Ser (*N*=37)	Ser/Ser (*N*=5)
Sex (M/F)	29/25	17/20	1/4
Age	40.2 (16.6)	39.8 (15.4)	44.4 (9.9)
Baseline HAM-D-17 score	21.2 (5.2)	20.1 (5.3)	17.2 (4.3)
Gastrointestinal side effects			
Number of patients	28	22	3
All side effects			
Number of patients	42	30	5

5-HT = 5-hydroxytryptamine; HAM-D-17 = 17-item Hamilton Rating Scale for Depression.

patients with the *1/*1 or *1/*10 genotype were termed normal metabolizers (NMs), and 22 patients with the *10/*10, *1/*5 or *5/*10 genotype were termed lower metabolizers (LMs) (Table 4). Figure 2 shows the effect of the CYP2D6 polymorphism on the incidence of gastrointestinal side effects. Cox regression analysis showed that LMs of CYP2D6 had a significantly higher risk of developing gastrointestinal side effects than NMs ($P=0.043$; hazard ratio (HR), 1.821; 95% CI, 1.019–3.254).

There were no significant differences in the incidence of discontinuation between NMs and LMs ($\chi^2=1.029$, df=1, $P=0.310$). No significant differences were demonstrated for the onset weeks, onset doses, onset concentrations, and cumulative numbers of gastrointestinal side effects or all side effects between NMs and LMs (Table 4).

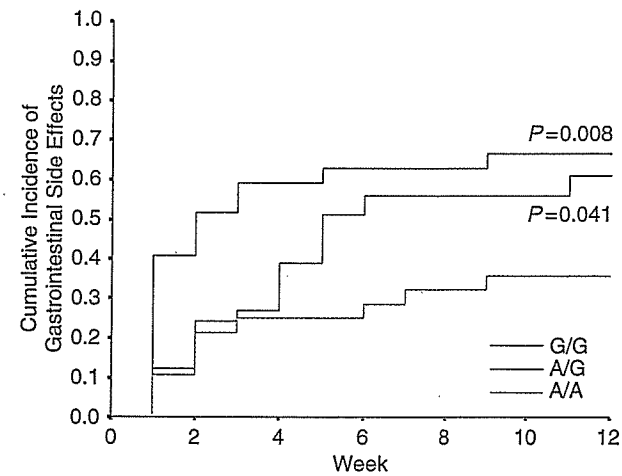

Figure 1 Effect of the A-1438G polymorphism of the 5-HT2A receptor gene on the cumulative 12-week incidence of gastrointestinal side effects induced by fluvoxamine.

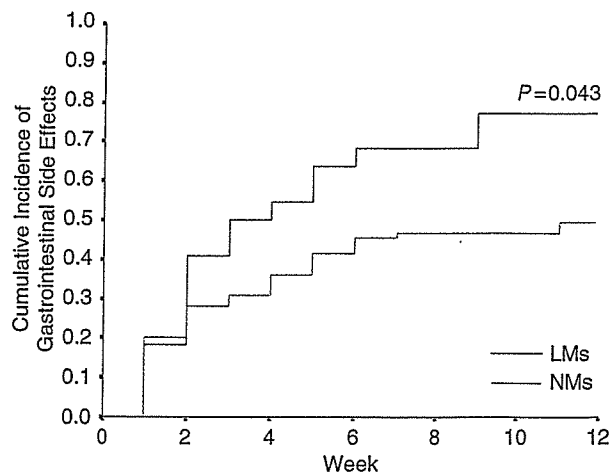

Figure 2 Effect of the CYP2D6 genotype on the cumulative 12-week incidence of gastrointestinal side effects induced by fluvoxamine.

Table 4 Characteristics of the Demographic Data and Fluvoxamine-Induced Side Effects by Comparison of the CYP2D6 Genotype Groups

	CYP2D6 genotype (phenotype)[a]		
	*1/*1, *1/*10 (NMs) (N=75)		*1/*5, *10/*10, *5/*10 (LMs) (N=22)
Sex (M/F)	35/40		12/10
Age	39.1 (16.1)		43.8 (14.2)
Baseline HAM-D-17 score	20.3 (5.3)		21.4 (5.0)
Discontinuation	17		8
P		0.267	
Gastrointestinal side effects			
Number of patients	37		17
Onset week	3.1 (2.7)		3.4 (2.6)
P		0.723	
Onset dose (mg)	64.9 (50.5)		58.8 (47.6)
P		0.679	
Onset concentration (ng/ml)	29.7 (44.8)		27.4 (30.3)
P		0.855	
Cumulative number of side effects	2.7 (2.3)		2.8 (1.9)
P		0.849	
All side effects			
Number of patients	58		20
Onset week	2.7 (2.4)		3.3 (3.2)
P		0.357	
Onset dose (mg)	56.0 (37.3)		51.3 (44.0)
P		0.638	
Cumulative number of side effects	5.3 (4.5)		6.8 (7.7)
P		0.285	

[a]Analysis stratified by the two genotype groups.
CYP = CytochromeP450; NMs = normal metabolizers; LMs = lower metabolizers; HAM-D-17 = 17-item Hamilton Rating Scale for Depression.

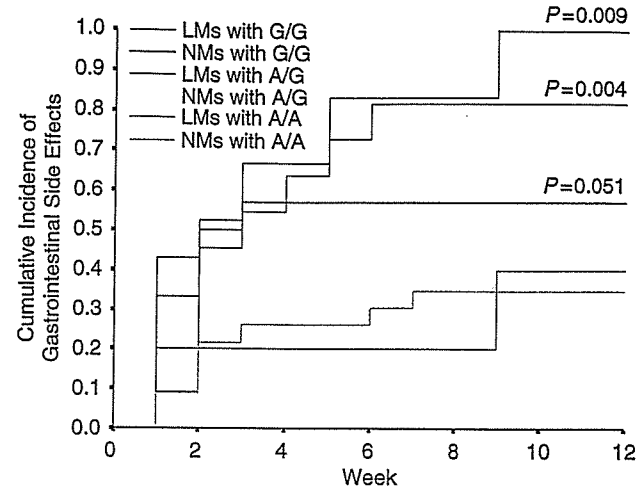

Figure 3 Combination effect of the A-1438G polymorphism of the 5-HT2A receptor gene and the CYP2D6 gene polymorphism on the cumulative 12-week incidence of gastrointestinal side effects induced by fluvoxamine.

Table 5 Combination Effect of the A-1438G Polymorphism of the 5-HT2A Receptor and the CYP2D6 Phenotype

	Hazard ratio	95% CI	P
Sex	0.65	0.368–1.146	0.136
Age	0.986	0.968–1.005	0.148
Baseline HAM-D-17 score	1.045	0.988–1.106	0.124
Combination of 5-HT2A and CYP2D6 polymorphisms			
LMs with A/A	0.859	0.179–4.122	0.849
NMs with A/G	1.681	0.717–3.939	0.232
LMs with A/G	4.147	1.558–11.038	0.004*
NMs with G/G	2.491	0.997–6.223	0.051
LMs with G/G	4.242	1.444–12.459	0.009*

*Statistically significant.
5-HT = 5-hydroxytryptamine; CYP = CytochromeP450; HAM-D-17 = 17-item Hamilton Rating Scale for Depression; NMs = normal metabolizers; LMs = lower metabolizers.

Combination Effects of 5-HT2A Receptor and CYP2D6 Gene Polymorphisms

The above results indicated that both the A-1438G polymorphism of the 5-HT2A receptor gene and the CYP2D6 gene polymorphism had significant effects on the incidence of gastrointestinal side effects. Therefore, Cox regression was used to analyze the combination effect of the two polymorphisms on the gastrointestinal side effects. Figure 3 shows the combination effect of the 5-HT2A receptor and CYP2D6 gene polymorphisms on the incidence of the gastrointestinal side effects. The Cox regression analysis showed that LMs of CYP2D6 with the G/G genotype had a 4.242-fold higher risk of developing gastrointestinal side effects ($P=0.009$) and LMs with the A/G genotype had a 4.147-fold higher risk of developing gastrointestinal side effects ($P=0.004$) than NMs with the A/A genotype (Table 5). NMs with the G/G genotype had a 2.491-fold higher risk of developing gastrointestinal side effects ($P=0.051$) than NMs with the A/A genotype (Table 5). Sex, age, and baseline HAM-D-17 scores showed no significant effects on the risk of developing gastrointestinal side effects.

DISCUSSION

In this study of a Japanese sample population, it was first demonstrated that the A-1438G polymorphism of the 5-HT2A receptor gene might predict the incidence of gastrointestinal side effects induced by fluvoxamine in depressed patients. Murphy *et al* (2003) reported that discontinuation due to paroxetine-induced side effects was

strongly associated with the C/C genotype of the 5-HT2A gene T102C polymorphism, and that there was a significant linear relationship between the number of C alleles and the probability of discontinuation. Since T102C is in complete linkage disequilibrium with the A-1438G polymorphism, the results in Murphy *et al* (2003) are generally consistent with those reported in this study. However, there are some important differences between the results in the two studies. Although the probability of discontinuation due to any adverse events, including gastrointestinal side effects, differed significantly between the genotype groups in the former study, our results showed significant differences between the genotype groups for the incidence of gastrointestinal side effects, but not for the incidence of all side effects. Furthermore, the A-1438G polymorphism had no significant effect on discontinuation in this study. Our results may indicate that the A-1438G polymorphism is only strongly related to the gastrointestinal side effects induced by fluvoxamine. While the subjects in the former study were 65 years of age or older, the mean age of the subjects in this study was 40.2 ± 15.7 years. Since elderly patients are thought to have pharmacodynamic and pharmacokinetic profiles different from those of younger patients, the difference in age may explain the discrepancy between the two studies. In addition, the difference in medication, paroxetine *vs* fluvoxamine, may cause disagreement of the results, since the two SSRIs have been reported to have different pharmacodynamic and pharmacokinetic profiles (Bourin *et al*, 2001). On the contrary, Yoshida *et al* (2003) reported that the A-1438G polymorphism of the 5-HT2A gene had no significant effect on the incidence of nausea. Their results disagree with those presented in this study. This may result from differences between the two studies in the numbers of subjects and other methodological points such as the dosage schedules and periods of observation.

A postmortem brain study found that the C allele of T102C (in complete linkage disequilibrium with the G allele of A-1438G) was associated with lower messenger ribonucleic acid (mRNA) and lower protein expression than the T allele (Polesskaya and Sokolov, 2002). Parsons *et al* (2004) reported that the presence of the A allele of A-1438G significantly increased promoter activity compared to the G allele. However, a study by Bray *et al* (2004) failed to replicate the differences in mRNA expression. Since the possible role of A-1438G in promoter function remains unclear, further studies are needed to clarify why this polymorphism affects the incidence of gastrointestinal side effects induced by fluvoxamine.

Moreover, sleep disturbances (Landolt *et al*, 1999) and sexual dysfunction (Sargent *et al*, 1998) are preferentially associated with the 5-HT2A receptor and it has been reported that SSRI-induced gastrointestinal side effects are mediated by the 5-HT3 receptor (Bergeron and Blier, 1994). To our knowledge, there have been no previous studies investigating the relationship between polymorphisms of the 5-HT3A and 3B genes and the gastrointestinal side effects induced by SSRI. In the current study, the polymorphisms of the 5-HT3A and 3B genes had no significant effects on the onset of fluvoxamine-induced gastrointestinal side effects. Tremblay *et al* (2003) reported that an insertion/deletion polymorphism in the promoter region of the 5-HT3B gene had a significant effect on the incidence of nausea and vomiting induced by cancer chemotherapy and that the Tyr129Ser polymorphism of the 5-HT3B gene, detected in this study, did not affect these side effects. On the other hand, Kaiser *et al* (2004) reported that polymorphisms of the 5-HT3A receptor gene may not serve as pharmacogenetic predictors of antiemetic treatment with 5-HT3 receptor antagonists in cancer patients. Since it is possible that polymorphisms of the 5-HT3A and 3B genes other than those detected in this study have significant effects on the gastrointestinal side effects, further studies are needed to clarify the impact of polymorphisms of the 5-HT3 gene on SSRI-induced gastrointestinal side effects.

Similar to A-1438G of the 5-HT2A receptor gene, the CYP2D6 polymorphism also showed a significant effect on the incidence of gastrointestinal side effects. Cox regression analysis showed that the combination of the A-1438G genotype and the CYP2D6 genotype could strongly predict the incidence of fluvoxamine-induced gastrointestinal side effects (Table 5). Indeed, there were six LMs of CYP2D6 who had the G/G genotype of the 5-HT2A receptor gene, and all of them suffered from gastrointestinal side effects. Among 11 LMs of CYP2D6 who had the A/G genotype, nine (81.8%) suffered from gastrointestinal side effects. In clinical situations, taking account of these results, tailor-made pharmacotherapy for fluvoxamine based on genetic factors may be possible. For example, LMs with the G/G or A/G genotype should be treated by antidepressants other than SSRIs or should be treated at lower starting doses of fluvoxamine than the other patients. Kasper *et al* (1992) reported that an increased incidence of nausea is associated with higher plasma concentrations of fluvoxamine. Since it has been shown that the plasma concentrations of fluvoxamine depend on the CYP2D6 polymorphism, our results support the preceding study. However, Murphy *et al* (2003) reported that the CYP2D6 genotype did not influence the side effects from paroxetine. Gerstenberg *et al* (2003) also reported that the number of mutated CYP2D6 alleles was not related to the development of nausea induced by fluvoxamine. CYP2D6 gene polymorphisms are known to have ethnic differences; for example, the CYP2D6*10 allele, causing decreased enzyme activity, had a higher frequency in an Asian population (51%) (Johansson *et al*, 1994) than in a white population (2.8%) (Bertilsson and Dahl, 1996). These ethnic differences in the genetic polymorphisms may produce the discrepancy between the results in Murphy *et al* (2003) and those in the present study. However, similar to this study, the subjects in the study by Gerstenberg *et al* were all Japanese patients. In the former study, the patients were divided into three genotype groups by the number of CYP2D6 mutated alleles: *1/*1, *1/*5 or *1/*10, and *5/*10 or *10/*10, whereas, in this study, the patients were divided into two genotype groups by the degree of enzyme activity: *1/*1 or *1/*10 and *10/*10, *1/*5 or *5/*10. We previously reported that one *5 allele had a greater impact on the metabolism of haloperidol, a substrate of CYP2D6, than one *10 allele (Someya *et al*, 2003). Since *1/*10 has only one mutation causing decreased enzyme activity, it was supposed that the enzyme activity of *1/*10 was almost equal to that of the *1/*1 genotype in this study. These differences in analysis may explain the inconsistency between the two studies.

It was demonstrated that a pharmacodynamic factor such as the A-1438G polymorphism of the 5-HT2A receptor gene and a pharmacokinetic factor such as the CYP2D6 gene polymorphism had synergistic effects on the prediction of the gastrointestinal side effects induced by fluvoxamine in Japanese depressed patients. However, since there have been several previous studies that were inconsistent with our results, much research remains to be carried out to explain the discrepancies.

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ORIGINAL ARTICLE

The effect of 5-hydroxytryptamine 3A and 3B receptor genes on nausea induced by paroxetine

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We investigated the effect of 5-hydroxytryptamine 3A and 3B receptor (*HTR3A* and *HTR3B*) gene polymorphisms on nausea induced by paroxetine in Japanese psychiatric patients. Blood samples were collected from 78 individuals after at least 2 weeks treatment with the same daily dose of paroxetine. The patients visited every 2 weeks and the paroxetine dose was changed in response to their clinical symptoms. Nausea was assessed at each visit. The Tyr129Ser polymorphism of the *HTR3B* gene had a significant effect on the incidence of nausea ($P=0.038$). Logistic regression analysis also showed that patients with the Tyr/Tyr genotype had a 3.95-fold ($P=0.048$) higher risk of developing nausea than patients with the Ser allele. *HTR3A* gene polymorphisms and the *CYP2D6* gene polymorphisms had no significant effect on the incidence of nausea. The mean score of nausea severity was corrected by the Bonferroni test. *HTR3B* gene polymorphisms are significant predictors of paroxetine-induced nausea.
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Keywords: HTR3A; HTR3B; paroxetine; nausea; gene polymorphism

Introduction

Nausea is a severe side effect induced by selective serotonin reuptake inhibitors (SSRIs). In general, SSRIs are better tolerated than tricyclic antidepressants, although gastrointestinal side effects can have an incidence of up to 40%, which can be severe enough to lead to early treatment discontinuation.[1,2] Recently, 5-hydroxytryptamine 3 receptors (HTR3) have been considered to have an important role in SSRI-induced gastrointestinal side effects, since HTR3 antagonists cisapride and ondansetron were reported to reduce SSRI-induced gastrointestinal side effects.[3]

HTR3 is a ligand-gated ion channel that mediates fast synaptic neurotransmission.[4] Central and peripheral HTR3 have different structures and different properties.[5] HTR3 exist in the area called the chemoreceptor trigger zone of the medulla oblongata, and are thought to be involved with the vomiting reflex.[6] HTR3 are also distributed in the autonomic, enteric and sensory nervous systems.[7] They also regulate the control pain sensation, movement of the digestive tract and vomiting by prompting nerve depolarization.[8] In particular, modification of HTR3 on the small intestinal mucosa is one of the mechanisms for regulation of antineoplastic-induced nausea and vomiting.[9] We do not have any data about the binding affinity of paroxetine for the 5-HT3 receptors. However, HTR3 antagonists such as cisapride and ondansetron were used for the therapy of nausea induced by SSRIs, and SSRIs such as fluvoxamine display a relatively high affinity for HTR2A. Thus, we considered that there might be some

relationship between nausea induced by SSRIs such as paroxetine and the function of HTR3.

Murphy *et al.*[10] report that the T102C polymorphism of the *HTR2A* gene may predict the treatment discontinuation caused by paroxetine-induced side effects in older patients with depression. *HTR3A* and *3B* genes have been assigned to chromosome 11q23.1–q23.3,[11] and several genetic variations have been reported. Tremblay *et al.*[12] report that variations in the *HTR3B* gene predict the efficacy of antiemetic treatment in cancer patients. However, no previous studies have investigated the effects of *HTR3A* and *3B* gene polymorphisms on the occurrence of SSRI-induced nausea.

Cytochrome P450 (CYP) 2D6 has been shown to be involved in the metabolism of paroxetine, and CYP2D6 is known to have genetic polymorphisms that affect enzyme activity.[13] These observations suggest that the *CYP2D6* gene polymorphism may be a predictor for paroxetine-induced side effects. On the other hand, Gerstenberg *et al.*[14] showed that steady-state plasma concentrations of fluvoxamine are not associated with incidence of nausea, and that *CYP2D6* genotype does not affect nausea development. Therefore, further studies are needed to clarify whether *CYP2D6* gene polymorphisms affect SSRI-induced side effects.

In this study, we investigated the effects of pharmacodynamic factors, such as *HTR3A* and *3B* gene polymorphisms, and the effects of pharmacokinetic factors, such as *CYP2D6* genotype, on the occurrence of paroxetine-induced nausea in Japanese psychiatric patients.

Materials and methods

Subjects

This study was conducted at Niigata University Medical Hospital, Japan, and the study protocol was approved by the Hospital Ethics Committee. Each subject provided written informed consent before enrolment. The subjects comprised 78 Japanese psychiatric outpatients (28 males, 50 females) aged 38.4±13.8 years (mean±s.d., range 18–70 years.). Thirty-nine subjects had major depressive disorder, 25 had anxiety disorders, six had adjustment disorder, seven had a depressive disorder not otherwise specified, and one had other mood disorders. All patients were diagnosed according to DSM-IV-TR. The exclusion criteria were additional diagnoses of Axis I or II of DSM-IV-TR. Demographic data, medical history and laboratory data, including hematology, serology, electrolytes and urine analysis, were collected for each patient. Patients with obvious physical illnesses were excluded from the study. All patients were orally treated with paroxetine for their psychiatric illness. No patients were being treated with antiemetic medication during our study.

Study design

The patients visited the hospital every 2 weeks and side effects, including nausea, were assessed at each visit. The paroxetine dose was increased from 10 or 20 to 30 and 40 mg/day in response to clinical symptoms. We rated the side effects during the last 2 weeks and evaluated the severity of nausea according to our original scale, which included five graded items: 0, no nausea; 1, mild nausea for less than during the last 2 weeks; 2, mild nausea for more than 1 week during the last 2 weeks; 3, continuous, moderate nausea during the last 2 weeks; 4, continuous, severe nausea and vomiting during the last 2 weeks. Subjects with a score of 0 or 1 were defined as subjects without nausea, and those with a score or 2, 3 or 4 were defined as having nausea. The side effect raters were blind to the patients' genotypes.

Blood sampling

Blood sampling was performed using a Venoject® tube containing EDTA-Na (Terumo Japan, Tokyo, Japan) at week 1 for genotype detection and subsequently at the first appearance of nausea to measure the concentration of paroxetine. Blood samples were taken at 12 h after the final ingestion of paroxetine. Venous blood, 7 ml, were collected, and genomic DNA was extracted from the peripheral leukocytes by utilizing a QIAamp Blood Kit (QIAGEN, Valencia, CA, USA) within 2 h of collection.

Genotyping and determination of plasma concentration

Polymerase chain reaction (PCR) was used to determine the C195T and Pro16Ser genotypes of *HTR3A* gene according to the method of Niesler *et al.*[15] and the Tyr129Ser genotype of *HTR3B* gene according to the method of Tremblay *et al.*[12] *CYP2D6*10* alleles causing decreased enzyme activity were identified by the C188T mutation using a two-step PCR as described by Johansson *et al.*[16] A long-PCR analysis was used to detect the **5* allele causing a lack of enzyme activity as described by Steen *et al.*[17]

The plasma concentration of paroxetine was measured using column-switching high-performance liquid chromatography (HPLC) with ultraviolet detection. Paroxetine was extracted from plasma, to which cisapride had been added as an internal standard, with hexane–chloroform, and the extract was subjected to automated column-switching HPLC using a TSK BSA-C8 precolumn (Tosoh, Tokyo, Japan) for sample clean-up, and a TSK gel ODS-80TS column (Tosoh) for separation.

Statistical analysis

Statistical analysis was performed using SPSSII for Windows. Genotype and allele distributions were analyzed by the χ^2 test. The clinical and demographic characteristics, sex, age, daily dose and paroxetine concentration were compared among groups by the unpaired *t*-test. The mean score of nausea severity was compared among each genotype group by one-way analysis of variance, and *post hoc* analysis of the mean score of nausea severity was carried out by using Bonferroni's test. Logistic regression analysis was used to compare the probability of the incidence of nausea. The level of significance was set at $P<0.05$.

Results

The genotype frequencies of the *HTR3A*, *HTR3B* and *CYP2D6* genes are shown in Tables 1–3. All of these genetic variations were in Hardy–Weinberg equilibrium. *HTR3A* C195T polymorphism genotype was not detected in four patients. There were no differences in nausea between each diagnostic groups (e.g. major depressive disorders and anxiety disorders ($P=0.12$)). Therefore, we consider that the nausea observed in the present study was induced only by paroxetine. There were also no differences in *HTR3A*, *HTR3B* and *CYP2D6* polymorphisms between major depressive disorders and anxiety disorders ($P=0.341$).

Effects of HTR3A *and* HTR3B *gene polymorphisms*

The genotype distribution of *HTR3A* and *HTR3B* gene polymorphisms are shown in Tables 1 and 2. No significant differences were demonstrated for sex, age, and paroxetine daily dose and plasma concentration among each genotype group. Association analysis revealed that genotype frequencies of *HTR3A* Pro16Ser and *HTR3B* C195T polymorphisms did not significantly differ between subjects with and without nausea (Pro16Ser genotype: $\chi^2=0.912$, $df=2$, $P=0.634$; C195T genotype: $\chi^2=2.128$, $df=2$, $P=0.546$). There was a significant difference in genotypic distribution associated with *HTR3B* Tyr129Ser polymorphism between patients with and without nausea ($\chi^2=6.547$, $df=2$,

Table 1 Genotypic distribution and demographic data of the *HTR3A* Pro16Ser and C195T

	HTR3A Pro16Ser			*HTR3A C195T*		
	Pro/Pro	*Pro/Ser*	*Ser/Ser*	*C/C*	*C/T*	*T/T*
Sex (M/F)	23/34	5/13	0/3	15/22	11/16	1/9
P		*0.261*			*0.291*	
Age (±s.d.)	37.8 (13.8)	38.2 (14.2)	52.0 (4.4)	37.8 (13.8)	38.2 (14.2)	52.0 (4.4)
P		*0.411*			*0.370*	
Daily dose of PRX (±s.d.)	23.0 (10.5)	20.0 (9.7)	20.0 (17.3)	20.5 (9.7)	25.9 (10.5)	19.0 (12.9)
P		*0.408*			*0.205*	
Concentration of PRX (±s.d.)	66.4 (89.3)	28.9 (31.3)	22.3 (29.8)	42.8 (61.1)	65.8 (90.6)	52.9 (80.6)
P		*0.167*			*0.707*	
Nausea (+) ($n=15$)	12	3	0	9	5	1
%	(21.1%)	(16.7%)	(0.0%)	(24.3%)	(18.5%)	(10.0%)
Nausea (−) ($n=63$)	45	15	3	28	22	9
%	(78.9%)	(83.3%)	(100.0%)	(75.7%)	(81.5%)	(90.0%)
P		*0.634*			*0.546*	
Mean score of severity of nausea	0.21 (0.411)	0.17 (0.383)	0 (0)	0.1 (0.316)	0.19(0.396)	0.24(0.435)
P		*0.643*			*0.593*	

Table 2 Genotypic distribution and demographic data of the *HTR3B* Tyr129Ser

	HTR3B Tyr129Ser			
	Tyr/Tyr	*Tyr/Ser*	*Ser/Ser*	*Tyr/Ser+Ser/Ser*
Sex (M/F)	16/19	10/26	2/5	12/31
P		*0.264*		*0.103*
Age (±s.d.)	36.3 (11.5)	38.9 (15.1)	46.1 (16.6)	40.1 (15.4)
P		*0.099*		*0.267*
Daily dose of PRX (±s.d.)	21.4 (10.6)	21.9 (10.6)	27.1 (9.5)	22.8 (10.5)
P		*0.4*		*0.939*
Concentration of PRX (±s.d.)	37.6 (46.9)	66.4 (100.2)	95.0 (77.2)	71.1 (96.6)
P		*0.124*		*0.064*
Nausea (+) ($n=15$)	11	4	0	4
(%)	(31.4%)	(11.1%)	(0.0%)	(9.3%)
Nausea (−) ($n=63$)	24	32	7	39
(%)	(68.6%)	(88.9%)	(100.0%)	(90.7%)
P		*0.038**		*0.014**
Mean score of severity of nausea	0.54 (0.919)	0.14 (0.487)	0 (0)	0.12 (0.448)
P		*0.030***		*0.009***

*$P<0.05$, significant difference from patients with nausea (χ^2 text).
**$P<0.05$, significant difference among the three genotypes (one-way analysis of variance).

Table 3 Genotypic distribution and demographic data of *CYP2D6* genotype groups

	CYP2D6		
	**1/*1*	**1/*5, *1/10*	**5/*10, *10/*10*
Sex (M/F)	17/34	5/7	6/9
P		*0.807*	
Age (±s.d.)	38.6 (14.9)	39.7 (10.7)	36.7 (12.9)
P		*0.126*	
Daily dose of PRX (±s.d.)	21.4 (10.2)	24.2 (11.7)	23.3 (11.1)
P		*0.925*	
Concentration of PRX (±s.d.)	50.7 (76.3)	89.5 (114.4)	47.5 (51.2)
P		*0.287*	
Nausea (+) ($n=15$)	9	2	4
(%)	(17.6)	(16.7)	(26.7)
Nausea (−) ($n=63$)	42	10	11
(%)	(82.4)	(83.3)	(73.3)
P		*0.716*	
Mean score of severity of nausea	0.18 (0.385)	0.17 (0.389)	0.27 (0.458)
P		*0.725*	

Table 4 Logistic regression analysis of independent valiables to Nausea

Independent valiable	*Partial regression coefficients*	P	*Odd ratio (95% confidence interval)*
Sex	−0.040	0.111	1.115 (0.518–3.225)
Age	−0.793	0.041	1.200 (0.810–1.110)
Daily dose of Paroxetine	−0.538	0.320	0.447 (0.112–3.154)
HTR3B Tyr129Ser genotype	−0.148	0.048*	3.950 (1.009–15.455)

*$P<0.05$, statistical significance.

$P=0.038$). The proportion of Ser allele carriers (i.e., patients with either Tyr/Ser or Ser/Ser) was significantly higher in the group without nausea ($\chi^2=6.082$, $df=1$, $P=0.014$). There were significant differences in the severity score of nausea among the three genotypes (score: 0.54±0.91, 0.14±0.49 and 0±0, $df=2$, $P=0.03$).

The results of logistic regression analysis are shown in Table 4. The incidence with or without nausea was used in the analysis as an independent variable, and sex, age, daily dose of paroxetine and the genotypes of *HTR3B* Tyr129Ser were added as potential confounders. This analysis also showed that there was a significant association between nausea and *HTR3B* Tyr129Ser genotype. ($P=0.048$; $OR=3.95$; 95% $CI=1.009$–15.455).

Effect of CYP2D6 gene polymorphism

Five CYP2D6 genotypes were identified: **1/*1* ($n=51$), **1/*5* ($n=1$), **1/*10* ($n=11$), **5/*10* ($n=3$) and **10/*10* ($n=12$). The allele frequencies of the **5* and **10* alleles were 2.6 and 24.4%, respectively. Patients were divided into three genotype groups according to the number of mutated alleles: 51 patients with the **1/*1* genotype, 12 with the **1/*10* and **1/*5* genotypes and 15 with the **5/*10* and **10/*10* genotypes. No significant differences were demonstrated for sex, age, and paroxetine daily dose and plasma concentration between those three genotype groups (Table 3).

There were no significant differences in the incidence of nausea between the three genotype groups ($\chi^2=1.029$, $df=2$, $P=0.716$). We also divided patients into two genotype groups: 61 patients with the **1/*1* or **1/*10* genotype were termed normal metabolizers, and 17 patients with the **10/*10*, **1/*5* or **5/*10* genotype were termed low metabolizers. However, there were also no significant differences in the incidence of nausea between the two genotype groups.

Discussion

We screened for two polymorphisms in the *HTR3A* gene and one variant in the *HTR3B* gene as a pharmacodynamic factor, and *CYP2D6* gene polymorphisms (**1*, **5*, **10* alleles) as a pharmacokinetic factor. To our knowledge, the present study is the first demonstration that the *HTR3B* gene may predict the incidence of paroxetine-induced nausea in Japanese psychiatric patients.

Kaiser *et al.*[18] reported that polymorphisms of the *HTR3A* gene may not serve as pharmacogenetic predictors of antiemetic treatment with HTR3 antagonists in cancer

patients. We also found no relationship between *HTR3A* gene polymorphism and paroxetine-induced nausea. Tremblay *et al.*[12] reported that the Tyr129Ser polymorphism of the *HTR3B* gene did not alter the incidence of nausea and vomiting. However, in the present study, there was a significant relationship between the Tyr129Ser polymorphism of the *HTR3B* gene and paroxetine-induced nausea. On the other hand, Tremblay *et al.*[12] also reported that an insertion/deletion polymorphism in the promoter region of the *HTR3B* gene had a significant effect on the incidence of nausea and vomiting induced by cancer chemotherapy, although we did not examine this insertion/deletion polymorphism. This discrepancy may occur because of the difference in medication, that is, cancer chemotherapy versus paroxetine. We cannot account for this discrepancy between the previous and the present study because the function of the *HTR3B* gene polymorphism still remains unclear. To date, there are no *in vitro* data about the functional effects of the Tyr129Ser polymorphism in *HTR3B* gene. With regard to the functional effects of the other polymorphism in *HTR3B* gene, Cazzola *et al.*[19] report that a 6-bp deletion in the 5′UTR of L-ferritin mRNA is a cause of hereditary hyperferritinanemia-cataract syndrome, and Frank *et al.*[20] report that the deletion *−100_−102delAAG polymorphism may change the structure of mRNA compared to the wild type. However, the Tyr129Ser polymorphism of *HTR3B* gene had an amino-acid substitution in the coding region, and it was possible that the Tyr129Ser polymorphism of *HTR3B* gene affected the expression level of the B subunit either by itself, or because of linkage disequilibrium with other yet unknown functional variants, and this polymorphism of *HTR3B* gene may affect the occurrence of nausea by itself, or indirectly. Meanwhile, Murphy *et al.* [10] reported that the T102C polymorphism of the *HTR2A* gene could predict the treatment discontinuation caused by paroxetine-induced side effects in older patients with depression. In future studies, we should also examine the relationship between *HTR2A* gene polymorphism and paroxetine-induced nausea.

In our study, the *CYP2D6* gene polymorphism had no significant effect on the incidence of paroxetine-induced nausea. Murphy *et al.*[10] reported that the *CYP2D6* genotype did not influence side effects of paroxetine. Gerstenberg *et al.*[14] also reported that the number of *CYP2D6* mutated alleles was not related to the development of fluvoxamine-induced nausea. Our present results support these previous findings.

Kasper *et al.*[1] reported that there was a relationship between plasma concentration of fluvoxamine and incidence of nausea. Sawamura *et al.*[21] showed that plasma paroxetine concentration in patients with *CYP2D6*10* alleles was significantly higher than those without **10* alleles, and plasma paroxetine concentration in patients with **5* alleles also showed a tendency to be higher than those without **5* alleles. Therefore, we hypothesized that the *CYP2D6* gene polymorphism had a significant influence on paroxetine-induced nausea, but it did not affect paroxetine-induced nausea in the present study. Sindrup *et al.*[22] reported that the effect of CYP2D6 on metabolism was less prominent at steady-state than after a single dose of paroxetine, since CYP2D6 enzymatic activity seems to be easily saturated upon increasing paroxetine dose. This may be one of the reasons that there was no effect on paroxetine-induced nausea. Furthermore, the groups with two mutated alleles were expected to have the highest concentration among these three genotype groups, but the concentration in groups with two mutated alleles was lower than in groups with one mutated allele, and was the same as in groups with no mutated allele (Table 3). This result suggests that there are patients who had noncompliance with paroxetine in the group with **5/*10* and **10/*10* alleles, since they discontinued because of various adverse events other than nausea, as a result of a great increase in plasma paroxetine concentration.

We demonstrated that a pharmacodynamic factor such as the Tyr129Ser polymorphism of the *HTR3B* gene may be a predictor of paroxetine-induced nausea in Japanese psychiatric patients. Taking account of these findings, in clinical situations, it may be possible to tailor paroxetine pharmacotherapy based on genetic factors. However, since the results of some previous studies are not consistent with our current results, further study is needed to clarify these discrepancies.

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Duality of Interest

None declared

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Gender differences in prolactin elevation induced by olanzapine in Japanese drug-naïve schizophrenic patients

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Abstract

We investigated the effect of gender on plasma prolactin levels in 20 Japanese drug-naïve schizophrenic patients [10 male, 10 female, aged 25.4±10.3 (mean±S.D.), range=12–46 years] treated with olanzapine. Plasma prolactin levels were measured at baseline, and weeks 3 and 8 after starting titration of olanzapine. Comparisons of plasma prolactin levels between baseline and week 3, and between baseline and week 8 were made by repeated analysis of variance (ANOVA) and paired *t*-test. Two-way ANOVA showed a significant difference in olanzapine-induced prolactin changes between male and female patients ($P=0.037$). In male patients ($n=10$), the plasma concentration of prolactin at week 3 was significantly higher than at baseline ($P=0.016$), but there was no significant difference between the plasma concentration of prolactin at week 8 and at baseline or week 3 ($P=0.191$). In female patients ($n=10$), there was a significant change of prolactin between baseline and week 3 ($P=0.005$), and between baseline and week 8 ($P=0.047$). Our results indicate the possibility of gender differences in prolactin elevation induced by olanzapine in Japanese drug-naïve schizophrenic patients. These gender-based findings may be helpful for clinicians when deciding the frequency of follow-up visits once a patient starts olanzapine therapy.

Keywords: Antipsychotics; Gender difference; Olanzapine; Plasma prolactin level; Schizophrenia; Side effects

1. Introduction

Olanzapine is an antipsychotic agent that is widely used in Japan as well as Europe and America for treatment of schizophrenia and related illnesses. This drug causes not only glycolipid metabolism dysfunction and weight gain but also elevation of prolactin, which is one of the most common adverse effects induced by olanzapine in patients with schizophrenia (Chakos et al., 2001).

Prolactin is a polypeptide hormone essential for lactation and its production in the lactotroph cells of the anterior pituitary is regulated primarily by the inhibitory action of hypothalamic dopamine (Luciano, 1999). Hyperprolactinemia in women can result in galactorrhea, amenorrhea, irregular menses and anovulation, and in men, impotence and azoospermia, with or without lactation and gynecomastia (Marken et al., 1992). Antipsychotics may block dopamine receptors in the pituitary prolactin-secreting cells and prevent dopamine-induced reduction of prolactin release (Marken et al., 1992). Each antipsychotic has a different prolactin response. Risperidone has the highest propensity to elevate plasma prolactin levels (Kleinberg et al., 1999). Mean prolactin levels at a dose of 3 mg risperidone (27 ng/ml) are significantly higher than those of olanzapine or clozapine (Turrone et al., 2002). Elevation of prolactin induced by olanzapine is regarded as transient and mild compared to that induced by other antipsychotics, such as haloperidol and risperidone, which cause acute and persistent elevation of prolactin (Kinon et al., 2003b). It is also reported that olanzapine is not associated with persistent elevations of prolactin (Crawford et al., 1997).

In positron emission tomography (PET) studies, the degree of dopamine D_2 receptor (DRD2) occupancy predicts clinical improvement and hyperprolactinemia (Kapur et al., 2000). However, some reports have shown that olanzapine has a similar occupancy value to risperidone, while others suggest that dopamine DRD2 occupancy of olanzapine is lower than

Abbreviations: ANOVA, analysis of variance; BPRS, Brief Psychiatric Rating Scale; DRD2, dopamine D_2 receptor; DSM, Diagnostic and Statistical Manual of Mental Disorders; PET, positron emission tomography.

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that of risperidone (Pilowsky et al., 1996; Kapur et al., 1999). It is not clear whether the difference in DRD2 occupancy of antipsychotics can directly predict the degree of prolactin elevation.

The prolactin response to neuroleptic medication is greater in females than in males (Wode-Helgodt et al., 1977; Kuruvilla et al., 1992). Kinon et al. (2003a) reported that women had 2.6 times greater odds of developing hyperprolactinemia than men in patients being treated with risperidone. Crawford et al. (1997) showed that women treated with olanzapine might have a higher elevation of prolactin than men during the acute phase of treatment. However, there are few reports referring to gender differences in prolactin elevation induced by olanzapine in drug-naïve schizophrenic patients. Further, it has been shown that Caucasians have lower serum haloperidol concentrations and less prominent prolactin responses than Asians (Lin et al., 1988). Kinon et al. (2003a) suggested that non-Caucasian women have a higher prevalence of antipsychotic-induced hyperprolactinemia than Caucasian women. Therefore, we investigated the gender differences in prolactin elevation induced by olanzapine in drug-naïve Japanese schizophrenic patients.

2. Methods

2.1. Patients

This study was approved by the gene ethics committee of Niigata University School of Medicine, Japan. The subjects received an explanation of the study objectives and only those who gave written consent to participate were enrolled. There were 10 male [mean age (mean±S.D.)=24.9±11.5 years, range=15–46 years] and 10 female (mean age=25.6± 10.6 years, range=12–42 years) patients. Overall age was 24.5±9.4 years (range=12–46 years). All met DSM-IV criteria for schizophrenia (including schizophreniform disorder) with acute exacerbation, while those who were diagnosed with other DSM-IV Axis I and II disorders were excluded. All subjects were first-episode and drug-naïve schizophrenic patients. Also, all female subjects were premenopausal.

2.2. Dosing regimen

All subjects received 5 or 10 mg/day olanzapine as the starting dose, which was administered at 21:00 h, and visited the hospital every week after the first examination until week 8 (endpoint). The daily dosage of olanzapine was based on the clinical judgment of the investigator. Only some benzodiazepines were allowed as concomitant drugs. Patients with obvious physical illness, such as liver dysfunction or renal failure, were excluded.

2.3. Clinical assessments

The Brief Psychiatric Rating Scale (BPRS) was evaluated at baseline (week 0), week 3 and week 8. Since plasma prolactin levels are associated with sleep and meals (Franz, 1978; Molitch, 1995), fasting blood samples were collected after >4 h had elapsed after each patient had awoken, at baseline, week 3 and week 8. In females, blood sampling was not performed during menstruation, because prolactin levels are higher during mid-cycle and the second half of the menstrual cycle (Haddad and Wieck, 2004). Serum prolactin levels were assayed by enzyme immunoassay in the laboratory section of Niigata University Medical and Dental Hospital, Japan (normal range: male, 2.9–12.9 ng/ml; female, 2.7–28.8 ng/ml).

2.4. Statistical analysis

Statistical analysis was conducted using SPSS II for Windows. Analysis of variance (ANOVA) for repeated measures was used for comparison of BPRS total score response patterns at baseline, week 3 and week 8. Two-way ANOVA was used to analyze the gender difference in prolactin elevation at baseline, week 3 and week 8. Additionally, comparisons of plasma prolactin levels between baseline and week 3, between baseline and week 8, and between week 3 and week 8 were performed by the paired *t*-test. Significance was set at <0.05.

3. Results

3.1. Comparisons of demographic data between male and female patients

Table 1 shows the demographic data of the patients. All 20 patients completed this protocol. There was no significant difference in daily olanzapine dosage between male and female patients. There was no significant difference in mean age between male and female patients. Repeated ANOVA showed significant difference in BPRS total score at week 8 compared with baseline ($P<0.0001$). A significant difference in prolactin elevation was observed between baseline and week 3 or week 8 ($P=0.003$).

The dosage of olanzapine at week 8 was not significantly higher than at week 3 in both male ($P=0.082$) and female subjects ($P=0.351$).

3.2. Gender differences in prolactin changes

Two-way ANOVA showed a significant difference in olanzapine-induced prolactin changes between male and female patients ($F=5.104$, $P=0.037$, $df=1$). Furthermore, in

Table 1
Patient demographic data

	Baseline	Week 3	Week 8 (endpoint)
Total ($n=20$)			
Age 25.4±10.3 (12–46) (years)			
Olanzapine dose (mg/day)	0	8.8±2.2	9.5±1.5
Plasma prolactin level (ng/ml)	8.2±4.1	18.5±10.9	17.2±15.9
Males ($n=10$)			
Olanzapine dose (mg/day)	0	8.5±2.4	9.5±1.6
Plasma prolactin level (ng/ml)	7.7±2.4	12.9±5.0	11.1±7.5
Females ($n=10$)			
Olanzapine dose (mg/day)	0	9.0±2.1	9.5±1.6
Plasma prolactin level (ng/ml)	8.7±5.4	24.1±12.4	23.2±19.9

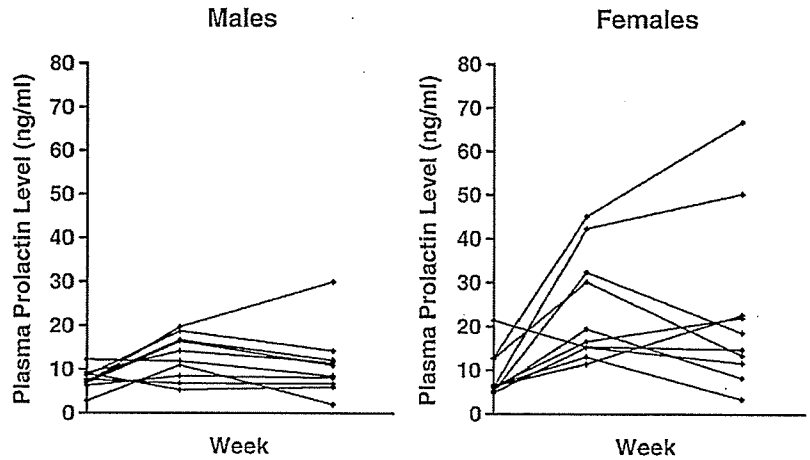

Fig. 1. Gender differences in prolactin elevation induced by olanzapine. Upper limits of plasma prolactin levels in male and female patients.

comparing the percentage coefficient of variation on plasma prolactin level at week 8 between male and female patients, the coefficient in female subjects (85.8%) was higher than in male (67.6%). Therefore, we suggest that the variability in plasma prolactin level during the 8 weeks might be, in part, attributable to female gender. In the 10 male patients, while the plasma concentration of prolactin at week 3 was significantly higher than at baseline ($t=-2.955$, $P=0.016$, $df=9$), there was no significant difference between the plasma concentration of prolactin at week 8 and at baseline or week 3 ($t=-1.414$, $P=0.191$, $df=9$) (Fig. 1). In the 10 female patients, there was a significant difference in prolactin between baseline and week 3 ($t=-3.705$, $P=0.005$, $df=9$) and between baseline and week 8 ($t=-2.298$, $P=0.047$, $df=9$) (Fig. 1).

4. Discussion

Our results show female patients had remarkable prolactin elevation after only 8 weeks of olanzapine treatment. It is important that clinicians should not overlook such patients, and because prolactin levels may elevate above upper normal limit at week 8, it is important not to prematurely conclude a normal range of prolactin level at week 4 in female patients during olanzapine therapy. Prolactin elevation, even if transient, may cause amenorrhea and discontinuation olanzapine therapy; therefore, we suggest that short-term monitoring of prolactin levels is particularly needed in female patients. Furthermore, there was a greater prolactin elevation, less adaptation to prolactin elevation, and a greater degree of unpredictability in prolactin concentrations in females at week 8. Thus, it is reasonable to propose that female patients be considered for more intensive therapeutic drug monitoring (TDM) measures during olanzapine therapy, compared to male patients with schizophrenia. These gender-based findings may assist clinicians in deciding the frequency of follow-up visits once a patient starts olanzapine therapy.

It is suggested that gender differences in prolactin elevation may exist during the acute phase of olanzapine therapy in drug-naïve schizophrenic patients. Olanzapine-induced prolactin elevation in women may be more persistent than in men. Volavka et al. (2004) reported that antipsychotics show major differences in their effects on prolactin, and that risperidone has clearly the most robust effect. Crawford et al. (1997) reported that olanzapine is not associated with persistent elevation of prolactin. However, in the acute phase of their study, it appeared that the olanzapine-induced prolactin changes were more persistent in female patients. Thus, our results agree with their study, in that the gender difference in prolactin elevation was seen in the acute phase of olanzapine treatment. In the present study, two female subjects, in particular, had marked increases in prolactin between weeks 3 and 8. However, they were treated with olanzapine 10 mg/day for 8 weeks. We believe that a significant degree of the variability of prolactin changes in the female subjects may not be related to the difference in olanzapine dosage. In a PET study, Pohjalainen et al. (1998) investigated gender differences in the striatal DRD2 binding characteristics in 33 healthy men and 21 healthy women and showed that women had lower DRD2 affinity than men. There may be a gender difference in the sensitivity to atypical antipsychotics in hypothalamic–pituitary prolactin regulation, and hypothalamic control of prolactin secretion may differ in male and female subjects.

Other factors may also play an important part in the prolactin response in female patients. Wudarsky et al. (1999) compared olanzapine-induced prolactin elevation in adolescent (mean age=14.1±2.3, range=9.1–19 years) and adult schizophrenic patients. They reported that adolescent patients may be more sensitive to the prolactin-elevating effects of olanzapine. Some studies suggest that there are age-related decreases in dopamine levels and uptake (Adolfsson et al., 1979; van Dyck et al., 1995). However, the relationship between aging and plasma prolactin level has not been clarified. Olanzapine levels were not measured in our study. It is possible that the differences in prolactin levels between male and female subjects were due to greater plasma olanzapine levels in female subjects despite the lack of any difference in oral dosage. In our study, the prolactin response may have been more sensitive than previous reports because our subjects were drug-naïve, first-episode,

schizophrenic patients. Therefore, the gender difference may have been more clearly detected.

It has been shown that there is a significant ethnic difference in the prevalence of antipsychotic-induced hyperprolactinemia (Kinon et al., 2003a). We investigated the prevalence of olanzapine-induced hyperprolactinemia. In the present study, the frequency of prolactin elevation was 45% (9/20; five male and four female subjects). This frequency was similar to that (~50%) found by Kinon et al. (2003a). The impact of olanzapine on prolactin elevation may be smaller than that of risperidone and other antipsychotics. Therefore, any interethnic difference in prolactin elevation induced by olanzapine may not be detected.

In this study, there was no significant difference of olanzapine dosage between male and female patients. Thus, it may be reasonable to postulate the likelihood of pharmacodynamic tolerance in male subjects because prolactin elevation was less than in females. After starting olanzapine therapy, male patients with prolactin elevation within a month may have normalized prolactin levels within 2 months, and clinicians should not modify the medication immediately when they notice hyperprolactinemia in male patients. Female patients with high plasma prolactin levels within a month may have still higher elevation afterward, and clinicians should carefully monitor their prolactin levels for >2 months. Thus, for both male and female patients, olanzapine-induced prolactin changes should be monitored for >8 weeks.

5. Conclusion

We are further investigating by follow-up study after 8 weeks of olanzapine therapy. As there are few reports of gender differences in prolactin changes in the long-term phase of olanzapine treatment, further studies are needed to clarify the extent of olanzapine-induced prolactin changes.

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REVIEW

Could endogenous substrates of drug-metabolizing enzymes influence constitutive physiology and drug target responsiveness?

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Integration of genomic data from pharmacokinetic pathways and drug targets is an emerging trend in bioinformatics, but is there a clear separation of pharmacokinetic pathways and drug targets? Should we also consider the potential interactions of endogenous substrates of drug-metabolizing enzymes with receptors and other molecular drug targets as we combine pharmacogenomic datasets? We discuss these overarching questions through a specific pharmacogenomic case study of the cytochrome P450 (CYP)2D6, serotonin and dopamine triad. Importantly, CYP2D6 may contribute to the regeneration of serotonin from 5-methoxytryptamine by virtue of its catalytic function as a 5-methoxyindolethylamine O-demethylase. Moreover, serotonergic neurons provide a regulatory feedback on dopaminergic neurotransmission. Hence, we hypothesize that independent of its role as a pharmacokinetic gene, *CYP2D6* may nuance the regulation of serotonergic and dopaminergic neurophysiology. Additionally, we reflect upon the contribution of hyperspecialization in biomedicine to the present disconnect between research on pharmacokinetics and drug targets, and the potential for remedying this important gap through informed dialogue among clinical pharmacologists, human geneticists, bioethicists and applied social scientists.

Keywords: 5-methoxytryptamine, CYP2D6, dopamine, drug targets, endogenous substrates, hyperspecialization, interdisciplinary studies, pharmacokinetics, serotonin

future medicine

"We are not students of some subject matter, but students of problems. And problems may cut right across the borders of any subject matter or discipline." Karl R Popper, philosopher of science [1].

Integration of pharmacogenomic data: context & rationale

Pharmacogenetics is traditionally defined as the study of the genetic contribution to interindividual and population-to-population variability in drug efficacy and safety [2,3]. Interest in pharmacogenetics has its origins in the 1950s with single gene analyses of drug-metabolizing enzymes [4]. In recent years, these early studies have been criticized for neglecting the contributions of other genes located in the biological pathway under investigation and the interactions of these genes with the gene of interest. Furthermore, such single-gene studies do not take into consideration the fact that pharmacological variability involves complex gene–environment interactions [5]. Hence, there has been a major expansion in the scope of inquiry in pharmacogenetics research, in large part facilitated by high-throughput genomic technologies that became available during and subsequent to the Human Genome Project [6,7]. It is increasingly recognized in pharmacological research that a comprehensive understanding of drug response necessitates multigene studies that integrate environmental and social factors such as diet, gender, age and socio-economic status [8]. With the advent of large population databases such as the UK Biobank, the Estonian Genome Project, GenomEUtwin, or CARTaGENE, it is now becoming feasible to conduct genome-wide studies that investigate the role and interaction of a diversity of genomic, social and environmental factors in drug response [9–11].

Recognition of the need for a broader scope of analysis has thus shifted attention from pharmacogenetics to pharmacogenomics, a term first introduced into the research literature in 1997 in an editorial in *Nature Biotechnology* [3,12]. Although there is still no consensus on the distinction between these two terms, most researchers now agree that pharmacogenomics employs a genome-wide survey of human genetic variation in relation to drug treatment outcomes. The pharmacogenomic approach creates a need, however, for studies involving large sample sizes to attain sufficient statistical power. In order to balance such competing concerns about the scope of molecular genetic analysis and the feasibility of recruiting a reasonable number of subjects in clinical pharmacogenomic studies, a 'candidate pathway' strategy has been advocated [13]. In this approach, all or most genes positioned on a biological pathway are included

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in genotype–phenotype association analyses [13,14], remedying concerns about sufficient inclusiveness of candidate genes in the survey, while also enabling the studies to have adequate sample size and statistical power.

An emerging theme is the need for the integration of genomic data from multiple candidate genes on pharmaceutically relevant biological pathways in order to obtain a biomarker signature to individualize drug therapy [15]. The integration of different pharmacogenomic datasets is traditionally framed around candidate genes in pharmacokinetic pathways, such as drug metabolism and transport, and molecular drug targets, such as receptors (Figure 1) [14,16]. Although application of genetic variation data to a joint study of pharmacokinetics and drug targets may explain a greater portion of variability in drug effects, thus far there has been little conceptual work on exactly how this integration should be implemented. In particular, two overarching questions remain unanswered:

- If it makes sense to combine genetic variation in pharmacokinetics and drug targets, is this simply a matter of a linear addition of these two elements; that is, can we ignore interactions between them?
- Is there a clear separation between pharmacokinetic and drug target variation? Specifically, could endogenous substrates of drug-metabolizing enzymes influence constitutive physiology and drug target responsiveness?

These questions were raised and discussed in a workshop at the Annual Meeting of the Pacific Rim Association for Clinical Pharmacogenetics and the International Conference on Pharmacogenetics held in Changsha, China, in June 2006, as a satellite symposium for the 15th World Congress of Pharmacology organized by the International Union of Basic and Clinical Pharmacology [101].

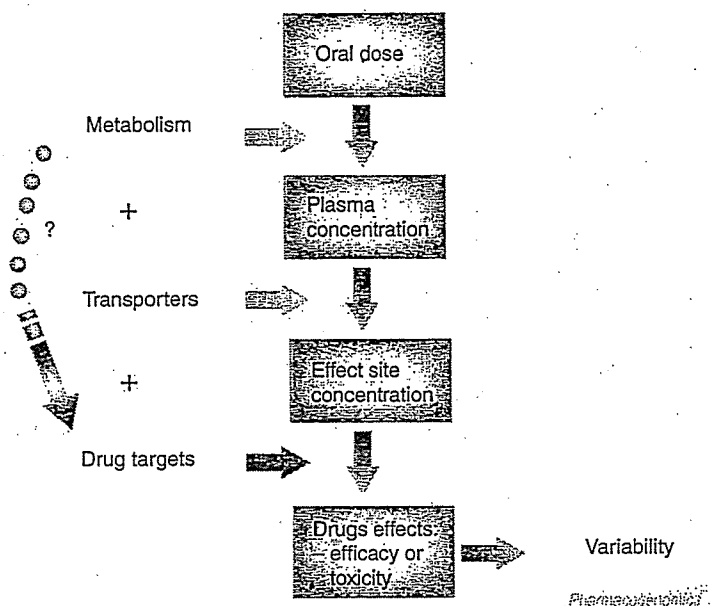
Here, we discuss these important overarching issues through a specific pharmacogenomic case study of cytochrome P450 (CYP)2D6, its putative endogenous substrate, 5-methoxytryptamine (5MT), and their potential interactions with serotonin and dopamine neurotransmitter systems. Additionally, we describe the process of fragmentation of knowledge space through disciplinary hyperspecialization and reflect on how this may impede the essential interdisciplinary collaboration and synthesis required to fully explain pharmacogenomic variability in pharmacokinetics and drug targets.

CYP2D6 genetic variation & neuroactive endogenous substrates

It is a widely held notion that drug-metabolizing enzymes (e.g., CYPs) evolved as a result of plant–animal warfare to detoxify a broad array of foreign chemicals (xenobiotics) [16]. Although the history of modern drug development only dates back some 100 years, drug-metabolizing enzymes have been in existence very much longer, potentially in archea and bacteria that existed over 2 billion years ago [16,17]. The CYP superfamily of enzymes, therefore, serves multiple functions beyond drug metabolism such as contributing to the synthesis and disposition of endogenous substrates (e.g., fatty acids and eicosanoids) essential for normal physiology [16,18]. From a conceptual standpoint, it should not be surprising that some CYPs may contribute to individual differences in constitutive physiological function as well as to drug effects on biological systems that are subject to regulation by endogenous substrates of drug-metabolizing enzymes [19].

Since the discovery of the *CYP2D6* genetic polymorphism in the 1970s by Robert Smith in London, UK [20] and Michel Eichelbaum in Bonn, Germany [21], there has been much speculation about the presence of endogenous substrates for CYP2D6 [22]. An intriguing observation of personality differences between extensive (EMs) and poor metabolizers (PMs) of CYP2D6 provided the first line of clinical evidence [23]. In a Swedish sample of healthy subjects, Bertilsson and colleagues found that PMs of CYP2D6 displayed a tendency for ease in decision-making, preference for extreme responses and lack of hesitation, traits that are collectively associated with a tendency for impulsivity [23]. A second replication study conducted in a Spanish healthy volunteer sample showed that PMs are more anxiety-prone than EMs [24]. To this end, it is interesting to note that both anxiety and impulsivity are behavioral characteristics that are in part attributed to a diminished serotonergic neurochemical tone [25,26]. Such differences in behavioral traits between CYP2D6 EMs and PMs are subtle in nature, and they do not necessarily indicate a clinical pathology or marked deviation from the normal range of human behavior. More recent investigations in other populations of healthy volunteers or patients had mixed results with some [27,28], but not all [29,30], studies suggesting a personality difference between EMs and PMs. Clearly, human personality is a complex phenotype that is

Figure 1. The pharmacological cascade of biological events starting from administration of an oral dose to clinical manifestation of drug efficacy and toxicity.



The biological elements that can mediate interindividual variability in drug efficacy and safety include pharmacokinetic pathways (e.g., drug metabolism and transport) and molecular targets for drugs. We note that intestinal drug transporters may also influence plasma drug concentrations. A key question, however, is whether genetic variation in these biological variables is simply additive in nature, or do the variables interact with each other?

subject to genetic, environmental and socio-cultural influences; it is not an easy task to discern the attendant role of a single genetic factor such as *CYP2D6*. Nonetheless, the observation of a 'hyposerotonergic cluster' of personality traits in Swedish and Spanish subjects remains an intriguing research finding [23,24].

Were it merely based on the observations of personality differences noted above in some (if not all) human populations, the suggested presence of an endogenous neuroactive substrate for CYP2D6 would have only been an interesting hypothesis. However, both preclinical and *in vitro* studies in human brain samples have subsequently shown that *CYP2D6* is expressed in the brain [31–34]. Importantly, CYP2D6 in the human brain appears to have a neuronal origin [32,33]. To clarify, *CYP2D6* messenger RNA displays a broad distribution in the human brain including pigmented cells of the substantia nigra, basal ganglia, cortex, hippocampus and cerebellum [32]. Furthermore, CYP2D6 protein is detected in the large principal neurons in the cortex, hippocampus and cerebellum, although more detailed investigations at specific brain regions are required to confirm these findings [32]. It is conceivable that trace amounts of CYP2D6 protein in neurons, at concentrations far beyond the level of quantification in post-mortem brain samples, may be clinically important under *in vivo* conditions. This may in part be due to spatial factors, that is, the presence of CYP2D6 at the site of psychotropic drug action in the brain and immediate physical proximity to neurophysiological pathways.

Trace amine receptors are a novel family of G-protein-coupled-receptors (GPCRs) that are distinct from the classical biogenic amine neurotransmitter receptors where serotonin, dopamine and norepinephrine serve as endogenous ligands [35]. Tryptamine, a trace amine that occurs in the brain, was initially postulated to be deaminated by CYP2D6 [36]. However, subsequent *in vitro* studies have shown that deamination of tryptamine is mediated by monoamine oxidase-A and aldehyde reductase instead of CYP2D6 [37]. Other *in vitro* studies found that CYP2D6 is responsible for the hydroxylation of tyramine to dopamine [38]. The latter reaction displayed a high Michaelis-Menten constant (K_M > 50 µM), hence it has been suggested that tyramine is unlikely to be a physiologically significant endogenous substrate for CYP2D6 [22].

Employing a battery of *in vitro* strategies, ranging from recombinant *CYP2D6*, hepatic microsomes from *CYP2D6*-transgenic mice, human liver microsomes, and a specific monoclonal antibody directed at CYP2D6, Yu and colleagues screened for endogenous substrates of CYP2D6 [39]. Interestingly, they found that CYP2D6 contributes to regeneration of serotonin from 5MT, another endogenous trace amine, as a highly specific, high-affinity, high-capacity 5-methoxyindolethylamine *O*-demethylase [39,40].

5MT is thought to be derived physiologically from two sources, namely, by deacetylation of melatonin by arylacylamidase [41] and by methylation of serotonin (5-hydroxytryptamine) via hydroxyindole *O*-methyltransferase [42]. It remains to be determined what proportion of the physiological pool of serotonin in synaptic nerve terminals or within serotonergic synaptic clefts is contributed by CYP2D6-mediated pathways from melatonin and 5MT. Nonetheless, the observation by Yu and colleagues provides a mechanistic basis for involvement of CYP2D6 in regeneration of serotonin from 5MT, and by extension, in serotonergic physiology and neuropsychiatric disorders [40].

5MT is detected in the pineal gland and the serotonergic raphe nuclei in the rat, but what is not clear are the patterns of colocalization (both spatially and temporally) of CYP2D6 and 5MT in various brain regions. The latter is essential for a deeper understanding of biological significance of the link between CYP2D6 and 5MT. In humans, 5MT is present in the pineal gland only in trace amounts (up to 12 pmol/g) [43]. It is conceivable that even small amounts of 5MT- or melatonin-derived serotonin may importantly supplement the endogenous serotonin pool; this mechanism may gain significance in the event of an already existing pharmacological challenge (e.g., administration of a serotonin receptor antagonist) or a pathophysiological state that causes a reduction in physiological serotonin stores (see also the Outlook and the Expert commentary sections for future projections along this line of research inquiry).

CYP2D6 variation, constitutive physiology & drug target responsiveness

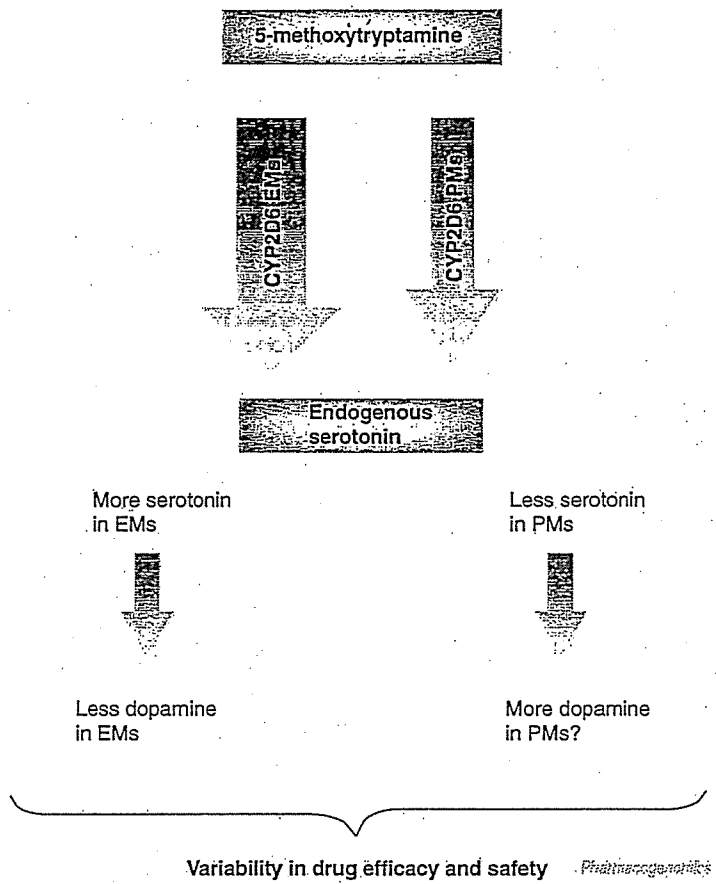
Could *CYP2D6* variation influence baseline physiology and by extension, drug target responsiveness? Preliminary empirical clinical data come from a recent and careful investigation by Kirchheiner and colleagues [44]. In a sample of 25 healthy male volunteers, all nonsmokers and drug naive, they detected significantly higher constitutive (baseline) serotonin concentrations in platelets from the CYP2D6 ultrarapid metabolizers (UMs; n = 11) compared with EMs (n = 11) and PMs (n = 3); mean values of serotonin were 462, 399 and 292 ng per 10^9 platelets, respectively [44]. Kirchheiner and colleagues suggest (and we agree) that this observation can be explained by regeneration of serotonin from 5MT by CYP2D6 (as platelets have an endoplasmic reticulum) and/or by CYP2D6 genotype-dependent differences in constitutive serotonin pool formed, particularly in the liver, by CYP2D6 [44]. This observation may potentially have a biological basis, since Kirchheiner and colleagues were able to reproduce their findings in the same group of healthy volunteers 2 and 6 weeks following the original investigation [44]. Follow-up studies in independent samples are warranted to confirm these findings.

Future empirical *in vitro* research is undoubtedly necessary to determine the extent to which CYP2D6-mediated 5MT metabolism contributes to serotonin concentration in different organs and neurochemical pathways. Nonetheless, we submit that it is essential to reflect upon several theoretical corollaries in the context of pharmacokinetics and pharmacodynamics. For example, consider cases when pharmacological agents are administered to downregulate the serotonin system. PMs may potentially exhibit a tendency for lower endogenous serotonergic activity at baseline. Moreover, if drugs used in such cases are also metabolized by CYP2D6, PMs will be subject to a double jeopardy: not only will they likely exhibit a lower baseline serotonergic activity (a potential risk factor for depression and psychiatric diseases), their systemic or target organ drug exposure may exceed therapeutic thresholds thereby posing a risk for side effects. Depending on whether the drug under investigation has active metabolites and whether or not a biological system is targeted for up- or down-regulation, the final clinical impact of *CYP2D6* genetic variation on pharmacokinetics and drug targets within the serotonin system will be further complicated.

Yet another dimension is that of physiological development: the fact that biological pathways and networks change over time between conception and attainment of functional maturity, and subsequently, with senescence. An example relevant to discussion of the serotonergic pathway is the current concern regarding the use of serotonin-selective reuptake inhibitor antidepressants in adolescents with major depressive disorders. Not only is there concern that these drugs may be associated with an increased risk of self-harm, which may or may not be a risk of therapy that is specific to this age group, but there is some question as to whether the drugs are effective at all in this patient population (i.e., are the components of the serotonergic pathway fully functional and thereby subject to therapeutic modulation at this developmental stage?). These developmental considerations can be further compounded by genetic variations in *CYP2D6*. However, for our purposes, these points illustrate the importance of theoretical reflection on the ways in which drug-metabolizing enzymes, their endogenous substrates and molecular drug targets can potentially interact in a nonlinear fashion. Given that exploratory bioinformatics analysis is a key component of pharmacogenomics, attention should be given to interactions of pharmacokinetic pathways and drug targets during interpretations of study outcomes.

Although CYP2D6-mediated hydroxylation of tyramine to dopamine is not considered to have a large physiological relevance (K_M > 50 µM) [22], modulation of serotonin concentration via 5MT and CYP2D6 may have

Figure 2. A hypothetical framework for regeneration of serotonin from 5MT by CYP2D6 and the attendant plausible neurochemical and clinical consequences of *CYP2D6* genetic variation (e.g., as extensive or poor metabolizers).



For example, in poor metabolizers of CYP2D6, regeneration of serotonin from 5MT may be impaired, potentially leading to a subtle decrease in serotonergic function. Due to reciprocal physiological interactions between serotonin and dopamine pathways (i.e., a higher serotonergic activity leads to downregulation of dopamine neurotransmission), *CYP2D6* genetic variation may conceivably have a secondary impact on dopaminergic neurophysiology as well.
5MT: 5-methoxytryptamine; CYP2D6: Cytochrome P450 2D6.

a secondary impact on dopaminergic neurotransmission. The rationale for this possibility stems from the reciprocal physiological regulation of the serotonin and dopamine neurotransmitter systems in certain brain regions. For example, serotonergic neurons projecting from the dorsal raphe nuclei exert a tonic inhibitory control on the nigrostriatal pathway through the 5-HT_{2A} subtype of serotonin receptors located on the dopaminergic neuronal soma in the substantia nigra and the nerve termini in the striatum. In effect, stimulation of the 5-HT_{2A} receptors on the nigrostriatal pathway results in a decrease in dopamine release in the striatum. Furthermore, CYP2D6 in the brain is functionally associated with the dopamine transporter and shares similarities in substrates and inhibitors (e.g., d-amphetamine), further suggesting a potential role for CYP2D6 in dopaminergic neurotransmission, although the precise mechanism of this association remains elusive [34]. A hypothetical conceptual framework depicting the projected interactions of 5MT, CYP2D6, serotonin and dopamine systems is presented in Figure 2.

Joint study of serotonin system & pharmacokinetic genes: is this happening in practice?

The postulated role of CYP2D6 in the metabolism of endogenous neurotransmitters, the CYP2D6-related differences in platelet serotonin levels and personality, as well as the contribution of CYP2D6 in the metabolism of many psychotropic drugs, prompted us to evaluate the extent to which the link between drug target genes and genes influencing the pharmacokinetics of drugs and endogenous substrates has actually been studied in humans. To this end, we chose the serotonin system as a model case study due to its immediate relevance for 5MT, as described above. A search of the biomedical literature (PubMed Medline [102]) was performed using the keywords 'serotonin' and 'polymorphism'. Only reports in English that evaluated associations between any serotonin system-related gene (e.g., receptors, transporters, and so on) and a clearly defined clinical phenotype in human subjects were included. Reviews, case reports and meta-analyses were excluded.

Although the number of association studies involving the serotonin system increased annually between 1995 and 2005 (from 14 per annum in 1995, to 150 in 2005 [Figure 3]), only four studies [45–48] of a total of 875 reported over the preceding decade evaluated serotonin system-related genes together with genes involved in pharmacokinetics (Table 1). Notably, the established or putative endogenous substrates of respective pharmacokinetic genes were not mentioned or discussed in any of these studies. Only one study analyzed the interaction of pharmacodynamic- and pharmacokinetic-related genes (Table 1). The synergistic effect of 5HT_{2A} and *CYP2D6* gene polymorphisms on the occurrence of fluvoxamine-induced gastrointestinal side effects was reported by Suzuki and colleagues [45]. Their findings, analyzed with Cox