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

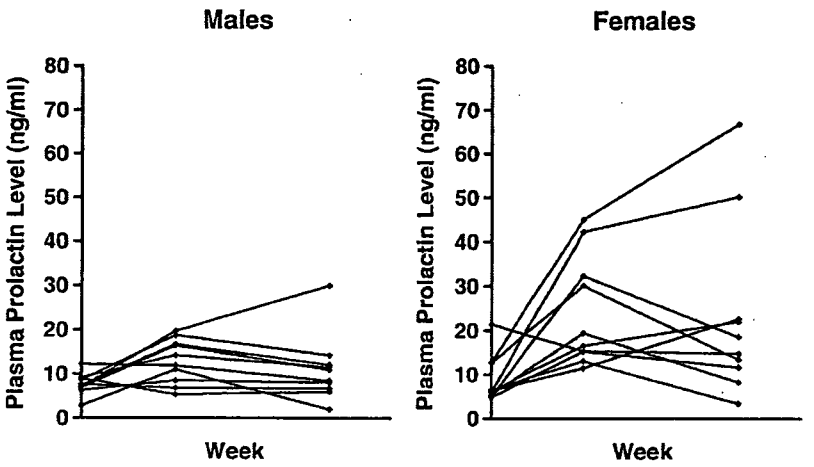

Fig. 1. Gender differences in prolactin elevation induced by olanzapine. Upper limits of plasma prolactin levels in male and female patients.

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

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.

"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].

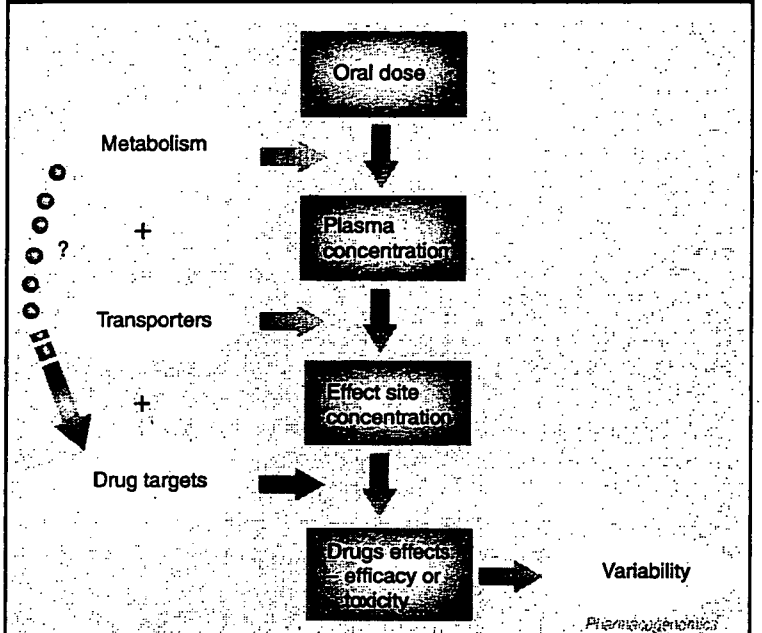
Here, we discuss these important overarching issues through a specific pharmacogenomic case study of cytochrome P450 (CYP)2D6, its putative endogenous substrate, 5-methoxytryptamine (5MT), and their potential interactions with serotonin and dopamine neurotransmitter systems. Additionally, we describe 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 indude 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

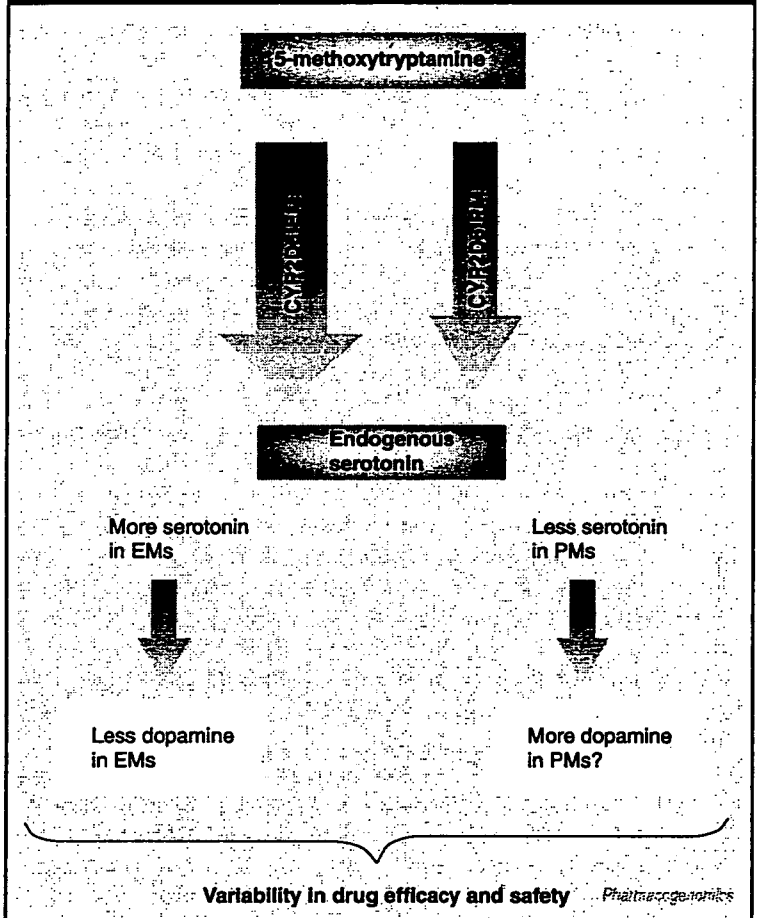
Could *CYP2D6* variation influence baseline physiology and by extension, drug target responsiveness? Preliminary empirical clinical data come from a recent and careful investigation by Kirchheiner and colleagues [44]. In a sample of 25 healthy male volunteers, all nonsmokers and drug naive, they detected 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.

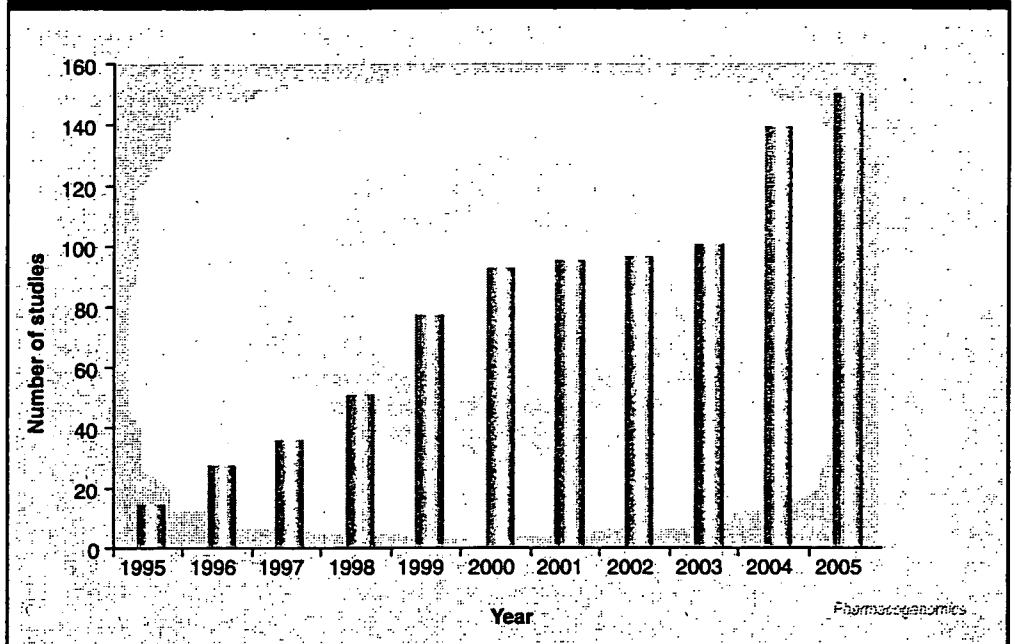
a secondary impact on dopaminergic neurotransmission. The rationale for this possibility stems from the reciprocal physiological regulation of the serotonin and dopamine neurotransmitter systems in certain brain regions. For example, serotonergic neurons projecting from the dorsal raphe nuclei exert 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 5-HT_{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

Figure 3. Proliferation in the number of published clinical pharmacogenetic association studies of the serotonin system candidate genes from 1995 to 2005.



The year 1997 benchmarks the first introduction of the term 'pharmacogenomics' into the research literature [3,12]. Despite a marked increase in the number of pharmacogenomic association studies concerning the serotonergic drug targets, only four studies actualy performed a tandem analysis of genes within the serotonin system and pharmacokinetic pathways (see also **Table 1**).

regression analysis, showed that CYP2D6 'low metabolizers' (**1/*5*, **10/*10* or **5/*10* carriers) with the $5HT_{2A}$ 1438G/G or A/G genotype had a higher risk of developing gastrointestinal side-effects compared with CYP2D6 'normal metabolizers' (**1/*1* or **1/*10* carriers) with $5HT_{2A}$ 1438A/A genotype. In the other three studies [46–48], the combinatory effects of both pharmacokinetic and drug target genes were not evaluated, possibly due to the results showing significance for only one gene.

It should be noted that our findings on the serotonin system as described above may not necessarily reflect the situation for all drug target genes. However, clinical pharmacogenetic studies evaluating both pharmacokinetic and pharmacodynamic aspects in tandem remain in the minority [49]. A possible reason for not evaluating the combinatory effects of pharmacokinetic and drug target genes or not including both of these gene groups in a protocol might be the loss of statistical power. To obtain sufficiently large numbers of study subjects with well-characterized and reliable phenotype data may require large multicenter studies, a task that is not easily feasible for individual academic research groups, either from an economic or a logistic point of view. Collaborative projects involving many groups using the same protocol may in the future solve some of these problems. Apart from genetic predisposition, the role of environmental and other nongenetic factors such as age, gender and previous drug history should be taken into account, further complicating or constraining study design and feasibility.

Hyperspecialization in biomedicine & fragmentation of knowledge commons in pharmacogenomics

One of the fundamental principles of modern science, and contemporary academia more generally, is the notion that research is to be shared within the academic community so that findings can be objectively and dispassionately tested and then accepted or rejected. An environment predicated on the free flow and sharing of knowledge, academia can be usefully thought of as a 'knowledge commons' [50,51]. But this commons is clearly not open for everyone; specific disciplines have their own bodies of knowledge, research questions, literatures, and even languages. Modern academia, and for our purposes the biosciences, is thus better understood as a plethora of knowledge commons, some of which will

share 'boundaries' or broad research interests, such as pharmacology, molecular genetics and clinical medicine.

Within particular disciplines, specializations will arise that, over time, develop their own focused literature and research questions (e.g., pharmacokinetics); at the interface of different disciplines, interdisciplinary specialties may be born (e.g., pharmacogenomics) [52]. Such disciplinarily or interdisciplinary specialization is likely a natural and beneficial process and one that allows for focused and productive scientific inquiry. However, the downside is separate literatures and languages, such that it becomes increasingly difficult to read across and learn from other specialties to solve more complex 'big-picture' research questions; research areas become fragmented [53]. It is our contention that such is the case with research on pharmacogenomics of pharmacokinetic pathways and drug targets (Table 1). These two specialties should, but do not, collaborate sufficiently. Pharmacokinetics is a more established discipline, while the genetic variation in drug targets is a relatively recent concern. However, as exemplified in the case of CYP2D6 and its putative endogenous substrates, knowledge from both specialties is necessary to understand complex interactions between pharmacokinetic and drug target processes. The fragmentation of bioscience research in pharmacogenomics is thus creating impediments to the advancement of knowledge and ultimately the development of beneficial diagnostic products to aid in selection of drug dosages and/or the type of prescription.

A solution to this, as hinted at earlier, lies at the interface or boundary between disciplines. We suggest that interdisciplinary research is needed that, working in collaboration with specialists in pharmacokinetics and molecular genetics, synthesizes the knowledge from these two areas as it relates to CYP2D6, metabolism of endogenous substrates and drug targets. As exemplified by other interdisciplinary fields of inquiry, such as bioethics (a broad and often contested field of study inhabited by scholars from philosophy, law, medicine, the social sciences and the applied sciences) [54], attempts to effectively synthesize diverse knowledge sets will be a source of much tension. On the other hand, the interdisciplinary space also presents opportunities for innovation and resolution of otherwise intractable research questions. For our purposes, such an interdisciplinary perspective can help address the issue of how best to bring together 'knowledge, capital and morality' in pharmacogenomics-guided personalized medicine and drug development [55–61]. We suggest that it is only when researchers place themselves in that interdisciplinary space and acknowledge the attendant semantic and methodological uncertainties, that they can begin to dispassionately learn from other disciplines (e.g., bioethics, the social sciences and human genetics).

Over the past decade, new graduate-training programs were implemented in several universities in North America and Europe that offer diploma or doctoral degrees in 'interdisciplinary studies'. Despite these isolated advances, organizational and governance structures within academia still remain as discipline-bounded entities. To the extent that scientific control is mediated by peer-review evaluations [62], there are further barriers to interdisciplinary inquiries; these relate to tightly-defined review committees and governance structures attached to national research funding agencies and biomedical journals. A broader representation of expertise within these peer-review committees would be an important step forward in promoting interdisciplinary and integrated research, not only in pharmacogenomics, but also across the allied health sciences, humanities and social sciences.

Expert commentary

There is a need for new strategies to identify the genes relevant for individual and population differences in drug efficacy and safety. Hence, integration of genomic data from pharmacokinetic pathways and drug targets is a timely and much needed research strategy in clinical pharmacogenomics. However, few studies to date have taken into account or integrated these two important domains of pharmacological variability. In this review, we discussed the potential involvement of drug-metabolizing enzymes in endogenous substrate metabolism, and its clinical relevance, with the emerging example of CYP2D6 and serotonin interaction and potential corollaries for the dopamine neurotransmitter system.

Over the past two decades, several converging lines of evidence have collectively suggested that neuroactive endogenous substrates of CYP2D6 may have clinical significance. These observations include personality differences between CYP2D6 EMs and PMs in some populations (e.g., in Sweden and Spain), expression of *CYP2D6* in the human brain with a primarily

Table 1. Summary of clinical pharmacogenomic association studies of the serotonin system genes where one or more drug-metabolizing enzyme (or other pharmacokinetic) candidate gene was analyzed in tandem.

Study	Serotonin system drug target genes reported	Pharmacokinetic-related genes reported	Sample size	Most significant associations	Source of funding	Other remarks
Murphy *et al.* (2003)	*HTR2A*	*CYP2D6*	246	*HTR2A* 102 C/C genotype related with paroxetine side effects and discontinuation	Organon Pharmaceuticals, Inc.; NARSAD; The Nancy Pritzker Network; and VA Medical Research (Sierra Pacific MIRECC)	Caucasians and ethnic minorities, elderly patients with major depression
Huang *et al.* (2005)	*HTR2A, MAOA*	*CYP2A6*	1518	CYP2A6 haploinsufficiency increases likelihood of continuing smoking in teenagers	The Welcome Trust; Medical Research Council	Caucasians, South Asians, African–American
Hedenmalm *et al.* (2006)	*HTR2A, SLCA4*	*CYP2D6, CYP2C9, CYP2C19*	20	No association between SSRI-induced EPS and *CYP2D6, CYP2C19, CYP2C9, HTR2A* or *5-HTTLPR* polymorphisms	The Swedish Research Council	Swedish patients with EPS
Suzuki *et al.* (2006)	*HTR2A, HTR3A, HTR3B*	*CYP2D6*	100	CYP2D6 low metabolizers (**1/*5, *10/*10* or **5/*10* carriers) with the *HTR2A* -1438 G/G or A/G genotype had higher risk of developing gastrointestinal side effects compared with CYP2D6 normal metabolizers (**1*1* or **1/*10* carriers) with *HTR2A* -1438 A/A genotype	Japan Society for the Promotion of Research	Japanese patients with major depression

Different study attributes are shown in column headings.

The Medline searches were structured to span the time period starting 1995 to August 2006. This interval includes September 1997 that signifies, according to some investigators [3,12], the introduction of the term 'pharmacogenomics' into the medical literature.

5-HT_{2A}: Serotonin receptor 2A; 5-HTTLPR: Serotonin transporter polymorphism; CYP: Cytochrome P450; EPS: Extrapyramidal symptoms; MAOA: Monoamine oxidase A; NARSAD: The National Alliance for Research on Schizophrenia and Depression; SSRI: Selective serotonin reuptake inhibitor.

neuronal origin, and discovery of 5MT as an endogenous substrate for CYP2D6. More recently, these data have been complemented by *in vivo* studies conducted by Kirchheiner and colleagues, who observed a higher platelet serotonin concentration in subjects with a *CYP2D6* UM genotype.

The idea that CYP2D6 may influence drug target responsiveness has several corollaries. For example, it provides a mechanistic rationale to consider epistatic interactions between *CYP2D6* and serotonin system genes. The contribution of *CYP2D6* to drug-related phenotypes can be mediated, as noted earlier, not only by a traditional pharmacokinetic influence but also through impact on serotonergic neurophysiology by regeneration of serotonin from 5MT. In addition, the CYP2D6–5MT–serotonin link further underscores the importance of *CYP2D6* as a susceptibility gene in association studies of neuropsychiatric complex diseases, such as major depression and anxiety disorders.

Clinical validation of polygenic models, including gene–gene interactions between pharmacokinetic and drug target genes, will require prospective large-scale clinical trials of uniformly treated and systematically characterized patients, high-throughput genomic methods, sophisticated bioinformatics analyses and recognition of genetic admixture in human populations [63]. Such studies hold great promise to yield a new panel of integrated molecular diagnostics (e.g., genotypes) that can be used to improve drug therapy by reducing toxicity and increasing efficacy. These studies can also help determine the added value of pheno- or genotyping, even though it must be said that no detailed documentation or adequate cost-effectiveness analysis exists for many (if not most) pharmacogenomic tests used at the point of patient care today.

Outlook

There is increasing evidence that endogenous substances such as 5MT are metabolized by CYP2D6 in the brain, presumably leading to a subtle modulation of serotonin levels. The regeneration of serotonin neurotransmitters in the serotonin–melatonin cycle by polymorphic CYP2D6 enzyme in the human brain could provide a reasonable explanation for the differences in personality observed between CYP2D6 EMs and PMs in a sample of Swedish and Spanish healthy subjects, with the PMs being more anxiety- and impulsivity-prone and less successfully socialized. However, the influence of CYP2D6 and its putative endogenous substrates on personality is complex and may not uniformly hold in all human populations [30]. Furthermore, what is presently unclear is the co-occurrence (spatially and temporally) of CYP2D6 and 5MT in various brain regions. The latter piece of information is essential for a deeper understanding of biological significance of the link between CYP2D6 and 5MT.

Although the contribution of CYP2D6 to regeneration of serotonin from 5MT may be modest or negligible at baseline (constitutive) conditions, we hypothesize that this pathway may become a significant 'reserve' mechanism in the event of depletion of serotonin due to disease and/or drugs that antagonize serotonergic receptors. Future study designs in this field of research should consider, we submit, the contribution of CYP2D6 to serotonergic neurophysiology at constitutive states and during a 'challenge' or dynamic study design paradigm, while administering agents that antagonize serotonergic receptors or related molecular targets. Many other drug-metabolizing enzymes, such as aldehyde dehydrogenase and alcohol dehydrogenase, are expressed in the brain, and are likely to have important roles in the function of the brain or response to medications [64,65].

An additional challenge is that pharmacogenomic-related changes in neurotransmitters, and especially trace amines, are likely to occur on a finer scale than changes in drug concentrations due to a given drug biotransformation enzyme. However, these 'finer' changes may still be associated with considerable functional consequences due to amplification of signals via downstream signal transduction pathways. The ability to quantify fine changes may be difficult but this is unlikely to be an intractable problem given ongoing advances in analytical (and other) technologies. Endogenous substrates of drug-metabolizing enzymes may also open a new research avenue to phenotype polymorphic pharmacokinetic pathways (e.g., CYP2D6) using such endogenous substrate and metabolite concentrations in accessible physiological fluids.

We suggest that researchers in pharmacogenomics must integrate knowledge on different sources of pharmacological variability, whether they be genetic, environmental, social or cultural in nature. A richer and more comprehensive mode of pharmacogenomics policy analysis in public and private dimensions of medical research that is appropriately responsive to the diversity of unmet patient needs and stakeholder viewpoints is much needed and timely

[2,52,60,66–70]. These policy analyses should also address the conflicts of interests or breaches in interdisciplinary dialogue due to epistemological distances between public and private medical research spheres, as well as among scientists, policy makers and patients [71–75,103]. This recognition will ultimately also contribute towards building a more certain and ethical future for pharmacogenomics, wherein functional personalized medicines can be delivered in a manner that is both effective and equitable [57,70,76].

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Highlights

- Clinical pharmacogenomic studies evaluating synergistically pharmacokinetics (e.g., drug-metabolizing enzymes) and pharmacodynamics (e.g., molecular drug targets) remain in minority. Therefore, interdisciplinary research involving specialists in both areas is needed.
- Although many genes encoding proteins involved in the metabolism, transport, and mechanism of action of drugs are known to exhibit polymorphism in humans, use of this knowledge in routine clinical practice is limited. Most studies have focused in the past on the effect of a single gene on drug response. This approach neglects the fact that drug-response phenotypes, like most disease phenotypes, are complex polygenic traits also determined by nongenetic factors.
- There is converging evidence on the involvement of drug-metabolizing enzymes in endogenous substrate metabolism; a notable example is the role of cytochrome P450 (CYP)2D6 in regeneration of serotonin from 5-methoxytryptamine.
- The presence of the polymorphic *CYP2D6* in the brain, where it is expressed at high levels in specific areas and cell-types, indicates that it may play an important role not only in the metabolism of drugs, but also in modulating the levels of neurotransmitters locally at the site of drug action.
- Informed dialogue and reasoned discussions among applied pharmacologists, human geneticists and social scientists are crucial for the integration of genomic, environmental, social and other sources of variability in pharmacokinetics and molecular drug targets. We suggest that this is also an ethical and moral obligation for the equitable advancement of population health through research funded either by public and/or private resources.

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Association between multidrug resistance 1 (*MDR1*) gene polymorphisms and therapeutic response to bromperidol in schizophrenic patients: A preliminary study

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Abstract

The drug-transporting P-glycoprotein transports drugs against a concentration gradient across the blood–brain barrier back into the plasma and thereby reduces the bioavailability in the brain. Polymorphisms in the *MDR1* gene regulating P-glycoprotein expression can be associated with differences in drug disposition in the brain. The present study was therefore designed to examine whether the major polymorphisms of *MDR1* gene, *C3435T* and *G2677T/A* are related to therapeutic response to neuroleptics in the treatment of schizophrenia. Subjects consisted of 31 acutely exacerbated schizophrenic inpatients treated with bromperidol (6–18 mg/day). Plasma drug concentrations were monitored and clinical symptoms were evaluated using the Brief Psychiatric Rating Scale (BPRS) before and 3 weeks after the treatment. The *C3435T* and *G2677T/A* genotypes were determined by a polymerase chain reaction method. Schizophrenic symptoms were allocated into 5 clusters: positive, excitement, cognitive, negative, and anxiety–depression symptoms. Patients were *C/C* in 12, *C/T* in 12 and *T/T* in 7 cases for *C3435T* genotype and *G/G* in 3, *G/T* or *A* in 17 and *T* or *A/T* or *A* in 11 cases for *G2677T/A* genotype. There were a tendency of difference, but not statistically different, in the percentage improvement or the improved scores of 5 sub-grouped symptoms after the 3-week treatment between *C3435T* genotypes and between *G2677T/A* genotypes. Multiple regression analyses including age, body weight, gender and drug concentration showed significant correlations between the percentage improvement and the improved scores of cognitive symptoms and *C3435T* genotypes. The present results suggest that the *C3435T* polymorphism is associated with some therapeutic response to bromperidol in schizophrenic patients, possibly by different drug concentration in the brain.

Keywords: Blood–brain barrier; Bromperidol; *C3435T*; *MDR1*

1. Introduction

P-glycoprotein, which is encoded by *MDR1* gene, is involved in the acquisition of multidrug resistance phenotypes not only in cancer cells but also in normal tissues such as brain, kidney, liver, and intestine (Thiebaut et al., 1987). Its major physiologic role is to serve as a barrier to entry and as an efflux mechanism for xenobiotics and cellular metabolites (Cordon-Cardo et al., 1989). Not only may P-glycoprotein limit intestinal drug absorption to constrain oral drug bioavailability, but rate of P-glycoprotein efflux transport can also mediate brain penetration of lipophilic drugs (Ambudkar et al., 1999; Benet et al., 1999). This is based on several kinetic studies showing large differences in brain concentration between the knockout animal, *mdr1a* (−/−) and *mdr1a/1b* (−/−) mice and normal animal, *mdr1a* (+/+) and *mdr1a/1b* (+/+) mice (Rao et al., 1999). Therefore, inter-individual variability of P-glycoprotein function in the brain contributes to this variability of clinical response to neuropsychiatric agents.

Abbreviations: BPRS, Brief Psychiatric Rating Scale; *MDR1*, multidrug resistance 1.

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The antipsychotic drug, bromperidol is a close chemical structure analogue to haloperidol. Similar to the action of haloperidol, bromperidol has a relatively specific and high affinity for dopamine D_2 receptor and antagonizes stereotyped behavior and agitation induced by apomorphine and amphetamine, dopamine receptor agonists, in rats (ED_{50}<0.05 mg/kg) (Poldinger et al., 1977; Malfroid et al., 1987; Denijs, 1980). Also other studies suggest that the pharmacological property of bromperidol is mainly characterized by its antidopaminergic property (Niemegeers et al., 1987; Schotte et al., 1995). The overall efficacy of bromperidol has been reported to be similar to or slightly better than that of haloperidol (Poldinger et al., 1977; Malfroid et al., 1987). Bromperidol may have a faster onset of action and a more activating effect than haloperidol (Woggon, 1978). Bromperidol undergoes *N*-dealkylation, hydroxylation and carbonic reduction yielding reduced bromperidol (Wong et al., 1983). Reduced bromperidol is known as a major metabolite in plasma and undergoes oxidation back to bromperidol and probably by *N*-dealkylation (Wong et al., 1983). Several in vitro studies have suggested that bromperidol metabolism is dependent on CYP3A4 (Tateishi et al., 2000; Sato et al., 2000).

There is a striking overlap between CYP3A4 substrates and P-glycoprotein substrates (Patel and Mitra, 2001). Our previous reports have shown that steady-state plasma concentration of bromperidol is altered by itraconazole (Iida et al., 2001; Furukori et al., 1999), a potent inhibitor of CYP3A4 (von Moltke et al., 1996; Isoherranen et al., 2004) and P-glycoprotein (Karyekar et al., 2003), or by carbamazepine (Otani et al., 1997), an inducer of CYP3A4 (Ogg et al., 1997; Egnell et al., 2003) and probably P-glycoprotein (Lazarowski et al., 2004), suggesting an potential involvement of CYP3A4 and/or P-glycoprotein or both in bromperidol disposition.

Hoffmeyer et al. (2000) suggested that a single-nucleotide polymorphism in exon 26 of the *MDR1* gene (*C3435T*) was associated with a lower level of intestinal *MDR1* expression. Moreover it has been reported that another single-nucleotide polymorphism in exon 21 of the *MDR1* gene (*G2677T/A*) is also linked with a lower function of P-glycoprotein (Siegmund et al., 2002). However, the role of P-glycoprotein in pharmacokinetics or pharmacodynamics has not been fully proven in the psychiatric field, yet. Therefore, the effect of the *MDR1* gene polymorphisms on clinical response to bromperidol was examined in 31 acutely exacerbated schizophrenic patients.

2. Methods

2.1. Selection exclusion criteria

Men and women inpatients aged 18 to 65 years with diagnosis of schizophrenia (DSM-IV) (American Psychiatric Association, 1994), more than 18 points of Brief Psychiatric Rating Scale score (BPRS) described by Bech et al. (1986), and no medication including antipsychotic agents for at least one month were eligible for inclusion. BPRS consisted of 18 items and is classified from 0 to 4 for each item. Patients with clinically significant abnormal laboratory or electrocardiographic findings, histories of mental disorder other than schizophrenia, epilepsy, alcoholism or drug abuse, or clinically significant organic or neurological disease were excluded. This study was approved by the Ethics Committee of Hirosaki University Hospital, and written informed consent to participate in this study was obtained from the patients or their families before the study.

2.2. Patients

Thirty-three acutely exacerbated patients (17 males, 16 females) participated in the study on admission. The mean±S.D. of age, body weight and duration of illness were 37.3±12.8 years, 59.7±13.1 kg and 119±101 months, respectively. All patients fulfilled the DSM-IV criteria for schizophrenia (3 disorganized type, 18 paranoid type, 1 catatonic type and 11 undifferentiated type). It was confirmed that none had received any medication for at least 1 month by interviews with patients or their families.

2.3. Protocol

On the first night of admission, the only medication allowed was flunitrazepam 2–4 mg. Next morning, blood samplings (10 ml) were performed at 8 a.m. after 30 min. rest. Assessment of pretreatment clinical status using Brief Psychiatric Rating Scale (BPRS) for schizophrenic symptoms and Udvalg for Klinicke Undersøgelser (UKU) side effects rating scales for side effects (Lingjaerde et al., 1987) was performed by two well-trained psychiatrists. Thereafter, bromperidol (Impromen®, Yoshitomi Pharmaceutical, Osaka, Japan) was administered in two equally divided doses at 8 a.m. and 8 p.m. for 3 weeks. Patients were randomly allocated to one of the three fixed doses in single-blind manner: 6 mg/day (n=10), 12 mg/day (n=13) and 18 mg/day (n=10). No other drugs were given except biperiden 6 mg/day (n=16) for moderate extrapyramidal side effects, flunitrazepam (2 mg/day, n=11 and 4 mg/day, n=17) for insomnia and sennoside (12–48 mg/day, n=8) as a laxative for constipation. Patients' compliance was confirmed by nursing staff. During bromperidol treatment, blood samplings and clinical assessments by BPRS and UKU scales were conducted at weekly intervals in the same manner as performed on the second day of admission.

2.4. Analyses for MDR1 genotypes

For the determination of *MDR1* genotype, DNA was isolated from peripheral leukocytes by a guanidium isothiocyanate method. The *C3435T* and *G2677T/A* alleles