also, however, untapped opportunities to increase adoption and acceptance of genomic technologies at the point of patient care. In particular, the recognition that pharmacogenomics is an experimental science creates an avenue for a systematic policy response to the ethical imperative to prospectively pursue genetically customized therapies before regulatory approval of pharmaceuticals.

Visions of Pharmacology as an Experimental Science: An Ethical Obligation to Conduct Prospective Pharmacogenomic Studies?

In general, the drug development process spans between 10 and 15 years from the discovery of a new drug molecule to regulatory approval for the drug to be marketed to the public. Understandably, a lag period is anticipated before new therapeutics developed with the use of -omics technologies, such as pharmacogenomics or proteomics, will be available in the clinic. For drugs that are presently in clinical use, one might expect that pharmacogenomics would have been already adopted prospectively in phase 4 clinical trials (ie, postmarketing studies of large patient populations) as there has been a dramatic increase in the availability of -omics technologies in biomedical research laboratories over the past decade.[6,11] It is interesting to note, then, that there is an acute shortage of prospective clinical studies designed to individualize drug labels, that is, formally limit a drug's target population to those people with a certain genotype.[8,15,31]

To date, most pharmacogenomic studies have been conducted in clinical trials designed for another purpose: to demonstrate efficacy or safety for drug registration by regulatory bodies such as the US Food and Drug Administration (FDA). The highly structured time frames in these trials may not always permit adequate scientific rigor or flexibility for exploratory research oriented toward genetic test development for individualization of drug therapy. In certain cases, this may lead to an ad hoc retrospective sampling of clinical trial data (eg, only when or if a compound displays toxicity after introduction into the market), even though, as noted earlier, prospective study designs are entirely feasible in pharmacology. By contrast, an abundance of discovery-oriented research (ie, remote from direct clinical applications to customize drug therapy) with genomic technologies is taking place for identification of new drug targets or proof of concept in early-phase clinical trials.[15] But this early-phase upstream basic research does not necessarily guarantee the eventual downstream access to genetic testing or delivery of personalized medicines at the point of patient care.[15,31–33] A number of concerns, such as small market sizes in narrowly defined therapeutic fields, have been presented in the past as an explanation for the obvious trepidation associated with the prospective development of pharmacogenomic tests at the point of care.[31,32,34]

We suggest that the motivations for prospective clinical pharmacogenomic applications to proactively influence drug labels and prescriptions may also be shaped by the type of pharmaceutical associated with specific pharmacogenomic tests. In 2004, of the 113 new drug applications (ie, marketing approval) approved by the FDA, only 17 (15%) were considered significant improvements compared with already marketed products.[35] Although there is much to be celebrated in terms of singular success stories on selected innovative medicines developed by the pharmaceutical industry, many of the pharmacotherapies introduced into the market every year are 'me-too' drugs, displaying comparable efficacy and safety profiles with already existing medicines (Figure 1).[35–37] These me-too drugs may be economically very profitable and in some cases will even constitute 'blockbusters' that generate billions of dollars in revenue. But for our purposes, it is important to note that in the context of customized therapeutics, me-too drugs (whether blockbuster or not) may adversely influence motivations for pharmacogenomic testing in the clinic in ways that were previously unanticipated.

Consider a hypothetical therapeutic area (eg, statins to reduce blood cholesterol or selective serotonin reuptake inhibitor antidepressants) that is characterized by an abundance of me-too drugs, with 60 to 80% of the available drugs exhibiting a similar pharmacologic mode of action or efficacy or safety profile. A pharmacogenomic test for a me-too drug may be equally predictive of treatment outcomes for most, if not all, drugs within the same me-too category, redistributing the financial gains made on the diagnostic test from an individual pharmaceutical company holding the pharmacogenomic patent to multiple firms that manufacture similar me-too drugs. Hence, the past and present focus on me-too drug development may serve as a barrier to both innovation in pharmacotherapy and the development of targeted therapies in conjunction with pharmacogenomic tests.

Another hitherto overlooked consideration is the significant reduction over the past decade in the duration of tenure and increased turnover of chief executive officers (CEOs) in various multinational corporations. For example, in a survey of CEO succession at the world's largest 2,500 publicly traded companies, Lucier and colleagues found that 14.2% of

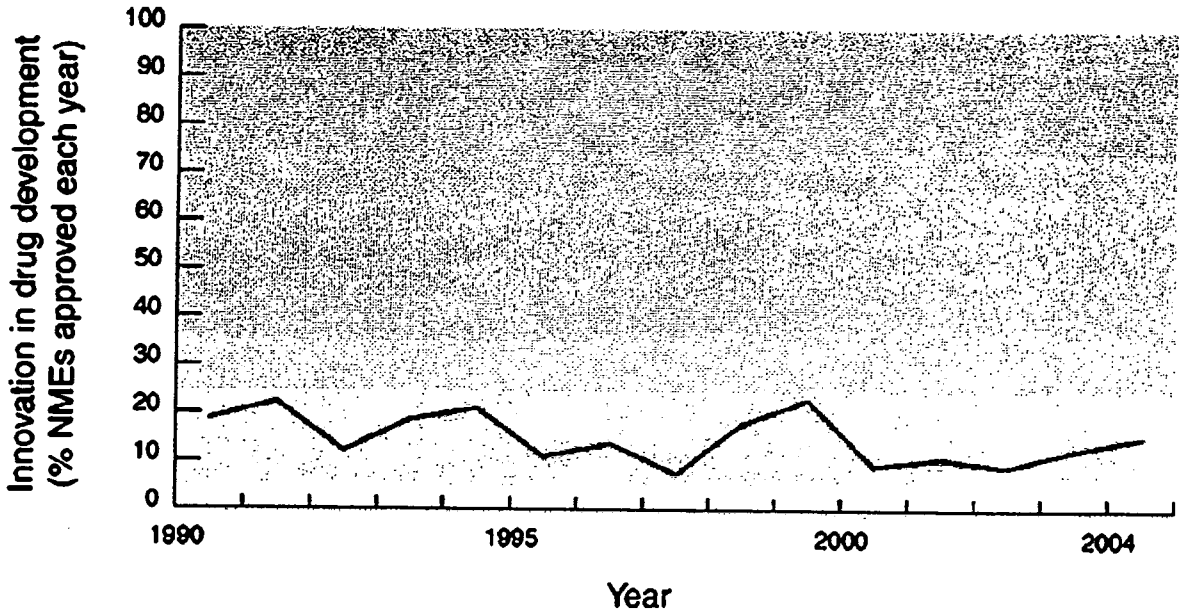

Figure 1 New drug applications (NDAs) approved in calendar years 1990–2004 by the US Food and Drug Administration (FDA) and the new molecular entities (NMEs) subjected to priority regulatory review while offering a significant improvement compared with marketed products in the treatment, diagnosis, or prevention of a disease. Innovation in drug development, as defined by the percentage of these breakthrough NMEs in relation to all NDAs approved in each calendar year, remained low for more than a decade. This further underscores the importance of recognizing (1) pharmacology and pharmacogenomics as experimental lines of scientific inquiry and (2) the attendant ethical obligation to prospectively pursue pharmacogenomics-guided drug development models (instead of the traditional 'wait-and-see' approach) that can improve innovation rates in drug development. Reproduced with permission from Ozdemir V.[15] 2

CEOs left office in 2004, a 300% increase in CEO departures since 1995.[38] Within the health care sector in 2004, CEO dismissals rose to 16.2%.[38] Nearly a third of all CEO resignations in 2004 were related to failure to meet demands for financial returns by increasingly impatient shareholders. Notably, the CEOs removed for inadequate performance had a median tenure of 5.2 years in the United States; in Europe, the situation was more difficult, with poorly performing CEOs remaining only for a median of 2.5 years. According to Lucier and colleagues, corporations "have reached a tipping point, in which power in the corporation is permanently shifting away from chief executives." In this climate of risk-averse and demanding shareholders and CEOs increasingly anxious about maximizing returns on a quarter-by-quarter basis, new pharmacogenomic technologies are being implemented.[39,40] Thus, it is difficult to reconcile the short-lived (2.5–5.2 years) tenure of the CEOs with new health technologies (eg, -omics biomarker platforms) that require long-term investment before tangible financial returns can be observed.

What incentives, then, can be put in place for corporate directors (as well as shareholders) to voluntarily exhibit socially responsible commitments to genomic technologies to achieve targeted therapeutics that, while potentially reducing short-term revenues,[34] may increase long-term retention of products (ie, safe and effective drugs) in the market? In the case of new genomic technologies, important social structural aspects,[15,32,33,38–40] such as those discussed above (eg, increased executive turnover and shareholder demands in favor of expediency), that can impact commercial or academic pharmacogenomic research and professional conduct may be dismissed or mistakenly ignored in the framing and future projections of these technologies.[41,42] To this end, a multidisciplinary learned society, such as the American Federation for Medical Research (AFMR), would be uniquely positioned to play a pivotal leadership role in facilitating dialogue across different professional languages and norms at the intersections of social sciences, research governance in public and private sectors, and professional practice of clinical

pharmacology and human genetics research to best realize the dream of pharmacogenomics-guided personalized medicines.

Regardless of the various sociologic, technology-based, or commercial factors and motivations that impede or facilitate the development of pharmacogenomic tests at the point of care, the fact is that the traditional model of drug development, with its focus on finding 'the next blockbuster drug,' is increasingly viewed as no longer realistic or viable.[37] Often overlooked is the fact that most recent blockbuster drugs were likely the 'lower-hanging fruits' resulting from rational and scientific drug development in the second half of the twentieth century. Further, many blockbuster drugs initially developed for broad use in the population have, on prescription in larger patient samples, been withdrawn from the market because of serious toxicity, a lack of effectiveness, or adverse drug-drug interactions. In effect, drug development without accompanying clinical biomarkers to customize prescriptions amounts to a statistical time bomb: when drug exposure exceeds the 1,000 to 3,000 patients collectively enrolled in typical premarketing clinical trials, members of the broader patient population who do not reflect the 'average' biologic or demographic attributes of trial participants are invariably exposed, leading to adverse drug-related events.

Exposing patients in clinical trials or during the postmarketing phase to partially preventable risks becomes a more acute and palpable social and ethical concern, especially when we consider that pharmacology is an experimental science amenable to proactive and prospective biomarker applications long before drug-related problems emerge. We submit that it is essential for both drug developers and regulators to adopt a longer-term vision that projects beyond the immediate goal of obtaining regulatory approval toward an enhancement of the entire life cycle and quality of a medicinal product. That is, prompt and timely introduction of new drugs to patients should be balanced against their sustainable use in the clinic, without postregistration withdrawal.[43]

Introducing noncustomized drugs in the clinic does not, in the long run, benefit many of the key actors in knowledge-based economies, whether they are patients or industry shareholders. Any costs incurred for postmarketing safety monitoring of drugs, such as frequent liver or kidney function tests, are ultimately transferred from the drug manufacturer to the patients and the payors.[44] Looking through the lens of global public health,[45] unfavorable perceptions about the societal commitment of a drug manufacturer on a given product withdrawn from the clinic will also have multiple detrimental effects on other compounds in their drug development pipeline: employee morale may suffer, thereby seriously undermining corporate initiatives to develop an equitable and attractive workplace environment that will retain highly trained and costly staff, whereas the broader mission of creating public benefit and ultimately safeguarding corporate and fiduciary responsibilities toward shareholders will be jeopardized.[46–48]

Future Outlook

As noted by David and Foray, commenting on the evolution of knowledge-based economies and civil societies, "[d]iscoveries in many domains are...made in the course of unplanned journeys through information space."[49] The genealogy of scientific progress can be even more complex in the case of interdisciplinary dialogues and experiments. Simply 'chunking' pharmacogenomics and human genetics together in conceptual proximity as two identical disciplines would be inadequate for a balanced reconciliation of their nuanced differences in science policy. Nor would such an approach acknowledge how these two fields might, in turn, impact both real and perceived expectations, for example, on sample size requirements in studies on the development of genetic tests for customization of drug therapy. More in-depth and realistic projections of their codevelopment as a new hybrid and intellectually richer discipline necessitate self-reflection that extends beyond the classic disciplinary boundaries. Hence, although the fields of clinical pharmacogenomics and human genetics research are increasingly coalescing through technology and knowledge transfer, it is critical to discern the ways in which discipline-specific traditions, tacit knowledge, and expectations of practitioners may influence the course of scientific dialogue and collaboration at their disciplinary boundaries and interdisciplinary junctions.

As academic institutions move increasingly toward serving a dual role as engines for economic growth and a knowledge commons (research and teaching),[50–52] future public policy debates on pharmacogenomics, genetic testing, and personalized medicine will need to be reframed to incorporate these subtle but significant characteristics (see Table 1). Ultimately, the recognition that pharmacology is an experimental science should also elevate the ethical standards and accentuate the moral obligation to develop pharmacogenomic or other biomarkers prospectively before obtaining marketing approval. For drugs that have already been in clinical use, an equal effort should be made to facilitate

their targeted use for individuals and patient populations. Blockbuster drugs may increase the profits in selected cases, but they also unethically concentrate the risks of drug development in specific groups and communities.[53]

The expansion in scope of scientific research enabled by new genomic technologies may soon result in fragmented but more diversified and narrowly defined therapeutic fields or markets for drugs that will ultimately benefit patients while also shaping the varied expectations for long-term and sustainable growth in the pharmaceutical industry. This expansion also creates new control points and sociotechnical actors in academic research governance. By contextualizing genomic technologies as important technical and social sources of momentum that unites human geneticists and pharmacologists, one sees the future of personalized medicine or clinical pharmacogenomics contingent on often indeterminate or multifactorial events.[54,55] Yet while the future remains undecided and uncertain, there is arguably an actual ethical responsibility on the part of regulatory scientists, human genetics, molecular medicine, pharmacogenomics, and social science researchers to engage in a sustained interdisciplinary, open, accountable, and transparent dialogue aimed at the development of shared standards and science policies that demonstrate optimal methodologic rigor to favorably advance discoveries and serve the best interests of patients' and public health.

In increasingly overspecialized, hypercompetitive, and fragmented biomedical research with semantic and disciplinary discontinuities,[56,57] the only assurance for continuity and objectivity in interdisciplinary fields of inquiry (eg, pharmacogenomics) will thus depend on certain human qualities in scientific professional practice and, more broadly, in public health research. These qualities include an open recognition of our own discipline-specific biases and shortcomings, giving credence to (at least noticing) hitherto disenfranchised professional viewpoints and the boundaries surrounding each discipline or individual scientific methodologies.[58,59] Reductionist conceptual juxtapositions of one discipline next to another (ie, pharmacology and human disease genetics presented as pharmacogenomics) or borrowing technologies from one discipline and applying in another without adequate reflection, in the best of circumstances, may only lead to multidisciplinary summation of scientific inquiries. But this is not necessarily equivalent to interdisciplinary synthesis and reasoned reconciliation of norms at disciplinary intersections. It is only when we comfortably place ourselves in that interdisciplinary space and acknowledge the attendant semantic and methodologic uncertainties that we can begin to dispassionately learn from other disciplines while building a more certain and ethical future for pharmacogenomics, personalized medicine, and equitable public health policies.

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We dedicate this article to our colleagues, Professors Werner Kalow and Laszlo Endrenyi (University of Toronto). Their interest in drivers of human cooperation at a population or group level and evolutionary biology helped shape our thinking on socially responsible corporate and academic research governance and their importance for equitable adoption of genomic medicine at the point of patient care.

All authors contributed to the development, interpretation, and synthesis of the ideas presented herein (from July 2004 to November 2006). The original idea for the asymmetry of inquiries between human genetics and pharmacology was conceived and contextualized by Ozdemir, Someya, Preskorn, and Friis at clinical pharmacology grand rounds at the Niigata University, Japan (April 2005), the National Institute of Mental Health–New Clinical Drug Evaluation Unit meeting (June 2005, FL), and the Summer Scholars Training Program at the VA Long Beach Healthcare System (August 2005). Ozdemir, Williams-Jones, Graham, Gripeos, Glatt, Reist, Szabo, and Lohr have discussed the need for targeted therapies, biomarker discovery, and validation before regulatory approval of pharmaceuticals, as well as the attendant importance of interdisciplinary dialogues for ensuring the application of targeted therapies that can actually serve the agenda of improving public health (in seminars at the University of California-San Diego Working Group on Personalized Medicine in Psychosis and, in part, at the Canadian Bioethics Society meeting in Halifax, October 2005). The ideas on framing pharmacology as an experimental science and how this may impact ethical, sociologic, and moral corollaries relating to the need for prospective clinical pharmacogenomic investigations were interpreted and synthesized to their final form by all authors.

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Dose-Dependent Effects of the 3435 C>T Genotype of *ABCB1* Gene on the Steady-State Plasma Concentration of Fluvoxamine in Psychiatric Patients

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Abstract: This study investigated effects of the 3435 C>T genotype of the adenosine triphosphate–binding cassette subfamily B member 1 (ABCB1, MDR1) gene on the steady-state plasma concentration of fluvoxamine (FLV).

Methods: Sixty-two psychiatric patients were treated with different doses (50, 100, 150, and 200 mg/d) of FLV. Blood samples were collected after at least 2 weeks of treatment with the same daily dose to obtain steady-state concentrations of FLV, and 3435 C>T genotype was determined by polymerase chain reaction.

Results: FLV concentration-to-dose ratio was significantly different among 3435 C>T genotype groups at the 200 mg/d dose ($P = 0.019$). A post-hoc analysis revealed that FLV concentration-to-dose ratio was significantly higher in the TT genotype group as compared with the CC genotype group at the 200 mg/d dose (median value of concentration-to-dose ratio (ng/mL)/(mg/d), 0.861 vs 0.434, $P = 0.026$). FLV concentration-to-dose ratio was significantly higher in the CT + TT genotype group than the CC genotype group at the 200 mg/d dose (median value of concentration-to-dose ratio (ng/mL)/(mg/d), 0.618 vs 0.434, $P = 0.031$). At 50, 100, and 150 mg/d dose, FLV concentration-to-dose ratios were not significantly different among 3435 C>T genotype groups. At 50, 100, and 150 mg/d dose, no significant differences were found in FLV concentration-to-dose ratios between the CT + TT genotype group and CC genotype group.

Conclusions: This study suggests that pharmacokinetics of FLV depend on *ABCB1* gene polymorphism only at the 200 mg/d dose.

Key Words: ABCB1 (MDR1/P-glycoprotein), gene polymorphism, antidepressants, SSRIs, plasma fluvoxamine concentration

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INTRODUCTION

Fluvoxamine (FLV) is a selective serotonin reuptake inhibitor (SSRI), which is used not only for the treatment of depression but also for the treatment of a variety of other psychiatric disorders, such as panic disorder, social anxiety disorder, and obsessive-compulsive disorder. Several studies have shown cytochrome-P450 (CYP) 1A2[1,2] and CYP2D6[2,3] to have a significant impact on FLV pharmacokinetics. However, other studies reported that CYP1A2[4] and CYP2D6[4,5] had no major effects on plasma FLV concentrations. The specific factors involved in the pharmacokinetics of FLV have not been clearly identified.

The adenosine triphosphate (ATP)-binding cassette subfamily B member 1 (ABCB1), which is also known as MDR1 or P-glycoprotein, is an integral membrane protein of 170 Kd and belongs to the ATP-binding cassette superfamily of membrane transporters. It serves as a potent ATP-dependent efflux pump for a wide variety of lipophilic compounds. Overexpression of ABCB1 in tumor cells confers the commonly known phenomenon of multidrug resistance against antineoplastic agents.[6,7] ABCB1 is also expressed in normal tissues such as liver, kidney, and intestine where it contributes to the elimination of xenobiotics and drugs into bile and urine or limits drug absorption from the gastrointestinal tract.[8,9]

Hoffmeyer et al[10] reported that a single nucleotide polymorphism (3435 C>T) in exon 26 of *ABCB1* gene was associated with duodenal expression of ABCB1 and associated function in humans. Carriers homozygous for this polymorphism (TT) showed more than a 2-fold lower ABCB1 expression and higher digoxin plasma concentration than the CC group. Several studies also have reported that the T allele of 3435 C>T relates to higher concentrations of drugs such as digoxin,[10,11] cyclosporine A,[12,13] and tacrolimus.[14,15]

Penetration of amitriptyline into the brain was enhanced in mice lacking P-glycoprotein.[16] The 3435 C>T genotype was also associated with the occurrence of nortriptyline-induced postural hypotension.[17] These studies suggested that ABCB1 might affect the pharmacokinetics of antidepressants. However, there have been few clinical studies investigating effects of the functional status of ABCB1 on pharmacokinetics of antidepressants. In this study, we investigated effects of the *ABCB1* gene polymorphism on steady-state plasma concentration of FLV in patients treated with different doses of FLV.

MATERIALS AND METHODS

Subjects

This study was approved by the Ethics Committee on Genetics of Niigata University School of Medicine.

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All patients received an explanation of the objectives of the study, and only those who gave written consent to participate in this study were enrolled. Demographic data; medical history; and laboratory data including hematology, serology, electrolytes, and urine analysis were collected for each patient. Patients with obvious physical illness were excluded. Patients older than 65 years of age and those younger than 20 years of age were excluded. Smokers (≥20 cigarettes/day) were also excluded because smoking is known to induce the CYP1A2 enzyme and increases the metabolisms of drugs eliminated by this enzyme. Patients concomitantly treated with other drugs, except some benzodiazepines, were excluded. The subjects included 62 Japanese outpatients (25 females and 37 males). The mean age ± standard deviation (SD) was 36.2 ± 11.9 years. Their diagnoses were major depressive disorder (n = 55), dysthymic disorder (n = 1), depressive disorder not otherwise specified (n = 1), bipolar disorder (n = 1), anxiety disorder (n = 1), bulimia nervosa (n = 1), and adjustment disorder (n = 2), according to the criteria of the *Diagnostic and Statistical Manual of Mental Disorders,* Fourth Edition, Text Revision.[18] The subjects received FLV in 2 equally divided doses at 9:00 and 21:00.

Blood Sampling

Patients were maintained on the same daily doses of FLV for at least 2 weeks to obtain steady-state concentrations of FLV. Blood sampling was done using a Venoject tube with ethylene tetraacetic acid (EDTA)-Na (Terumo Japan, Tokyo, Japan) 12 hours after the last dosage. Within 2 hours of collection, samples were centrifuged at 3,000 *g* and aliquots of plasma were drawn out in pipettes for determining plasma levels of fluvoxamine with samples stored at –80°C until assayed.

Determination of Drug Plasma Concentration

Plasma FLV concentration was measured using column-switching high-performance liquid chromatography with ultraviolet detection. Drugs in plasma to which cisapride had been added as an internal standard were extracted with hexane-chloroform. The extract was subjected to an automated column-switching high-performance liquid chromatography using a TSK BSA-C8 precolumn (Tosoh, Tokyo, Japan) for sample cleanup and a TSK gel ODS-80TS column (Tosoh) for separation.

Calibration curves (n = 6) were linear over the concentration ranging from 1.2 to 150 ng/mL ($r > 0.999$) for FLV. Intraday (n = 6) and between-days (n = 6) coefficients variations determined at 3 different concentrations (2.5, 38, and 150 ng/mL) were less than 4.7% and 3.9%, respectively. Recoveries and their coefficients variations (n = 6) determined at 3 different concentrations (1.25, 25, and 100 ng/mL) were 94.9–97.3%, and less than 1%, respectively. The limit of quantification (signal/noise ratio = 5) was 1.0 ng/mL.

Genotyping

Genomic DNA was extracted from peripheral blood using a QIA-amp Blood Kit (Qiagen, California). 3435 C>T of *ABCB1* were genotyped using the TaqMan 5'-exonuclease assay. Primer and probe sets were designed and synthesized by Applied Biosystems (Foster City, California). Polymerase chain reaction amplification was preformed using TaqMan 2x Universal Master Mix, No AmpErase UNG (Applied Biosystems), 5 ng of DNA, 0.9 μmol/L of each primer, and 200 nmol/L of each probe in total volume of 5 μL. Thermal cycler conditions were 95°C for 10 minutes, 40 cycles of 92°C for 15 seconds, and 60°C for 1 minute. Fluorescence and allelic discrimination were measured with a ABI PRISM 7900HT Sequence Detection System using SDS 2.0 software (Applied Biosystems).

Statistical Analysis

Statistical analysis was conducted with the SPSS II software for Windows (SPSS Japan Inc, Tokyo, Japan). A Mann-Whitney test was used to detect differences in FLV concentration-to-dose ratio between 50 mg/d and 200 mg/d dose treatment groups. Comparison of mean age among 3435 C>T genotypes was performed by analysis of variance (ANOVA) with the Bonferroni's test used as a post-hoc test. Differences in gender distribution among 3435 C>T genotypes were compared with the χ^2-test. Differences in FLV concentration-to-dose ratio among 3435 C>T genotypes were compared using the Kruskal-Wallis analysis with the Scheffe's test used as post-hoc test. Differences in FLV concentration-to-dose ratio between 2 genotype groups were compared by a Mann-Whitney test. A nonparametric ranking sum test (Friedman test) was used to detect differences in FLV concentration-to-dose ratio among 4 doses of FLV. Level of statistical significance was set at $P < 0.05$.

RESULTS

Of the 62 patients, 15 patients had data of FLV concentration at 1 dose, 15 patients had it at 2 different doses, 12 patients had it at 3 different doses, and 20 patients had it at 4 different doses of FLV. Median value of FLV concentration-to-dose ratio was 0.282 (50 mg/d, n = 49), 0.381 (100 mg/d, n = 42), 0.477 (150 mg/d, n = 40), and 0.554 (ng/mL)/(mg/d) (200 mg/d, n = 30); it significantly increased with increasing daily dose (50 gm/d vs 200 mg/d, $P < 0.001$).

3435 C>T genotype frequency was 0.31 (n = 19) for CC, 0.55 (n = 34) for CT, and 0.15 (n = 9) for TT. Genotype frequency was not significantly different from the values expected from the Hardy-Weinberg equilibrium ($\chi^2 = 0.99$, $P = 0.32$, df = 2).

In the 50, 100, 150, and 200 mg/d dose treatment groups, there were no significant differences among 3435 C>T genotype groups regarding mean age ($P = 0.23$, 0.35, 0.27, and 0.09, respectively; Table 1) or gender distribution ($P = 0.08$, 0.18, 0.29, and 0.08, respectively; Table 1). Furthermore, there were no significant sex-related differences in plasma FLV concentration at any doses (data not shown).

FLV concentration-to-dose ratio was significantly different among 3435 C>T genotype groups at the 200 mg/d dose ($P = 0.019$; Table 1). A post-hoc analysis revealed that FLV concentration-to-dose ratio was significantly higher in the TT genotype group than that in the CC genotype group at 200 mg/d dose ($P = 0.026$; Table 1). FLV concentration-to-dose

TABLE 1. Demographics and FLV Concentration-to-Dose Ratio (ng/mL)/(mg/d) of Subjects Classified by *ABCB1* Genotypes

	50 mg/d			100 mg/d		
	CC	CT	TT	CC	CT	TT
Number of subjects	13	29	7	12	25	5
Female/male	6/7	8/21	5/2	6/6	9/16	4/1
Age (year ± SD)	38.3 ± 12.7	37.7 ± 12.1	36.6 ± 12.0	37.8 ± 10.7	35.7 ± 11.5	28.6 ± 5.0
FLV concentration*						
Median	0.268	0.250	0.350	0.391	0.337	0.556
Range	0.088–0.762	0.032–0.776	0.210–0.832	0.105–0.825	0.058–1.888	0.277–2.172
Kruskal-Wallis test		$P = 0.15$			$P = 0.24$	
	150 mg/d			200 mg/d		
	CC	CT	TT	CC	CT	TT
Number of subjects	11	23	6	10	16	4
Female/male	6/5	8/15	4/2	5/5	6/10	4/0
Age (year ± SD)	35.4 ± 8.4	36.9 ± 12.3	28.3 ± 4.5	36.5 ± 6.2	35.2 ± 13.2	27.3 ± 4.6
FLV concentration*						
Median	0.411	0.433	0.548	0.434	0.603	0.861†
Range	0.196–1.009	0.104–2.241	0.255–2.858	0.188–0.667	0.088–2.042	0.588–3.395
Kruskal-Wallis test		$P = 0.55$			$P = 0.019$	

FLV, fluvoxamine; *ABCB1*, ATP-binding cassette subfamily B member 1 gene; SD, standard deviation.
*Concentration-to-dose ratio (ng/mL)/(mg/d).
†Post-hoc analysis revealed that plasma FLV concentration was significantly higher in the TT group than that in the CC group ($P = 0.026$).

ratio was significantly higher in the CT + TT genotype group than in the CC genotype group at the 200 mg/d dose (median value of concentration-to-dose ratio (ng/mL)/(mg/d), 0.618 vs 0.434, $P = 0.031$).

At 50, 100, and 150 mg/d dose, FLV concentration-to-dose ratios were not significantly different among C3435T genotype groups (Table 1). At 50, 100, and 150 mg/d dose, there was no significant difference in FLV concentration-to-dose ratio between the CT + TT genotype and CC genotype groups ($P = 0.96$, 0.91, and 0.55, respectively).

Table 2 shows FLV concentration-to-dose ratios of 20 patients tested at all 4 doses. In these 20 patients, FLV concentration-to-dose ratios significantly increased with increasing daily dose (Table 2). At 50, 100, 150, and 200 mg/d dose, FLV concentration-to-dose ratios were not significantly different among 3435 C>T genotype groups (Table 2). At 50, 100, 150, and 200 mg/d dose, subjects with the TT genotype of 3435 C>T had higher FLV concentration-to-dose ratios than those from the CC + CT genotype group ($P = 0.038$, 0.12, 0.13, and 0.038, respectively).

DISCUSSION

This study suggested that the 3435 C>T genotype in *ABCB1* had a significant effect on steady-state plasma concentration of FLV only at a treatment dose of 200 mg/d. Subjects with the TT genotype of 3435 C>T showed a significantly higher FLV concentration-to-dose ratio than that of subjects with the CC genotype at the 200 mg/d. Although not statistically significant, heterozygous (CT) subjects tended to have higher FLV concentration-to-dose ratios than that of CC subjects at the 200 mg/d dose. This result was consistent with those of Hoffmeyer et al's findings that showed subjects with the CC genotype of 3435 C>T had high intestinal ABCB1 expression and low digoxin concentration in contrast to TT subjects with low intestinal ABCB1 expression and high

TABLE 2. FLV Concentration-to-Dose Ratio (ng/mL)/(mg/d) of 20 Patients Tested at All 4 Doses

ABCB1 Genotype	50 mg/d		100 mg/d		150 mg/d		200 mg/d	
	Median	[Range]	Median	[Range]	Median	[Range]	Median	[Range]
All (n = 20)	0.284*	[0.048–0.832]	0.474*	[0.085–2.172]	0.526*	[0.104–2.858]	0.580*	[0.088–3.395]
CC (n = 5)	0.252	[0.164–0.338]	0.359	[0.132–0.528]	0.411	[0.196–0.751]	0.501	[0.187–0.667]
CT (n = 11)	0.250	[0.048–0.550]	0.458	[0.085–0.899]	0.464	[0.104–0.986]	0.572	[0.088–1.091]
TT (n = 4)	0.476	[0.310–0.832]	0.626	[0.467–2.172]	0.731	[0.490–2.858]	0.861	[0.588–3.395]
Kruskal-Wallis test	$P = 0.091$		$P = 0.18$		$P = 0.25$		$P = 0.072$	

FLV, fluvoxamine; *ABCB1*, ATP-binding cassette subfamily B member 1 gene.
*FLV concentration-to-dose ratio significantly increased with increasing daily dose ($P < 0.001$, Friedman test).

digoxin concentration, whereas heterozygous (CT) subjects presented with intermediate levels.[10] Our additional analysis of 20 patients tested at all 4 doses also suggested that TT subjects have higher FLV concentration-to-dose ratio compared with CC or CT subjects, although the number of subjects was small and dose-dependent effects of the 3435 C>T genotype could not be detected clearly.

Two studies[2,3] using a single oral dose of 50 mg FLV reported that the area under the plasma or serum concentration-time curve (area under the curve, or AUC) of FLV was significantly associated with CYP2D6 activity. A significant correlation between AUC of FLV and CYP1A2 activity was found in 1 study using a single oral dose of 50 mg FLV.[1] These studies suggested that pharmacokinetics of FLV depended on CYP2D6 and CYP1A2 activity at lower doses such as 50 mg FLV. However, a 2003 study[4] using 200 mg/d FLV did not find effects of CYP1A2 and CYP2D6 on the pharmacokinetics of FLV, and the possible explanation for this result was thought to be saturation of CYP1A2 and CYP2D6 at high doses of FLV. Saturation kinetics of CYP2D6 have been demonstrated for other drugs such as paroxetine.[19] CYP1A2 has also been characterized by saturation kinetics for other drugs such as theophylline[20] and caffeine.[21] Several other studies[22–24] have shown nonlinear kinetics for FLV, which is considered to be caused by the saturation of CYP1A2 and CYP2D6. In our study, FLV concentration-to-dose ratio also increased with increasing treatment dose. The 3435 C>T genotype had a significant effect on plasma FLV concentration only at the highest dose of FLV, 200 mg/day, likely because of an increasing number of subjects with saturated CYP1A2 and CYP2D6 at this higher dose.

An in vitro study[25] suggested that FLV had an ABCB1 inhibitory effect. Therefore, ABCB1 may be partly involved in nonlinear kinetics of FLV, and ABCB1 inhibitory effect of FLV may be different among *ABCB1* genotype groups. However, our study did not investigate these points.

As mentioned earlier, several reports have suggested that CYP2D6 is involved in the metabolism of FLV. Several mutated alleles of the *CYP2D6* gene causing absent activity such as *3, *4, and *5, and decreased activity such as *10, have been reported. Accordingly, in subjects having mutated alleles of CYP2D6, the CYP2D6 pathway may be easily saturated at relatively lower doses, and ABCB1 has a greater impact on FLV metabolism. Further studies analyzing genotype of *ABCB1* with that of *CYP2D6* are needed to investigate FLV pharmacokinetics in details.

The 3435 C>T polymorphism genotyped in our study causes no amino acid change. There may be other functional polymorphisms in significant linkage disequilibrium with 3435 C>T polymorphism that affect the pharmacokinetics of FLV. For instance, the G2677A/T polymorphism in exon 21 causes an amino acid change and is associated with expression of P-glycoprotein.[26,27] This polymorphism is found to be in significant linkage disequilibrium with 3435 C>T polymorphism.[26] Further studies investigating other polymorphisms including G2677A/T are needed.

In addition, it would be interesting to investigate effects of *ABCB1* gene polymorphisms on FLV pharmacokinetics at doses higher than 200 mg/d because high doses of SSRIs are occasionally needed to obtain a therapeutic effect in the treatment of psychiatric disorders such as obsessive-compulsive disorder.

CONCLUSION

This study suggests that pharmacokinetics of FLV depend on 3435 C>T genotype of *ABCB1* at high doses such as 200 mg/d. However, sample size of the present was quite small, and only 4 subjects carried the TT genotype of 3435 C>T in the 200 mg/d treatment group. Further studies with a larger sample size are needed to clarify the extent of involvement of *ABCB1* gene polymorphism on FLV pharmacokinetics.

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PHARMACOKINETICS AND DISPOSITION

Terbinafine increases the plasma concentration of paroxetine after a single oral administration of paroxetine in healthy subjects

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Abstract
Objective Paroxetine is believed to be a substrate of CYP2D6. However, no information was available indicating drug interaction between paroxetine and inhibitors of CYP2D6. The aim of this study was to examine the effects of terbinafine, a potent inhibitor of CYP2D6, on pharmacokinetics of paroxetine.
Methods Two 6-day courses of either a daily 150-mg of terbinafine or a placebo, with at least a 4-week washout period, were conducted. Twelve volunteers took a single oral 20-mg dose of paroxetine on day 6 of both courses. Plasma concentrations of paroxetine were monitored up to 48 h after dosing.
Results Compared with the placebo, terbinafine treatment significantly increased the peak plasma concentration (C_{max}) of paroxetine, by 1.9-fold (6.4±2.4 versus 12.1±2.9 ng/ml, $p<0.001$), and the area under the plasma concentration-time curve from zero to 48 h [AUC (0–48)] of paroxetine by 2.5-fold (127±67 vs 318±102 ng/ml, $p<0.001$). Elimination half-life differed significantly (15.3±2.4 vs 22.7±8.8 h, $p<0.05$), although the magnitude of alteration (1.4-fold) was smaller than C_{max} or AUC.
Conclusion The present study demonstrated that the metabolism of paroxetine after a single oral dose was inhibited by terbinafine, suggesting that inhibition of CYP2D6 activity may lead to a change in the pharmacokinetics of paroxetine. However, further study is required to confirm this phenomenon at steady state.

Keywords Paroxetine · Terbinafine · CYP2D6 · Interaction

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Introduction

Paroxetine is one of the selective serotonin transporter inhibitors (SSRI) and is widely used in the treatment of mental disorders, including depression, panic disorders, and obsessive compulsive disorder [1, 2]. Paroxetine is almost completely absorbed following oral administration. However, the drug undergoes extensive first pass metabolism [3, 4]. As a result, less than 50% of a single dose of paroxetine reaches the general circulation. Paroxetine is eliminated by metabolism involving oxidation, demethylation, and conjugation [5].

An early in vivo study using healthy volunteers demonstrated a cosegregation between paroxetine and sparteine metabolism, and that steady-state plasma concentration of paroxetine in poor metabolizers (PM) was higher than extensive metabolizers (EM) [6, 7]. Moreover, an in vitro study demonstrated that quinidine and quinine, both of which are potent inhibitors, inhibit paroxetine demethylation in human liver microsomes from EM, but did not inhibit that from PM [8]. Based on these findings, it is evident that paroxetine is primarily metabolized by the cytochrome CYP2D6. However, there are few in vivo data,

indicating that paroxetine concentration is influenced by CYP2D6 modulators despite the fact that numerous in vivo and in vitro studies used paroxetine as a potent inhibitor of CYP2D6 [9–12].

Terbinafine is an orally active allylamine antifungal agent with a primarily fungicidal action in vitro [13, 14]. Clinical trials have demonstrated that orally administered terbinafine is effective in the treatment of dermatophyte infection of the skin [15]. Several in vitro studies demonstrated that terbinafine has a potent inhibitory effect on CYP2D6 activity [16–18]. Several in vivo studies indicated that terbinafine inhibited dextromethorphan [19] and desipramine [20], both of which are substrates of CYP2D6, suggesting an inhibitory effect of terbinafine on CYP2D6 in vivo. Moreover, several case reports demonstrated two cases who suffered from desipramine toxicity [21] and nortriptyline toxicity [22] induced by terbinafine. Terbinafine increased CYP2D6-mediated amitriptyline and nortriptyline concentration for at least 6 months [23], suggesting terbinafine inhibited CYP2D6-mediated metabolism of these antidepressants. Therefore, it is more likely that terbinafine affects the disposition of paroxetine. To our knowledge, however, there is no information about drug–drug interaction between terbinafine and paroxetine. The aim of this study was to confirm the effects of terbinafine on the pharmacokinetics of paroxetine.

Methods

Subjects

Twelve healthy Japanese volunteers (nine males, three females) were enrolled in this study. Their mean±SD of age (range) was 24.8±2.5 (20–35) years and mean body weight was 58.3±8.5 (46–75) kg. The Ethics Committee of Hirosaki University School of Medicine approved this study protocol, and written informed consent had been obtained from each participant before any examinations.

Study design

A randomized crossover study design was conducted at intervals of 4 weeks. One capsule containing either 125 mg of terbinafine or a matched placebo with 240 ml of tap water was given once daily at 0800 hours for 6 days. Compliance of thetest drug was confirmed by pill-count. No other medications were taken during the study periods. No meal was allowed until 4 h after dosing (1300 hours). The use of alcohol, tea, coffee and cola was forbidden during the test days.

Sample collections

Blood samplings (10 ml each) for determination of paroxetine were taken into heparinized tubes just before and 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 36 and 48 h after the administration of paroxetine. Plasma was separated immediately and kept at −30°C until analysis. At the same time as the blood samplings, blood pressure and heart rate were measured. Any adverse events were reported by each subject.

Assay

Plasma concentrations of paroxetine were measured using a high-performance liquid chromatography method developed in our laboratory. In brief, extraction procedure was as follows: to 2,000 μl of plasma sample was added 500 μl of 0.5 M NaOH, 100 μl of internal standard solution (trifluperidol 200 μg/ml) and 100 μl of methanol. Thereafter, the tubes were vortex-mixed for 10 s and 5μml of n-heptane–chloroform (70:30, v/v) was added as extraction solvent. After 10 min of shaking, the mixture was centrifuged at 2,500 *g* for 10 min at 4°C, and the organic phase was evaporated in vacuo at 40°C to dryness (TAITEC VC-960, Shimadzu, Kyoto, Japan). The residue was dissolved in 500 μl of mobile phase, then 400 μl were injected onto the HPLC system. The HPLC system consisted of Shimadzu LC-10AT high-pressure pumps, a Shimadzu CTO-10AVP column oven and a Shimadzu Work station CLASS-VP chromatography integrator (Kyoto, Japan), a Shimadzu SPD-10AVP (Kyoto, Japan) and a Shimadzu SIL-10ADVP (500-μl injection volume) (Tokyo, Japan) and a column (STR-ODS II C18 150×4.6, 3 μm) (Tokyo, Japan). The mobile phase was phosphate buffer (0.02 M, pH=4.6), acetonitrile and perchloric acid (60%) (57.25: 42.5: 0.25, v/v/v). The lowest limit of detection and quantification were 0.5 and 1.0 ng/ml, respectively, and the values of the intra-assay and inter-assay coefficient of variation were less than 10 % at all the concentrations (1.0–150 ng/ml) of calibration curves for paroxetine.

CYP2D6 genotypes

For the determination of *CYP2D6* genotype, DNA was isolated from peripheral leukocytes by a guanidium isothiocyanate method. Genotypings of CYP2D6 were performed using AmpliChip CYP450 Test DNA chip (Roche Diagnostics). The AmpliChip CYP450 Test provides materials for genotyping two cytochrome genes, encompassing 31 known mutations in the CYP2D6 gene, including duplication and gene deletion (CYP2D6*2, *3, *4, *5, *6, *7, *8, *9, *10AB, *11, *14A, *15, *17, *19, *20, *25, *26, *29, *30, *31, *35, *36, *40, *41, *1XN,

*2XN, *4XN, *10XN, *17XN, *5XN, *41XN). The alleles without activity were *3, *4, *5, *6, *7, *8, *11, *14A, *15, *19, *20, *40. The alleles which lead to the decreased enzyme activity were CYP2D6*9, *10, *17, *29, *36, *41.

Date analyses of pharmacokinetics

The peak concentration (C_{max}) and concentration peak time (t_{max}) were obtained directly from the original data. The area under the plasma concentration-time curve [AUC (0–48)] was calculated with use of the lin-lin trapezoidal rule. AUC from zero to infinity [AUC(0–∞)] and elimination half-life were determined by non-compartment model with WinNonlin Professional software (Pharsight, Cary, N.C., USA). The terminal elimination rate constant (k_e) was determined by log-linear regression of the final data points (4). The apparent elimination half-life of the log-linear phase ($t_{1/2}$) was calculated as follows: $0.693/k_e$.

Statistical analysis

Data are shown as means±SD in tables and figures. Paired *t*-test was used for the comparison of the plasma drug concentrations between two phases, i.e., placebo and terbinafine. The comparison of $t_{max}$was performed using the Wilcoxon signed-sample test. A *p*value of 0.05 or less was regarded as significant. Geometric mean ratios to corresponding values in placebo phase with 95% confidence intervals were used for detection of significant differences. When the 95% confidence interval did not cross 1.0, the result was also regarded as significant. When the calculated 90% confidence intervals with logarithmic transformation of pharmacokinetic data (C_{max}, AUC(0–48) and total AUC) fell within, on average, 80–125% for the ratio of the paroxetine plus terbinafine to paroxetine only, we regarded the paroxetine plus terbinafine as bioequivalent to paroxetine only. SPSS 13.0J for Windows (SPSS Japan, Tokyo) was used for these statistical analyses.

Results

The subjects had the following CYP2D6 genotypes: *wt/wt* (4 subjects), **10/wt* (6), **10/*10* (1) and **5/*10* (1), respectively. No subjects regarded as poor metabolizers were included. These patients were divided into three groups according to the number of mutated alleles: no mutated allele in 4, one mutated allele in 6 and two mutated alleles in 2 subjects.

Appetite loss (n=4, n=6), abdominal disturbance (n=4, n=7), diarrhea (n=1, n=1), asthenia (n=5, n=7) and sleepiness (n=3, n=4) were observed in control and terbinafine phases, respectively. All of these side effects were mild to moderate and recovered at longest within 2 days after paroxetine doses.

Plasma drug concentration-time curves during both placebo and terbinafine treatments are shown in Fig. 1. Their pharmacokinetic parameters are summarized in Table 1, and individual data are shown in Table 2. There were three subjects whose plasma concentrations of paroxetine 48 h after paroxetine dosing in control phase were under the detectable quantification (1.0 ng/ml). Although we were not able to calculate accurate averages in 12 subjects, we calculated the average at 48 h in Fig. 1, using the half of limit of quantification (0.5 ng/ml) in the three subjects. Extrapolated AUC of paroxetine was 9.3% for control and 24.9% for terbinafine, respectively.

The C_{max} of paroxetine during terbinafine treatment was higher than the corresponding value during placebo by 1.87-fold [95% confidence interval (95% CI), 1.49, 2.57-fold]. The AUC (0–48) of paroxetine during trebinafine treatment was higher than placebo by 2.53-fold (1.85, 4.58-fold). The total AUC of paroxetine during trebinafine treatment was higher than placebo by 2.88-fold (1.99, 5.41-fold). Elimination $t_{1/2}$ of paroxetine during trebinafine was significantly longer than that during placebo [1.35-fold (1.14, 1.70-fold)]. No change was found in t_{max} [1.14-fold (0.99, 1.36-fold)].

Bioequivalence analyses showed that 90% confidence interval of log-transformed C_{max}, AUC (0–48) and total AUC between two treatments were 130–148%, 116–127 and 118–128%, respectively.

There was significant correlation between number of mutated alleles for CYP2D6 and Cmax (r_s=0.772, p<0.01), AUC(0–48) (r_s=0.724, p<0.05) and total AUC (r_s=0.724, p<0.05). Significant correlations were found between

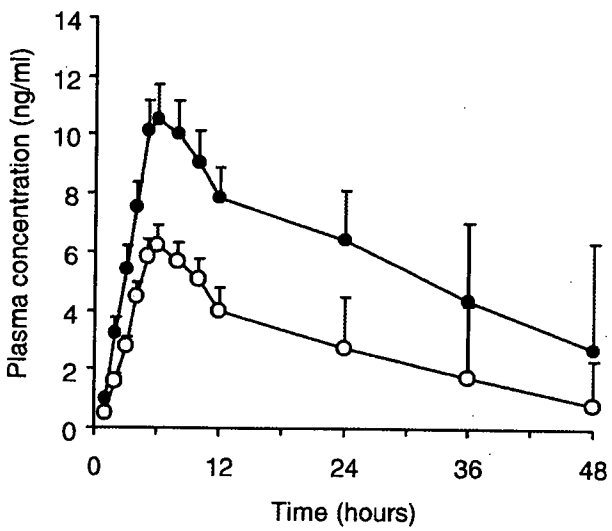

Fig. 1 Mean plasma concentration-time curves of paroxetine after a single oral 20 mg dose of paroxetine. *Open circles* indicate control and *solid circles* indicate terbinafine. *Error bars* indicate standard error

Table 1 Effects of terbinafinee treatment on paroxetine pharmacokinetic parameters after a single oral 20-mg dose of paroxetine in 12 healthy volunteers

Parameters	Control	Terbinafine	Ratio to control
C_{max}(ng/ml)	6.5±2.4	12.1±2.9***	1.92 (1.71, 2.22)
t_{max}(h)	5.0 (4.0–10.0)	6.0 (5.0–8.0)	
AUC (0–48) (ng.h/ml)	127±67	318±102***	2.64 (2.07, 3.64)
AUC (0–∞) (ng.h/ml)	150±78	443±172***	3.03 (2.46, 4.08)
Vd/F (l)	3,599±1,515	1,577±528***	0.47 (0.36, 0.76)
CL/F (l/h)	167±76	58±44***	0.35 (0.25, 064)
Elimination half-life (h)	15.3±2.3	22.7±8.8*	1.39 (1.13, 1.86)

Data are shown as mean±SD for pharmacokinetic parameters and geometric mean
(95% confidence interval) for ratio to control
Data for tmax are shown as median and range
C_{max}, peak concentration; t_{max}, time to peak concentration in plasma; AUC (0–48), are under plasma concentration-time curve from 0 to 48 h; AUC (0–∞), AUC from 0 to infinity; Vd/F, apparent volume of distribution; CL/F, apparent total clearance
*$p<0.05$, ***$p<0.001$

number of mutated alleles for CYP2D6 and ratio of terbinafine phase to placebo for Cmax ($r_s=-0.628$, $p<0.05$), AUC(0–48) and ($r_s=-0.774$, $p<0.01$), but not total AUC ($r_s=-0.477$, ns). Elimination half-life was not correlated with number of mutated alleles for CYP2D6 ($r_s=0.211$, ns).

There was no relationship between total AUC and the observed side effects after single dose of paroxetine. No difference was found in different sequence (placeco–terbinafine versus terbinafine–placebo).

Discussion

The results of this study showed a significant increase in plasma concentration of paroxetine (C_{max} and AUC) during terbinafine treatment. Additionally, the paroxetine pharmacokinetics were not regarded as bioequivalent according to EMEA and FDA guidance. These findings imply that terbinafine increases the bioavailability of paroxetine or inhibits the metabolism of paroxetine. Our result was in accordance with the previous studies of dextrometrophan [19] and desipramine [20]. Paroxetine is almost completely absorbed following oral administration. However, because paroxetine undergoes extensive first pass metabolism, less than 50% of a single dose of paroxetine reaches the general circulation, suggesting that the first pass effect of paroxetine is more than 50% [4]. Therefore, terbinafine might inhibit the first pass effect of paroxetine in this study.

Furthermore, prolongation of elimination of half-life was observed during terbinafine coadministration. This suggests that terbinafine inhibits metabolism of paroxetine. Previous studies confirmed the decrease in their metabolites, 2-hydroxydesipramine [20] and dextrophan [19], during terbinafine as well as the increase in the substrates of CYP2D6, suggesting that terbinafine inhibits biotransformation from despramine to 2-hydroxydesipramine and from dextrometrophan to dextrophan. Unfortunately, we were unable to measure the metabolites of paroxetine because of undetectable levels in both phases.

Numbers of mutated alleles for CYP2D6 correlated well with Cmax and AUC, but not with elimination half-life in this study. This finding suggests that CYP2D6 activity is associated with the first pass effect of paroxetine. Furthermore, we found a significant relationship between CYP2D6 genotype and the magnitude of this interaction. In subjects having lower CYP2D6 activity, the inhibitory effect of

Table 2 Characteristics of healthy subjects and their individual data

No.	Age	Gender	Weight	CYP2D6	C_{max}(ng/ml)		AUC (0–∞) (ng.h/ml)		Elimination half-life (h)	
					Control	Terbinafine	Control	Terbinafine	Control	Terbinafine
1	29	Male	75	*1/*10	4.7	10.9	88	448	16.2	38.4
2	25	Male	63	*2/*10	6.2	14.2	123	547	12.4	29.6
3	21	Male	64	*1/*1	3.2	6.8	66	99	11.7	8.8
4	21	Female	47	*1/*2	4.3	11.6	72	419	17.0	15.8
5	26	Female	49	*2/*5	6.4	10.2	129	350	13.8	16.6
6	25	Male	57	*1/*1	4.8	10.2	78	350	13.3	16.6
7	20	Male	52	*5/*10	12.3	16.2	304	830	16.1	30.5
8	24	Female	46	*10/*10	9.3	16.9	286	574	16.2	22.9
9	22	Male	54	*1/*10	6.4	10.9	166	406	15.6	18.5
10	24	Male	62	*2/*10	6.9	9.9	141	409	14.1	33.0
11	25	Male	64	*1/*1	6.3	14.9	161	385	18.9	15.7
12	35	Male	66	*1/*10	6.9	12.4	185	510	19.0	25.9

C_{max}, peak concentration; AUC (0–∞), AUC from 0 to infinity;

terbinafine is expected to have been smaller. This confirms that these interactions resulted from CYP2D6 inhibition.

An earlier in vivo study demonstrated that the interphenotype difference in metabolism was less prominent at steady state than after a single dose [7]. There is ongoing controversy as to whether or not the steady-state plasma concentration of paroxetine is different between CYP2D6 genotypes. A recent study indicated no relationship between CYP2D6 genotypes and steady-state plasma concentration of paroxetine [24], although there were significant differences between the CYP2D6 genotypes [25, 26]. Therefore, the magnitude of our interaction might be reduced after repeated doses. Thus, further studies are required to confirm our finding even at steady state.

Severely depressed patients tend to suffer from dermatophytosis in skin, hair and nails due to the difficulty in self-care. On the other hand, systemic fungal infections remain a major clinical problem in immunocompromised patients and such patients tend to have negative thinking [27]. From a clinical point of view, it is more likely that terbinafine is added to depressed patients treated with paroxetine. Thus, interaction between paroxetine and terbinafine should be kept in mind by physicians. Although there is no information about drug interaction between repeated doses of these drugs, dosage of paroxetine should be adjusted based on our result showing the increased exposure of paroxetine during terbinafine.

Limitations of this study included sampling time and washout period. We had a 4-week washout period in this study. Extrapolated AUC of paroxetine was 9.3% for control and 24.9% for terbinafine. If we took blood samples for a longer period (e.g., 72 h), more accurate parameters of paroxetine might have been calculated. In addition, a case report suggested that the effect of terbinafine on CYP2D6-mediated amitriptyline and nortriptyline concentration continues for at least 6 months [23]. Madani et al. [20] suggest that the inhibitory effect of terbinafine on CYP2D6 measured by desipramine continues for 4 weeks, while Abdel-Rahman et al. [19] suggest that CYP2D6 inhibition of terbinafine measured by dextrometrophan metabolic ratios in most subjects return to baseline at 4 weeks after discontinuation of terbinafine. Although half dose (125 mg) and shorter duration (7 days) than previous studies by Madani et al. (250 mg for 21 days) and Abdel-Rahman et al. (250 mg for 14 days) were used in our study and no difference was found in different sequences (placeco–terbinafine versus terbinafine–placebo), the inhibitory effect of terbinafine on CYP2D6 might remain until the control phase.

In conclusion, the present study showed that terbinafine increased paroxetine exposure, probably because of an increase in bioavailability through CYP2D6 inhibition. A change in regulation of CYP2D6 may lead to significant alteration of paroxetine pharmacokinetics.

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No association between CYP2D6 polymorphisms and personality trait in Japanese

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What is already known about this subject

- CYP2D6 mediates, to some extent, the synthesis of the neurotransmitters, serotonin and dopamine in the brain. A positive relationship between CYP2D6 activity and personality has been suggested, while other studies have failed to find a relationship.
- Therefore, because of these inconsistent findings, the association between CYP2D6 polymorphism and interindividual variability in personality has not yet been resolved.

What this study adds

- The detailed genotypes for *CYP2D6* were identified using DNA tip in a large number of Japanese subjects. However there were no correlations between scores of personality and *CYP2D6* genotypes.
- This study demonstrated that there was no significant association between CYP2D6 activity and personality trait in the Japanese population.

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Aims
The polymorphic enzyme CYP2D6 is expressed not only in liver but also in brain at low concentrations. CYP2D6 mediates, to some extent, the synthesis of the neurotransmitters, serotonin and dopamine. We investigated a possible association between the genetic polymorphism of CYP2D6 and individual personality trait.

Methods
Mentally and physically healthy volunteers were recruited ($n = 342$). Temperament and Character Inventory (TCI) and *CYP2D6* genotyping were performed in all subjects. We detected mutated alleles which were identified using the Amplichip CYP450 DNA chip.

Results
The number of phenotypes, assumed by genotype for ultrarapid metabolizers (UM), extensive metabolizers (EM), intermediate metabolizers (IM) and poor metabolizers (PM) were 4 (1.1%), 262 (76.6%), 75 (21.9%) and 1 (0.3%), respectively. There were no differences in scores for novelty seeking, harm avoidance, reward dependence or persistence among the *CYP2D6* phenotypes. The number of mutated alleles for CYP2D6 did not differ for scores of novelty seeking, harm avoidance, reward dependence or persistence. In subitem analyses, only RD3 (attachment) had a significant difference both in the *CYP2D6* phenotype ($P < 0.05$) and genotype ($P < 0.05$).

Conclusions
This study did not demonstrate a significant association between CYP2D6 activity and personality trait because of the small interindividual variability in CYP2D6 activity within the Japanese population.

Introduction

Cloninger *et al.* [1] demonstrated that human personality consists of seven dimensions including three temperament dimensions and four character dimensions, and on the basis of this model they developed the temperament and character inventory (TCI), a questionnaire for assessing personality traits. Of the temperament dimensions, which include novelty seeking, harm avoidance, reward dependence, and persistence, three have been assumed to be related to monoamine neurotransmitters, novelty seeking with dopaminergic activities, harm avoidance with serotonergic activities and reward dependence with noradrenergic activity.

It has been reported that CYP2D6 is expressed not only in the liver but also in the brain at low concentrations [2]. The CYP2D6 in the brain has been shown to interact with the dopamine transporter and it has been suggested that it plays a role in the catabolism and processing of neurotransmitters [3–5]. Genetic polymorphism in CYP2D6 has been associated with smoking behaviour and this modification may occur through the involvement of CYP2D6 in the dopaminergic pathway. In addition, CYP2D6 mediates the synthesis of the neurotransmitters, serotonin and dopamine, from tyramine and 5-methoxytryptamine [2]. Since these neurotransmitters are linked with personality trait according to Cloninger's model, it is likely that genetic polymorphism of CYP2D6 is associated with individual personality. However, the association is inconsistent. Several studies have suggested a positive relationship between CYP2D6 activity and personality [6–8], while another study failed to find the relationship [9].

Therefore, the aim of the present study was to clarify the possible relationship between genotypes of *CYP2D6* polymorphism and behavioural traits in a large sample of Japanese, as measured by the TCI.

Methods

This study was approved by the Ethics Committee of Hirosaki University School of Medicine. The subjects enrolled consisted of Japanese students and medical staff within the medical school ($n = 342$, male = 166, female = 176). Their mean age was 29.9 ± 11.4 years (range 18–69 years). After giving the subjects a full description of the study, written informed consent to participate was obtained from each of them. The subjects were asked to complete the 240 items of the Japanese version of the TCI, whose reliability and validity had been established by Kijima *et al.* [10]. The 240 items were allocated into four factors of temperaments (novelty seeking, harm avoidance, reward dependence and persistence) and three factors of characters (self-directedness, co-operativeness and self-transcendence), where self-transcendence consisted of self-forgetfulness, transpersonal identification and spiritual acceptance. Genotyping of CYP2D6 was performed using the AmpliChip CYP450 test® DNA chip (Roche Co., Tokyo, Japan). The AmpliChip CYP450 Test provides materials for genotyping the *CYP2D6* gene, encompassing 31 known mutations (CYP2D6*2, *3, *4, *5, *6, *7, *8, *9, *10AB, *11, *14, *15, *17, *18, *19, *20, *25, *26, *29, *30, *31, *35, *36, *40, *41, *1XN, *2XN, *4XN). The alleles which led to no enzyme activity are CYP2D6*3, *4, *5, *6, *7, *8, *11, *14, *15, *19, *20 and *40. The alleles which led to decreased enzyme activity are CYP2D6*9, *10, *17, *29, *36 and *41. CYP2D6*2 and *35 are regarded as normal enzyme activity. Based on the *CYP2D6* genotypes, subjects were grouped into four main phenotypes: poor metabolizer (PM) (two deficit alleles, e.g. *5/*5), intermediate metabolizer (IM) (either two reduced activity alleles or one reduced activity allele and one no activity allele, e.g. *4/*10, *5/*10, *10/*10), extensive metabolizer (EM) (one or more wild type alleles, e.g. *1/*1, *1/*2, *1/*5, *1/*10), and ultrarapid metabolizer (UM) (more than two copies of wild type, e.g. *1/*1XN, *1/*2XN). The mean scores for the seven factors of the TCI were compared among UM, EM and IM and between the number of mutated alleles using ANOVA. Because only one of our subjects was a PM, PM was excluded from the statistical analysis. The result was defined as being significant at $P < 0.05$.

Results

The genotypes of our subjects were *1/*1 ($n = 65$, 19.0%), 1/*2 ($n = 33$, 9.6%), *1/*4 ($n = 1$, 0.3%), *1/*5 ($n = 95$, 4.4%), *1/*10 ($n = 95$, 27.8%), *1/*14 ($n = 5$, 0.3%), *1/*36 ($n = 1$, 0.3%), *1/*41 ($n = 5$, 1.5%), *1/*1xN ($n = 2$, 0.6%), *1/*2xN ($n = 1$, 0.3%), *2/*2 ($n = 7$, 2.0%), *2/*4 ($n = 1$, 0.3%), *2/*5 ($n = 6$, 1.8%), *2/*10 ($n = 30$, 8.8%), *2/*14 ($n = 1$, 0.3%), *2/*41 ($n = 1$, 0.3%), *2/*2xN ($n = 1$, 0.3%), *4/*10 ($n = 1$, 0.3%), *5/*5 ($n = 1$, 0.3%)), *5/*10 ($n = 19$, 5.6%), *5/*36 ($n = 1$, 0.3%), *10/*10 ($n = 50$, 14.6%), *10/*14 ($n = 1$, 0.3%), *10/*41 ($n = 31$, 0.9%). The number of phenotypes, assumed by genotype, for UM, EM, IM and PM were 4 (1.1%), 262 (76.6%), 75 (21.9%) and 1 (0.3%), respectively. There were no differences in the mean scores for the seven TCI factors between the phenotypes, assumed by genotype (Table 1). In subitem analyses, only RD3 (Attachment) had a significant difference in *CYP2D6* phenotype ($P = 0.017$).

The number of *CYP2D6* genotypes based on the number of mutated alleles was 109 (31.9%) for zero

Table 1
Comparison of the temperament and character inventory (TCI) subitem scores among phenotypes assumed by *CYP2D6* genotypes

	UM ($n=4$)	EM ($n=262$)	IM ($n=75$)	PM ($n=1$)	Significance
Novelty seeking	18.3 (16.7, 19.8)	21.4 (20.9, 22.0)	21.5 (20.4, 22.5)	27	NS
Harm avoidance	19.3 (13.2, 25.5)	19.8 (19.0, 20.6)	20.0 (18.6, 21.2)	17	NS
Reward dependence	17.5 (15.9, 19.1)	14.7 (14.3, 15.1)	15.5 (14.7, 16.2)	15	NS
Persistence	4.8 (1.2, 8.3)	4.4 (4.1, 4.6)	4.2 (3.8, 4.6)	7	NS
Self-directedness	32.0 (24.1, 40.0)	26.1 (25.3, 26.9)	26.8 (25.2, 28.4)	27	NS
Co-operativeness	31.5 (27.3, 35.7)	28.0 (27.4, 28.6)	28.4 (27.2, 29.6)	32	NS
Self-transcendence	7.8 (4.5, 11.0)	10.7 (10.1, 11.3)	9.6 (8.4, 10.7)	13	NS

Data are mean (95% confidence interval); UM; ultra rapid metabolizer, EM, extensive metabolizer, IM; intermediate metabolizer, PM; poor metabolizer.

Table 2
Comparison of the temperament and character inventory (TCI) subitem scores among the number of mutated alleles for CYP2D6

	Number of mutated alleles			
	0 ($n=109$)	1 ($n=157$)	2 ($n=76$)	Significance
Novelty seeking	21.2 (20.3, 22.1)	21.6 (20.8, 22.3)	21.5 (20.5, 22.6)	NS
Harm avoidance	20.3 (19.1, 21.4)	19.4 (18.5, 20.4)	20.0 (18.7, 21.3)	NS
Reward dependence	14.6 (13.9, 15.2)	14.9 (14.4, 15.4)	15.4 (14.7, 16.2)	NS
Persistence	4.3 (3.9, 4.6)	4.5 (4.2, 4.8)	4.2 (3.8, 4.6)	NS
Self-directedness	26.4 (25.2, 27.7)	26.0 (25.0, 27.1)	26.8 (25.2, 28.4)	NS
Co-operativeness	27.8 (26.7, 28.8)	28.2 (27.4, 29.0)	28.5 (27.3, 29.7)	NS
Self-transcendence	9.7 (8.9, 10.6)	11.3 (10.5, 12.1)	9.7 (8.6, 10.8)	$P<0.05$

Data are mean (95% confidence interval).

alleles, 157 (45.9%) for one allele, and 76 (22.2%) for two alleles, respectively. The number of mutated alleles for CYP2D6 did not differ with the scores of TCI for six factors except self-transcendence which differed significantly. (Table 2). *Post hoc* analysis showed a significant difference between 0 mutated allele *vs.* 1 mutated allele ($P = 0.034$). In subitem analyses, only RD3 (attachment) had significant difference in *CYP2D6* genotype ($P = 0.034$).

Discussion

The results of this study showed that scores of TCI (novelty seeking, harm avoidance, reward dependence and persistence) were not different between *CYP2D6* phenotypes, as assumed by *CYP2D6* genotype in Japanese. There are three possibilities to explain these results. First, since we had only one PM in the present study, PM was not included in statistical analyses. In addition, four UMs were not enough to detect the difference. This is ascribable to a narrower variation of CYP2D6 activity than that in previous studies using Caucasians. Therefore, the relatively small difference in CYP2D6 activity in Japanese was not enough to detect differences in personality. Second, most of our subjects were medical students and medical staff. Although statistical analyses were not performed, it is possible that the characteristics of our subjects deviated and had small interindividual variability in personality trait. Last, although cross validation between the original TCI and its Japanese version was performed, there is a possibility