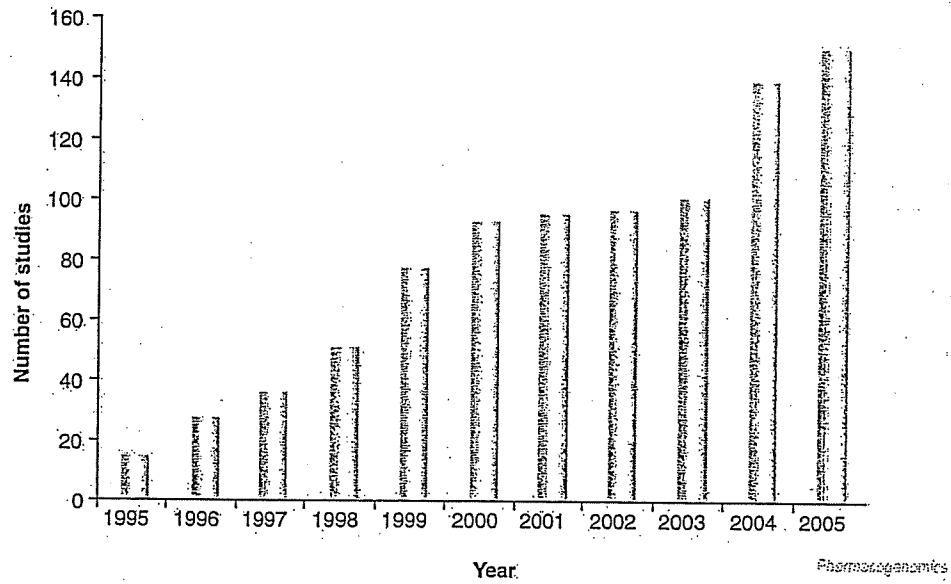
Figure 3. Proliferation in the number of published clinical pharmacogenetic association studies of the serotonin system candidate genes from 1995 to 2005.



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 actually 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

were detected by PCR–RFLP methods as described by Tanabe et al. (2001).

2.5. Assay

Plasma concentrations of bromperidol and reduced bromperidol were determined in duplicate using the high-performance liquid chromatographic (HPLC) methods developed in our laboratory (Yasui-Furukori et al., 2004a). The lowest limit of detection was 1.0 ng/ml, and interassay coefficient of variation (CV) was less than 5.1% for both compounds.

2.6. Data analyses and statistics

The 18 items in BPRS were divided into five subgroups, i.e., positive (exaggerated self-esteem, suspiciousness, hallucinations and unusual thought content), excitement (hostility, uncooperativeness and psychomotor agitation), cognitive (conceptual disorganization, specific motor disturbance and disorientation), negative (emotional withdrawal, psychomotor retardation and blunted affect) and anxiety–depression (somatic concern, anxiety [psychic], self-depreciation, anxiety [somatic] and depressive mood) symptoms as classified by Lindenmayer et al. (1995). The UKU items were also divided into three subgroups, i.e., psychic (concentration difficulties, asthenia, sleepiness, failed memory and depression), extrapyramidal (dystonia, rigidity, hypokinesia, hyperkinesia, tremor, akinesia and increased salivation) and autonomic (accommodation disturbances, reduced salivation, constipation, micturation disturbance, orthostatic dizziness and palpitation) side effects. A pretreatment score was subtracted from one obtained during the treatment and was recorded as drug-associated side effect. Scores of subgrouped side effects after 3-week treatment were used for statistical analysis. In only analysis of EPSs, the data in patients treated with biperiden were excluded after administration of the medication.

The comparisons of severity of illness (baseline BPRS scores), age, body weight, duration of illness and plasma concentrations of bromperidol and reduced bromperidol between *MDR1* genotypes were made by ANOVA followed by Tukey's test. Multiple regression analyses were conducted for correlations of age, gender, drug concentrations, co-administration of biperiden and *MDR1* genotypes with % improvement in 5 sub-grouped BPRS symptoms and UKU scores of 3 sub-grouped side effects. Dummy variables (male=0 and female=1) were used for analysis of gender effects. Because *C3435T* (Hoffmeyer et al., 2000) and *G2677T/A* (Siegmund et al., 2002) genotype proportionately expressed P-glycoprotein in intestine, dummy variables were used for analyses of *MDR1* genotype effects as follows: *CC*=2, *CT*=1 and *TT*=0 for *C3435T*, and *GG*=2, *GA* or *TA*=1 and *TT*, *TA* or *AA*=0 for *G2677T/A*. Effects of antiparkinson agents on therapeutic response were compared using *t*-test. A p value less than 0.05 was regarded as statistically significant. All analyses were performed using SPSS 12.0J for windows (SPSS Japan Inc., Tokyo, Japan).

3. Results

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 was no difference in clinical profiles such as age, gender or duration of illness between *C3435T* genotypes or between *G2677T/A* genotypes. Since significant difference in baseline BPRS scores was found between *C3435T* genotypes, statistical significance both in percentage improvement and improved scores were defined as clinically relevant findings.

Table 1
Clinical profiles, drug therapy and percent improvement in *C3435T* and *G2677T/A* genotypes

	C3435T			*G2677T/A*		
	C/C (n=12)	*C/T* (n=12)	*T/T* (n=7)	*G/G* (n=3)	*G/T* or *A* (n=17)	*T* or *A/T* or *A* (n=11)
Clinical profile						
Age (years)	37±12	34±13	41±13	46±9	34±13	39±13
Gender (M/F)	1	5/7	6/1	1/2	10/7	6/5
Duration of illness (month)	108±100	115±100	158±107	216±113	85±71	154±116
Baseline BPRS score	29±6	22±6	25±5*	31±2	25±8	24±5.1
Drug therapy						
Dose (mg/day)	11.4±5.0	9.3±3.9	16.3±2.7*	8.0±2.8	10.7±4.6	14.2±4.6
Plasma concentration (ng/ml)	6.7±5.0	5.7±3.1	11.1±2.9*	7.2±4.6	6.3±4.2	9.0±4.3
Prolactin response (ng/ml)	15.2±13.9	11.4±7.9	13.7±8.9	9.1±6.5	14.3±12.8	13.2±8.1
Percent improvement						
Total BPRS score	61±32	56±29	47±27	79±14	62±30	41±26
Positive	63±38	56±42	64±30	87±18	65±35	47±42
Excitement	74±30	77±26	61±47	83±24	76±27	63±44
Cognitive	89±21	85±33	19±104	100±0	89±20	35±92
Negative	47±52	32±51	39±38	72±21	48±46	16±51
Anxiety–deperssion	34±41	41±46	29±13	56±14	37±47	24±24

Data are shown as mean±S.D.
* p<0.01 among three *C3435T* genotypes.

Although there were significant differences in clinical profiles such as daily dose or plasma concentration of bromperidol between *C3435T* genotypes ($p<0.05$) and post hoc analyses showed significant differences in *C/T* vs. *T/T*, there were no differences in the percentage improvement or the improved scores of total BPRS scores or 5 sub-grouped symptoms after the 3-week treatment between *C3435T* genotypes (Table 1). Clinical profiles did not differ between *G2677T/A* genotypes, while significant difference in the improved score of total BPRS was found between *G2677T/A* genotypes and post hoc analyses showed significant differences in *G/G* vs. *T* or *A/T* or *A*. There were no differences in the percentage improvement or the improved scores of 5 sub-grouped symptoms after the 3-week treatment between *G2677T/A* genotypes (Table 1). Furthermore, no differences were found in side effects induced by bromperidol between *C3435T* genotypes or between *G2677T/A* genotypes.

Multiple regression analyses including age, body weight, gender, drug concentration and *MDR1* genotypes (*C3435T* and *G2677T/A*) showed significant correlations between *C3435T* genotypes and the percentage improvement or the improved scores of cognitive symptoms (Table 2). There were significant correlations between age and improved score of excitement symptoms and between body weight and percent improvement of excitement symptoms and between age and percent improvement of negative symptoms. No correlations were found in other symptoms vs. other variables. Neither total side effect nor sub-grouped side effects correlated with any clinical profiles including *MDR1* genotypes.

There were no differences in percent improvement or improved scores of total BPRS and 5 clusters including cognitive symptoms between subjects treated with biperiden and without biperiden.

4. Discussion

P-glycoprotein is found in the epithelial cells lining the luminal surface of many organs often associated with an excretory or barrier function, that is, the hepatic bile canalicular membrane, renal proximal tubule, villus-tip enterocyte in the small intestine, and the endothelial cells making up the blood–brain and blood–testes barriers (Cordon-Cardo et al., 1989). Oral administration of [^{3}H] ivermectinin in the knockout animal, *mdr1a* (−/−) mice and mdr1a (+/+) mice resulted in 87-fold higher levels of radioactivity in the brain of *mdr1a* (−/−) mice as compared with wild-type mice, whereas the levels in liver, kidney, small intestine and plasma were increased by less than 4-fold (Rao et al., 1999). This finding suggests that P-glycoprotein in blood–brain and blood–testes barriers has major impact on the disposition of P-glycoprotein substrates.

The results of this study showed that there were no correlations between *C3435T* variants and total BPRS score or 5 sub-grouped scores using simple correlation analyses. In cognitive impairment, patients with *TT* type had a trend of poor improvement as evaluated with improved scores and percentage improvement, although these were not statistically significant because of relatively large standard deviation. However, multiple regression analyses including plasma drug concentration showed that *C3435T* variant had significant association between the impaired scores and percentage improvement of cognitive symptoms. If lower expression and function of P-glycoprotein was present in blood–brain barrier in *TT* type as well as small intestine, a lower efflux function for bromperidol and hence higher drug concentration in brain might occur in *TT* type. In the light of the hypothesis that typical antipsychotic agents lead to worsening of cognitive function, higher drug concentration in brain might be associated with poor efficacy on cognitive

Table 2
Standard partial correlation coefficients between clinical profiles including MDR1 genotypes and percentage improvement or improved scores in the treatment with bromperidol

Variables	Standard partial correlation coefficients				
	Positive	Excitement	Cognitive	Negative	Anxiety–Depression
Percentage improvement					
Gender	−0.257	0.253	0.094	−0.052	0.053
Age	0.372	0.326	0.224	−0.490*	0.325
Body weight	0.184	0.569*	0.286	0.060	0.198
C3435T	0.075	0.219	0.464*	−0.064	−0.083
G2677T/A	0.162	0.101	−0.003	0.265	0.220
Drug concentration	0.017	0.020	0.030	0.031	0.085
Improved scores					
Gender	−0.390	0.010	−0.394	−0.226	−0.182
Age	0.392	0.465*	0.134	−0.295	0.107
Body weight	0.099	0.310	−0.006	0.049	0.038
C3435T	0.171	0.293	0.673**	0.358	0.034
G2677T/A	0.306	−0.113	−0.251	−0.085	0.227
Drug concentration	0.090	0.171	0.050	0.059	−0.138

* $p<0.05$.
** $p<0.01$.

function, although we had no evidence of higher drug concentration in the brain in *TT* type than other *MRD1* genotypes. However, the possibility that higher plasma drug concentration and hence excessive dopamine receptor blockade simply resulted in poor improvement of cognitive function cannot be excluded entirely, because significant difference in plasma bromperidol concentration was found between *C3435T* genotype in this study.

It is considered that large inter-individual variation in percentage improvement of cognitive function in this study led to lack of statistical difference between *C3435T* genotypes. The large inter-individual variation may be explained by data included by both patients with deterioration of cognitive function as side effect and patients with amelioration of cognitive function as efficacy, provided that the BPRS may have methodological problems.

A previous study suggested that a significant association was present between nortriptyline-induced postural hypotension and *C3435T* ($P=0.034$) in patients with major depression enrolled in a randomized antidepressant treatment trial of nortriptyline and fluoxetine (Roberts et al., 2002). The results suggest that homozygosity for *3435T* alleles is a risk factor for occurrence of nortriptyline-induced postural hypotension (OR=1.37, $P=0.042$, 95% CI: 1.01–1.86). These results support our finding because it is possible that postural hypotension is also induced by higher drug concentration in the brain.

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). Improved scores in total BPRS scores correlated significantly with *G2677T/A* variants while no correlations were found between *G2677T/A* variants and any symptoms using multiple regression analyses. Therefore it is less likely that *G2677T/A* variants are useful for prediction of efficacy when bromperidol is used in the treatment of schizophrenia.

It is known that antagonists to acetylcholine such as biperiden or triphexyphnydil impair cognitive function (Tracy et al., 2001). However, there was no difference in percent improvement of cognitive function between subjects treated with biperiden and without biperiden. Thus it is unlikely that coadministration of anticholinergic agents plays an important role in treatment of cognitive impairment.

Our previous studies have shown that dopamine D_2 receptors (*DRD2*) polymorphisms such as *Taq IA* and *−141C Ins/Del* are associated with positive symptoms (Suzuki et al., 2000) and anxiety–depression symptoms (Suzuki et al., 2001b), respectively, in schizophrenic patients treated with selective *DRD2* antagonists. In addition, we have reported that neuroleptic malignant syndrome is also linked with these *DRD2* polymorphisms (Suzuki et al., 2001a). These findings suggest that several clinical responses to selective *DRD2* antagonists are dependent upon inter-individual variation of target receptor function.

An in vitro study has shown that quetiapine and risperidone have stronger affinity to P-glycoprotein than other atypical antipsychotic drugs, suggesting that quetiapine and risperidone are substrates of P-glycoprotein (Boulton et al., 2002). However, there is no in vivo data indicating that quetiapine or risperidone as a substrate of P-glycoprotein is of clinical relevance except an in vivo data showing lack of impact of *MDR1* genotype on steady-state plasma concentration of risperidone and 9-hydroxyrisperidone (Yasui-Furukori et al., 2004b). Further information is required for antipsychotics to determine whether these *MDR1* genotypes are clinically relevant or not.

5. Conclusion

The present results suggest that the *C3435T* polymorphism is associated with some therapeutic response to bromperidol in schizophrenic patients. This finding is possibly explained by different drug concentration in the brain between *MDR1* genotypes. However, further replication studies are required to determine whether or not *MDR1* genotypes are clinically relevant in the treatment with antipsychotics because this study had a relatively small number of subjects.

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Case Report

Digitalis intoxication induced by paroxetine co-administration

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In February, 2005, a 68-year-old woman, who had no history of psychiatric illness, presented to our department with a major depressive disorder, with moderate sadness, inner tension, difficulty in concentrating, severe sleep impairment, appetite loss, and pessimistic thoughts. She had atrial fibrillation (AF) which had been treated with 0·25 mg digoxin daily and 1 mg warfarin daily for 2 years (INR 1·7). There were no abnormal physiological findings except a slightly raised serum creatinine concentration (106 μmol/L). She was admitted to the psychiatric ward. On day 3 of her admission, we initiated paroxetine 20 mg/day for depression. Nausea, vomiting, and dizziness began on day 5. Delirium with visual hallucinations and disorientation developed on day 7. She was not able to eat or walk on day 10. On day 11, we suspected digitalis intoxication (serum digoxin concentration 5·2 ng/mL, normal range 0·5–2·0 ng/mL; ECG showed many ventricular premature contractions and complete A-V block). There was no electrolyte disturbance. All medications were discontinued from day 12. As a rebound effect of digoxin discontinuation, bradycardia was observed from day 13 to day 15, and subsequently the ECG showed AF as the plasma concentration of digoxin was decreasing.

On day 21, digoxin 0·25 mg daily and warfarin 1 mg daily were reinstated. The delirium with disorientation recovered gradually and ameliorated fully by day 28. However, slight to moderate depressive symptoms remained She became bedridden during these events and developed aspiration pneumonia due to difficulty in swallowing despite recovery of her appetite on day 45. Primary treatment for the pneumonia with antibiotics was started, but her physical condition did not change. In May, 2005, she was moved to a medical ward for intensive care, and she died from pneumonia in June, 2005. We measured plasma concentrations of digoxin retrospectively (figure).

Negative affective states such as depression are associated with premature mortality and increased risk of coronary heart disease, type 2 diabetes, and disability. Patients with chronic medical illness and comorbid depression show substantial improvements in mood, social and emotional functioning, and disability after initiation of antidepression treatment.[1] Patients scoring in a higher quartile for anxiety and total psychological distress are at a greater risk of developing AF than those in lower quartiles.[2] Therefore, patients are likely to be prescribed antidepressants concomitantly with digoxin. In our patient, there were no symptoms suggesting digitalis intoxication during digoxin treatment alone. Plasma concentrations of digoxin increased during administration of paroxetine. Several symptoms disappeared with the decrease in plasma concentration of digoxin, showing that

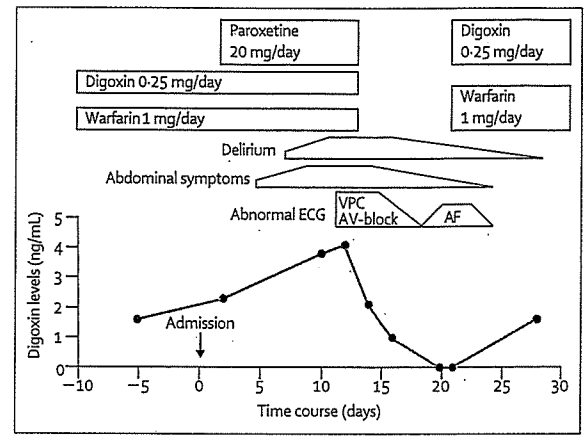

Figure: Clinical course of digitalis-paroxetine interaction
VPC, ventricular premature contraction; AF, atrial fibrillation.

concomitant administration of paroxetine with digoxin caused the digitalis intoxication. Several in-vivo studies have consistently suggested that paroxetine decreases CYP2D6 activity, resulting in drug interactions,[3] and an in-vitro study showed potent P-glycoprotein inhibitory activity with paroxetine.[4] Digoxin is regarded as a substrate of P-glycoprotein on the basis of several interaction studies.[5] The high digoxin concentrations during paroxetine administration in our case probably resulted from P-glycoprotein inhibition in the kidney. Since nausea, vomiting, and reduced appetite are not only early symptoms of digitalis intoxication but also some of the side-effects of selective serotonin-reuptake inhibitors (SSRI), it is difficult to distinguish early symptoms of digitalis intoxication from SSRI-mediated side-effects or symptoms of depression. Therefore, therapeutic drug monitoring of digoxin concentrations is necessary in the early stage of concomitant administration with SSRI. In addition, administration of citalopram or venlafaxine may be recommended in patients treated with digoxin because an in vitro study showed that inhibitory effects of these drugs on P-glycoprotein are weak.[4]

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Lithium response and Val66Met polymorphism of the brain-derived neurotrophic factor gene in Japanese patients with bipolar disorder

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Lithium is a first-line agent for the treatment of bipolar disorder. A significant association between the Val66Met polymorphism of the brain-derived neurotrophic factor gene and bipolar disorder has been reported. We investigated whether this polymorphism is associated with the response to lithium treatment in Japanese patients with bipolar disorder. Patients had been treated with lithium carbonate for more than 1 year, and the response was retrospectively evaluated. No significant differences were found in the genotype distribution or allele frequency between responders and non-responders. Our results suggested that the brain-derived neurotrophic factor Val66Met polymorphism might not greatly contribute to the efficacy of lithium in bipolar disorder. *Psychiatr Genet* **16:49–50 © 2006 Lippincott Williams & Wilkins.**

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Lithium is a first-line agent for the treatment of bipolar disorder (BPD). Although its therapeutic mechanisms remain poorly understood, recent studies suggested a potential role of the brain-derived neurotrophic factor (BDNF) (Hashimoto *et al.*, 2004). A significant association between the Val66Met single-nucleotide polymorphism (SNP) of the BDNF gene and BPD has been reported (Neves-Pereira *et al.*, 2002). This SNP affects activity-dependent secretion of BDNF in cultured neurons, and human memory and hippocampal function. Therefore, we investigated whether the Val66Met SNP of the BDNF gene is associated with the response to lithium treatment in Japanese patients with BPD.

Study participants were 161 patients with BPD [83 bipolar I disorders (BPI) and 78 bipolar II disorders (BPII)]. Consensus diagnosis was made according to the Diagnostic and Statistical Manual of Mental Disorders 4th edition criteria. They were composed of 76 male and 85 female patients, with age of 48.2 ± 12.8 (mean ± SD) years and a mean age at onset of 34.1 ± 11.7 years. All the participants were biologically unrelated Japanese. Patients had been treated with lithium carbonate and its serum concentration was maintained between 0.4–1.2 mEq/l at least for 1 year. After a complete description of the study, written, informed consent was obtained from every participant. The study protocol was approved by institutional ethics committees.

Response to lithium treatment was retrospectively determined according to the criteria described previously (Masui *et al.*, in press). Briefly, lithium responders were defined as those patients with less frequent and/or severe relapse, including no relapse, during the maintenance period of lithium treatment compared with the period before the initiation of lithium treatment. During the maintenance period, administration of antidepressants or antipsychotics was regarded as a relapse. The genotyping of the Val66Met SNP (rs6265) of the BDNF gene was determined by TaqMan 5′-exonuclease allelic discrimination assay.

Among 161 patients, 110 were determined as responders and 51 patients as non-responders. The genotype distribution for responders (Val/Val = 41, Val/Met = 55, Met/Met = 14) and non-responders (Val/Val = 16, Val/Met = 27, Met/Met = 8) was in Hardy–Weinberg equilibrium ($P = 0.50$ and $P = 0.54$, respectively, χ^2 test).

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No significant difference was found in the genotype distribution or allele frequency between the responders and non-responders ($P = 0.73$ and $P = 0.45$, respectively, χ^2 test). When a subtype of BPD (BPI or BPII) or sex was examined separately, there were no differences in genotype distributions or allele frequencies between the responders and non-responders.

Our results suggest that the Val66Met SNP of the BDNF gene is unlikely to be associated with lithium prophylaxis in Japanese patients with BPD. It is noteworthy that the significant association between this SNP and BPD has been demonstrated in Caucasian populations (Neves-Pereira *et al.*, 2002), although the subsequent studies in Asian populations failed to replicate it (Kunugi *et al.*, 2004). Therefore, the effects of this SNP might be different between ethnicities. The association between lithium prophylaxis and this SNP should be further tested in other ethnicities.

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Association Study between Vesicle-Associated Membrane Protein 2 Gene Polymorphisms and Fluvoxamine Response in Japanese Major Depressive Patients

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Key Words
Depressive disorder · Fluvoxamine response · Haplotype analysis · Vesicle-associated membrane protein 2

Abstract
Background: Vesicle-associated membrane protein 2 (VAMP2) is a key component of the synaptic vesicle docking/fusion machinery and its mRNA reportedly increases in the frontal cortex of rats following chronic antidepressant and electroconvulsive treatment. VAMP2 is therefore thought to be involved in the mechanism of action of antidepressants and may alter their efficacy. The purpose of this study was to investigate whether the *VAMP2* gene is associated with clinical responses to a specific antidepressant, fluvoxamine. ***Methods:*** A total of 106 patients with major depressive disorder were given fluvoxamine (50–200 mg/day) for 8 weeks and assessed for severity of depression using the Semi-Structured Interview Guide of the Hamilton Depressive Scale (SIGH-D; 17 items) at 0 and 8 weeks. We defined a clinical response as more than a 50% reduction in baseline SIGH-D within 8 weeks, and defined clinical remission as a SIGH-D score of less than 7 at 8 weeks. Genotyping was performed by PCR-RFLP. ***Results:*** Analysis of haplotype tagging single nucleotide polymorphisms as well as haplotype analysis did not reveal any significant associations. ***Conclusion:*** Our results suggest that the *VAMP2* gene is unlikely to play a major role in the efficacy of fluvoxamine.

Introduction

The selective serotonin reuptake inhibitors (SSRIs) are one of the first line drugs for treatment of major depressive disorder. Unfortunately, approximately 2 weeks are required for the onset of clinical effect, and only 60% of major depressive patients show a complete response to antidepressant treatment [1]. It is difficult for clinicians to predict which patients will respond to which drug based on clinical or biological features, although genetic factors are believed to play a major role in the variety of responses to treatment [2]. Many pharmacogenetic studies of the SSRI response have been undertaken, most of which have con-

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Table 1. Clinical characteristics of the patients in both definition groups

	Total patients	Males	Females	Age, years	Baseline SIGH-D	Fluvoxamine dose at 8 weeks mg/day	Previous episodes
Overall	106	49	57	43.2 ± 16.0	20.1 ± 6.5	122.0 ± 40.9	1.37 ± 0.56
Clinical response group[1]							
Responders	59	30	29	45.3 ± 16.6	21.5 ± 6.5	116.8 ± 43.4	1.34 ± 0.50
Nonresponders	47	19	28	41.0 ± 15.1	18.2 ± 5.9	128.9 ± 36.8	1.41 ± 0.59
p value		0.285		0.216	0.008	0.167	0.584
Clinical remission group[2]							
Remission	47	24	23	43.6 ± 14.7	19.2 ± 5.6	107.5 ± 43.2	1.34 ± 0.52
Nonremission	59	25	34	43.0 ± 17.0	20.7 ± 7.0	133.3 ± 35.5	1.39 ± 0.70
p value		0.373		0.694	0.23	0.002	0.698

Values for age, baseline SIGH-D, fluvoxamine dose and previous episodes are expressed as mean ± SD.
[1] Clinical response was defined as a 50% or greater decrease in the baseline SIGH-D score.
[2] Clinical remission was defined as a final SIGH-D score of less than 7.

centrated on mutations in the genes coding for pathways in the serotonergic systems [3–6]. However, no consistent pattern of results has been established [7]. Recently, researchers conducting SSRI pharmacogenetic studies have shifted their emphasis to genes other than those involved in serotonin concentration or receptor function [8, 9].

Vesicle-associated membrane protein 2 (VAMP2), which is located on synaptic vesicle membranes, is a key component of the regulated secretory pathway at nerve terminals and, along with syntaxin 1 and SNAP-25, forms the soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor (SNARE) complex [10–13]. Messenger RNA of *VAMP2* was recently reported to increase in the frontal cortex of rats following chronic antidepressant and electroconvulsive treatment [14]. Therefore, neuron secretory behavior alteration due to *VAMP2* gene expression was hypothesized to play a role in the mechanism of action of antidepressants and possibly to alter the clinical response of treated patients. In the present study, we investigated the effects of *VAMP2* gene polymorphisms on antidepressant responses to fluvoxamine in Japanese patients.

Materials and Methods

Subjects and Treatments

This study included 106 Japanese patients (49 males and 57 females; mean age ± standard deviation, 43.2 ± 16.0) who fulfilled the DSM-IV criteria for the diagnosis of major depressive disorder and whose scores on the 17 items of the Semi-Structured Interview Guide of the Hamilton Depressive Scale (SIGH-D) [15] were 12 or higher, indicative of their being in at least moderate depression [16]. In addition to these patients, 96 healthy volunteers, including unrelated medical staff and medical students, were recruited for linkage disequilibrium (LD) mapping. Patients with other axis I disorders, including schizophrenia, dementia, substance abuse, panic disorder, obsessive-compulsive disorder and generalized anxiety disorder, and those with axis II disorders diagnosed by the DSM-IV criteria were excluded by clinical interviews. All patients were free of psychotropic drugs in the past at least 1 month before beginning the study. After explaining the study to the subjects, written informed consent was obtained from each one of them. This study was approved by the Ethics Committee of the Nagoya University Graduate School of Medicine and Fujita Health University.

Fluvoxamine was administered two or three times a day for 8 weeks. The initial total dose was 50–100 mg/day and was gradually increased depending on the patient's condition. Patients with insomnia and severe anxiety were prescribed benzodiazepine drugs appropriately, but no other psychotropic drugs were allowed during the study.

Data Collection

Severity of depression symptoms was assessed using the 17 SIGH-D items. Assessments were conducted at baseline and 8 weeks after initiating antidepressant treatment. A single evaluator performed all the ratings for a single patient. A clinical response was defined as a 50% or greater decrease in the baseline SIGH-D score, and clinical remission was defined as a final SIGH-D score of 7 or less. The clinical characteristics of the patients, classified according to these definitions, are shown in table 1.

Single Nucleotide Polymorphism Selection and Genotyping

Genomic DNA was extracted from the peripheral blood of all subjects. For LD mapping, we selected single nucleotide polymorphisms (SNPs; rs8067606, rs1061032, rs2278637) distributed equally throughout the gene based on data from dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/; fig. 1). These SNPs were first genotyped in the 96 control samples to avoid redundant genotyping, and then LD blocks were determined with reasonable criteria based on 95% confidence intervals on the D′ values using Haploview v3.2 software (http://www.broad.mit.edu/mpg/haploview/). Next, haplotype-tagging SNPs (htSNPs) were selected within each LD block to provide 90% haplotype coverage. Finally, we genotyped each selected htSNP in the depressive samples using PCR-RFLP. Detailed protocols for the PCR-RFLP method are available upon request.

Statistical Analysis

Genotyping deviation from Hardy-Weinberg equilibrium was evaluated by a χ^2 test using the Haploview software. Marker-trait allele/genotype-wise association was evaluated using a conventional χ^2 test (SPSS version 11.0J, Tokyo, Japan) and haplotype-wise was evaluated using COCAPHASE software (http://www.rfcgr.mrc.ac.uk/~fdudbrid/software/unphased/). The COCAPHASE program performs log-likelihood ratio tests under a log-linear model for global p values. To estimate the haplotype frequencies of htSNPs, we used the expectation-maximization algorithm. Global p values were calculated for haplotype-wise analyses, and we also performed exploratory analysis of the possible correlations between response or remission, fluvoxamine treatment, and several clinical factors by logistic regression (SPSS). In these analyses, response classification was set as the dependent variable, and gender, age at the time of recruitment, fluvoxamine dose at 8 weeks, SIGH-D total score at the baseline, and htSNP genotypes were set as the independent variables. The significance level for all statistical tests was $p < 0.05$.

Results

After three SNPs were genotyped to evaluate the LD in the control samples, one LD block was defined and two SNPs (rs1061032 and rs8067606) were selected as the htSNPs for this gene (fig. 1). Genotyping these two htSNPs in all the depressive samples revealed that all the respective genotypic frequencies of these SNPs were in accordance with Hardy-Weinberg equilibrium.

In the allele/genotype-wise association analysis, the frequencies of each htSNP were not significantly different between fluvoxamine responders and nonresponders, or between remitters and nonremitters (table 2). In the haplotype-wise association analysis, we found no significant associations between fluvoxamine responders and nonresponders, or between remitters and nonremitters (table 3).

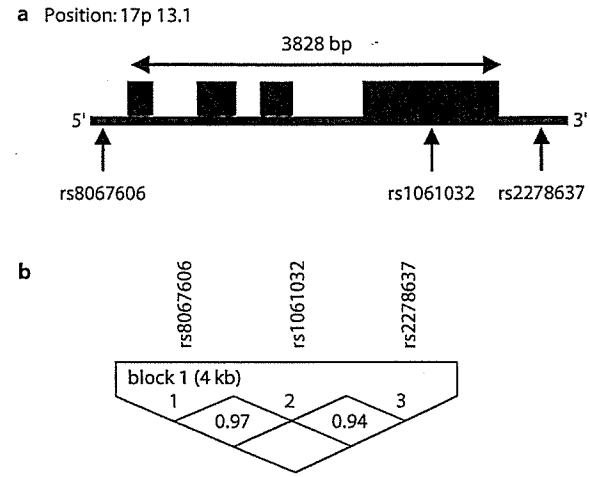

Fig. 1. Genomic structure and pairwise LD of *VAMP2*. **a** Genomic structure of VAMP2 and SNPs used in association analyses and LD mapping. The vertical bars represent exons of VAMP2, and the numbers under the arrows represent SNP IDs. **b** Pairwise LD of VAMP2 in the 96 controls. Each block was defined by a solid spine of the LD using Haploview v3.2. The numbers in the polygons represent D′, and the blank space represents complete LD.

In our exploratory logistic regression analysis, none of the htSNP genotypes correlated with clinical responses or remission (data not shown).

Discussion

In this study, the polymorphisms and estimated haplotypes of *VAMP2* were not associated with response or remission in fluvoxamine-treated Japanese depressive subjects. The exploratory logistic regression analysis revealed that none of the htSNP genotypes could serve as a predictor for clinical response or remission.

VAMP2 forms the SNARE complex at the presynapse, and SNARE proteins are central to the membrane fusion machinery. Membrane fusion is regulated by a Ca^{2+} sensor, and synaptotagmin is believed to function as a Ca^{2+} sensor, not only binding to the membrane in a Ca^{2+}-dependent manner but also interacting with SNARE complexes [17]. As VAMP2 interacts with various proteins that are essential for vesicular transport and/or fusion, other genes interacting with VAMP2 might play important roles in the response to antidepressants. We must therefore consider gene-gene interactions between *VAMP2* and these other genes [18].