ies—for example, dopamine D2 and D3 receptor variants (2)—there are no definitive predictors of response.

Pharmacogenetics has been driven by a candidate gene approach. This approach has the disadvantage that targets for study are limited by our current understanding of the mechanisms of drugs, and therefore, this method cannot identify unsuspected predictor genes. Approaches that are independent of prior functional hypotheses of gene action based on genome-wide surveys of SNPs are, however, now feasible. The genome-wide approach has its disadvantages, but one of the most important is that, with effectively random sets of SNPs, the low prior probability that any is truly associated with disease requires a stringent type I error rate to control the enormous potential for reporting false positives. One way to address this issue is to use very large (and therefore highly powered) studies in which such stringent statistical support might realistically be achieved. Another approach that is more economical in genotyping costs is to undertake multistage analysis in which candidate variants from a screening sample are validated by replication in other data sets (3). However, because the samples for pharmacogenomics require a large amount of clinical data and are preferably prospective, large samples are difficult to collect.

Another approach is to try to enhance the prior probability for a given gene by integrating pharmacogenomic data with other sources of data—for example, from studies of gene expression (4). Under the hypothesis that genes related to drug response may be regulated by exposure to that drug, genes in which expression is altered in animals exposed to that drug have a higher probability of being genuinely associated in a pharmacogenetic study than any random gene. If this is correct, genes in which expression is altered by drug exposure require less stringent statistical support.

We aimed to detect predictor genes for risperidone response in schizophrenic patients using this convergent approach (4). Specifically, we compared data from a pharmacogenetic study based on first-episode, previously drug-naive subjects with schizophrenia who were treated with risperidone with data from a pharmacotranscriptomic study based on mice exposed to the same drug. Moreover, candidate variants from genes implicated by convergent data were also tested for evidence for association to schizophrenia per se because variants that are related to drug response may also be related to disease risk (5). Evidence that this occurs can be considered an additional independent line of circumstantial support that the convergence between the pharmacogenetic and transcriptomics does not merely reflect chance.

Methods and Materials

Subjects and Collection of Clinical Data

We performed an open-labeled pharmacogenetic study involving 108 first-episode, previously antipsychotic-naive schizophrenic patients. All received risperidone monotherapy after enrollment. Details are described elsewhere (6,7). Briefly, patients were entered into the study if they 1) met DSM-IV-TR criteria for schizophrenia (and then remained in follow-up to at least 6 months), 2) were physically healthy and had all laboratory parameters within normal limits, and 3) had neither a current nor a past DSM-IV-TR diagnosis of mood disorders or substance abuse. Consensus diagnoses were made by at least two experienced psychiatrists on the basis of unstructured interviews with patients and families and review of medical records. Duration of untreated psychosis (DUP) was defined as the period from the onset of psychotic symptoms to that of first antipsychotic exposure. Sixty subjects were recruited from outpatient clinics, and 48 subjects were treated as inpatients.

Subjects received risperidone monotherapy (starting dosage: .5–4 mg/day, mean starting dosage: 2.5 mg), and dosage was adjusted in accordance with symptomatic response by trained psychiatrists (1–8 mg/day, mean dosage: 3.4 mg at 8 weeks) for 8 weeks. Patients with insomnia were prescribed brotizolam, .25 mg or .5 mg, at bedtime. No other psychotropic drugs were permitted.

Clinical symptoms were evaluated at the first visit and after 8 weeks of treatment by the use of the Positive and Negative Syndrome Scale (PANSS). Evaluations were carried out by qualified psychiatrists and psychologists (the interrater reliability was measured by intraclass correlation coefficient was .90, unpublished data).

The clinical characteristics of subjects that we used as potential covariates were selected from another report (8): sex (57 male, 51 female), age (mean 30.2 ± 9.5 years), DUP (1.5–32 months, mean 7.6 ± 7.1 months), and baseline PANSS total score (mean 83.0 ± 22.9).

Samples used in the schizophrenia case-control association analysis consisted of three sets: (1) JPN_1st: this was used for identifying genes of potential interest and comprised 540 patients with schizophrenia (275 male and 265 female; aged 43.3 ± 15.0 years) and 425 healthy controls (236 male and 189 female; aged 36.3 ± 13.9 years) from the Japanese population; 2) JPN_2nd sample (used to follow up genes of interest) comprised 545 patients with schizophrenia (282 male and 263 female; aged 50.7 ± 14.9 years) and 500 controls (279 male and 221 female; aged 40.8 ± 15.4 years) from the Japanese population; 3) Additional follow-up data for SNPs of interest were extracted from a UK genomewide association study (GWAS) of schizophrenia comprising 479 patients with schizophrenia and 2937 controls from the UK population (9).

Controls in the Japanese population were screened for past history of mental disorders. All individuals were unrelated. After explanation of the study, written informed consent was obtained from each subject. This study was approved by the Ethics Committee at Fujita Health University, University of Occupational and Environmental Health, Nagoya University Graduate School of Medicine, Osaka University Graduate School of Medicine and by multiple ethics committees across the UK where sample recruitment was performed.

Microarray Experiments

See also Methods in Supplement 1.

SNP Chip. Genomewide genotyping was carried out using Illumina Sentrix human 1 Genotyping BeadChip (109,363 SNPs randomly distributed throughout the genome) according to the manufacturer's instructions (Illumina, San Diego, California). Details are given in the Supplement 1.

Mouse Expression Chip. We compared mRNA levels of the prefrontal cortex (PFC) between control ($n = 3$) and risperidone-exposed mice (2.4 mg/kg given orally, once a day for 21 days, $n = 3$). Affymetrix Mouse Gene 1.0 St. Array, which profiles the expression of 28,853 genes (Affymetrix, Santa Clara, California), was used to measure the amount of mRNA.

The procedures involving animals and their care were conducted in conformity with the international guidelines, Principles of Laboratory Animal Care (National Institutes of Health Publication 85-23, revised 1985).

Experimental Procedures and Statistical Analysis

Study 1: Pharmacogenomics. Quality control (QC) regarding population stratification (Figure S1 in the Supplement 1),

Hardy-Weinberg equilibrium (HWE), genotyping rate, and minor allele frequency was conducted by PLINK (10). Details are described in Supplement 1.

After QC, 99 samples (51 males and 48 females) and 62,935 autosomal SNPs (a mean call rate of 99.2%, indicating a high rate of successful genotyping) were analyzed to evaluate the effect of each SNP on antipsychotic response to risperidone.

To evaluate the effect of each SNP on antipsychotic response to risperidone, multiple regression analysis was carried out with a dependent variable [% PANSS change = 100 × ((PANSS at week 0) − (PANSS at Week 8))/PANSS at Week 0] and independent variables that included sex, age, duration of illness, initial PANSS score, and the genotype of each polymorphism. Each genotype was assessed using dominant, recessive, and multiplicative genetic models, respectively.

To calculate the best empiric p values based on the most significant result in each genetic model, we generated 1 million simulated data sets by randomizing the PANSS changes (the covariates stay with the genotypes) with respect to the GWAS data. This approach retains the linkage disequilibrium (LD) relationships between SNPs, and therefore allows for the appropriate degree of nonindependence in the data sets. The same multiple regression analysis model as applied to the real data were applied to each SNP in each permuted data set, and the empiric significance for a SNP was the proportion of the simulated data sets in which the test statistic was equal to, or greater than, that observed in the true data set (11–15).

SNPs were annotated to the closest genes with an up- and downstream span of 20 kb by WGAViewer (16).

Study 2: Mouse Expression Assay. In the mouse expression assay, data sets passing QC were normalized using GeneChip Operating Software (Affymetrix) and the raw intensity values exported for further analysis. Only genes called present based on Affymetrix detection p value for the presence of each gene on either chip were included. A t test was performed to assess the statistical significance of genes in which expression differed between control and risperidone-exposed mice. Power analysis was carried out using PowerAtlas (17). Our data set provides expected discovery rate (corresponding to power) of .37, an expected proportion of true positives (PTPs) of .72, and an expected proportion of true negatives of .80 at alpha set at .05. A major aim of this study was to prioritize genes showing convergent evidence in the pharmacogenomic study, thus we consider a high PTP optimal.

These data were submitted to CIBEX (http://cibex.nig.ac.jp/index.jsp, accession number: CBX77).

Study 3: Checking Overlap Results Between Pharmacogenomics (Study 1) and Mouse Expression Assay (Study 2). We checked candidate SNPs from the genes that showed convergent evidence for relevance to risperidone action from Study 1 and Study 2. Candidate genes were defined as follows: 1) genes for which there was at least one SNP with p values less than 5.0×10^{-4} in the pharmacogenomic study and in which expression significantly differed between groups at $\alpha < .05$ and 2) genes with much stronger evidence for $p < 1.0 \times 10^{-4}$ but that had weaker evidence for association in the pharmacogenomic study ($p < .05$).

Study 4: Case-Control Association Analysis of Strong Candidate Genes from Pharmacogenomics and Mouse Expression Assay. The candidate SNPs from Study 3 were further assessed for evidence for association with schizophrenia. These SNPs were genotyped by TaqMan assay (Applied Biosystems, California) in the Japanese case-control samples. Genotypes for the SNP in *PDE7B* in the UK samples were extracted from the Affymetrix GWAS data (9) after confirmation of good-quality cluster plots.

Genotype deviation from HWE was evaluated by a goodness of fit chi-square test. Marker-trait association was evaluated for allele/genotype-wise using standard contingency tables (SPSS 15.0, SPSS, Tokyo, Japan).

For SNPs analyzed in multiple samples, we conducted a meta-analysis using a random-effects model. Heterogeneity was measured using a Q statistic test in the combined studies. Odds ratios (ORs) were pooled using DerSimonian and Laird methods. The significance of the pooled OR was determined using a Z test. All data were analyzed using an R package, meta (http://www.r-project.org/index.html).

Results

Possible Predictor SNPs for Risperidone Treatment: From Pharmacogenomic Result (Study 1)

Among the 62,935 SNPs we examined in the pharmacogenomics study, 51,550 SNPs were annotated to 14,655 genes (annotation span: 5′ or 3′ ± 20 kb). For a number of genes, we had multiple SNPs with p values less than 5.0×10^{-4} because of the high LD among genotyped markers. Where this occurred, we list only the strongest associated SNP from that gene (the top 10 hits and SNPs with p value less than 5.0×10^{-4} in Table 1 and Table S1 in Supplement 1, respectively).

Table 1. Predictor Genes in the Pharmacogenomics (Top 10)

Ranking	SNP	Chr	Position[a]	Closest Gene[b]	p Value (Pharmacogenomics)
1	rs2289273	3	10,388,601	*ATP2B2*	1.60×10^{-5}
2	rs234091	1	183,186,172	*FAM129A*	2.00×10^{-5}
3	rs241202	8	28,689,604	*INTS9*	3.20×10^{-5}
4	rs4340422	19	48,604,802	*TEX101*	5.00×10^{-5}
5	rs6682786	1	23,615,883	*TCEA3*	7.30×10^{-5}
6	rs1001220	7	72,748,539	*WBSCR22*	7.70×10^{-5}
7	rs3829241	11	68,611,939	*TPCN2*	8.90×10^{-5}
8	rs460473	16	22,740,528	*HS3ST2*	1.03×10^{-4}
9	rs9792264	8	135,640,117	*ZFAT*	1.10×10^{-4}
10	rs6443999	3	186,056,249	*VPS8*	1.17×10^{-4}

Chr, chromosome; SNP, single nucleotide polymorphism.
[a]Based on Ensemble *Homo sapiens* Version 54.36p (NCBI36).
[b]SNPs are annotated to the closest genes with ± 20-kb span.

Table 2. Overlap Genes Based on the Pharmacogenomcis ($p < 5.0 \times 10^{-4}$) with Mouse Expression Assay ($p < .05$)

Ranking	SNP	Chr	Position[a]	Closest Gene[b]	*p* Value (Pharmacogenomics)	*p* Value[c] (Mouse Expression)	Fold Change
1	rs2289273	3	10,388,601	*ATP2B2*	1.60×10^{-5}	.000710	.504
8	rs460473	16	22,740,528	*HS3ST2*	1.03×10^{-4}	.00600	.259
28	rs3775003	4	96,390,234	*UNC5C*	2.20×10^{-4}	.0132	1.85
32	rs196290	10	121,398,061	*BAG3*	2.81×10^{-4}	.0283	1.33
35	rs9389370	6	136,472,958	*PDE7B*	2.88×10^{-4}	.00806	.710
53	rs1356787	4	57,012,104	*PAICS*	4.26×10^{-4}	.0368	.660
54	rs4641299	1	117,284,884	*PTGFRN*	4.27×10^{-4}	.00160	.283

Chr, chromosome; SNP, single nucleotide polymorphism.
[a]Based on Ensemble *Homo sapiens* Version 54.36p (NCBI36).
[b]SNPs are annotated to the closest genes with ± 20-kb span.
[c]Comparison between risperidone-treated mice ($n = 3$) and saline-treated mice ($n = 3$).

We also looked specifically in our data for support for genes recently suggested as associated with iloperidone based on the only other available antipsychotic GWAS data set (18) and candidate genes implicated in earlier studies (2) including *DRD2*, *DRD3*, *HTR2A*, and others (Tables S2 and S3 in Supplement 1). No strong evidence for association to any of these was found in our pharmacogenomics data set.

Genes Influenced by Risperidone Exposure in Mouse PFC (Study 2)

We examined 22,556 probes in 12,706 genes in RNA extracted from the PFC of mice treated with either risperidone or with vehicle. Of these, 754 (5.9%) and 2227 (17.5%) genes had at least one probe that showed nominally significant differences at $p <$ .01 and .05, respectively, a rate much higher than chance. The top genes with *p* value less than 5.0×10^{-4} are presented in Table S4 in Supplement 1.

Overlapping Genes Between Pharmacogenomic and Mouse Expression Assays (Study 3)

We looked to see whether the pharmacogenetic data (excluding 14 SNPs that could not be annotated to the closest gene) and expression overlapped. Seven genes containing nominally significant alteration in expression in mice also contained SNPs with *p* value less than 5×10^{-4} (Table 2). The relation between PANSS changes and physical locations of each SNP and the genotype effects to risperidone response can be seen in Figures S2 and S3 and Table S5 in Supplement 1. In addition, we found seven genes that met the more stringent threshold for expression change in the mouse and that had at least one significant SNP ($p < .05$) in the pharmacogenomic data (Table 3). It should be stressed these SNPs were not strongly associated with treatment response ($p = .0047$-$.0472$).

Consequently 14 SNPs were further assessed for case-control association analysis in Study 4.

Examining Candidate SNPs as Susceptibility Factor for Schizophrenia (Study 4)

The 14 candidate SNPs in genes showing convergent evidence from Study 3 were further tested for association with schizophrenia (Table 4). For rs242056, a proxy for rs2071999 in *AJAP1*, the genotypes significantly deviated from HWE in controls ($p = .0016$). This SNP was therefore excluded.

Of the remaining 13 SNPs, a single SNP (rs9389370) in *PDE7B* showed a nominally significant association in the JPN_1st case-control sample ($p_{allele} = .026$, two-tailed). In an attempt to extend this putative association, we used two other samples. In the second Japanese sample, we obtained significant evidence for association (second set, $P_{allele} = .02$, one-tailed) and a nonsignificant trend in the UK sample ($p_{allele} = .07$, one-tailed) (Table 4). Meta-analysis of the two replication data sets showed significant evidence for association ($p_{JPN2nd+UK} = .008$, one-tailed). As expected, in all data sets combined, the evidence was stronger than observed in the screening sample alone ($p_{all} = .0014$, two-tailed, uncorrected; $p = .018$, 13 times Bonferroni correction for number of SNPs tested in Study 4) with no evidence for heterogeneity ($p = .56$; Table 5).

Discussion

Combined Analysis as a Tool for Prioritizing Candidate Genes for Pharmacogenomics and Susceptibility

Genomewide approaches to pharmacogenomics have the capacity to provide novel insights into mechanisms and predictors of drug response. However, a major concern of this approach, which is not specific to pharmacogenomics, relates to balancing the need to set a stringent threshold for the type I error rate against the desire to achieve power to detect findings at that threshold. Unless the genetic effect sizes in pharmacogenetics are substantially greater than is typical for complex diseases (19), the sorts of sample sizes currently available for studies of

Table 3. Overlap Genes Based on the Mouse Expression Assay ($p < 1.0 \times 10^{-4}$) with the Pharmacogenomics ($p < .05$)

Ranking	Gene	Probe ID	Fold Change	*p* Value (Mouse Expression)	*p* Value (Pharmacogenomics)	SNP ID
2	*Nr3c2*	1435991-at	5.86	2.23×10^{-6}	.0297	rs2070951
3	*Zbtb20*	1439278-at	4.94	5.04×10^{-6}	.0230	rs9883949
4	*St6gal2*	1434819-at	.23	6.52×10^{-6}	.0102	rs1448110
7	*Pip5k1b*	1450389-s-at	2.46	1.02×10^{-5}	.0472	rs1414944
8	*Epha6*	1421527-at	3.18	1.46×10^{-5}	.0047	rs727229
9	*Kcnh5*	1441742-at	.44	2.72×10^{-5}	.0305	rs10141458
24	*Ajap1*	1438662-at	.66	9.21×10^{-5}	.0208	rs2071999

SNP, single nucleotide polymorphism.

Table 4. Case-Control Analysis of the Candidate SNPs from the Pharmacogenomics and Mouse Expression Data

SNP	Sample	Proxy SNPs	Phenotype	N	Genotype			*p* Value		*p* Value	MAF
					M/M	M/m	m/m	Allele	Genotype	HWE	
ATP2B2	JPN_1st		Case	536	275	208	53	.676	.184	.14	29.3
rs2289273			Control	417	209	179	29			.26	28.4
HS3ST2	JPN_1st		Case	538	163	263	112	.408	.682	.76	45.3
rs460473			Control	407	117	196	94			.50	47.2
UNC5C	JPN_1st		Case	540	336	178	26	.249	.136	.70	21.3
rs3775003			Control	406	231	159	16			.07	23.5
BAG3	JPN_1st		Case	539	234	243	62	.609	.877	.93	34.0
rs196290			Control	407	183	180	44			.98	32.9
PDE7B	JPN_1st		Case	535	259	222	54	**.0255**	.0738	.53	30.8
rs9389370			Control	422	229	165	28			.81	26.2
	JPN_2nd		Case	536	278	200	58	**.0214**[a]	.0966	.018	29.5
			Control	500	281	183	36			.41	25.5
	UK		Case	478	181	226	71	.0672[a]	.327	.97	38.5
			Control	2,932	1203	1348	381			.91	36.0
PAICS	JPN_1st		Case	540	181	274	85	.662	.808	.27	41.1
rs1356787			Control	424	134	223	67			.10	42.1
PTGFRN	JPN_1st		Case	535	319	180	36	.222	.219	.13	23.6
rs4641299			Control	413	255	141	17			.65	21.2
NR3C2	JPN_1st		Case	534	295	206	33	.284	.509	.71	25.5
rs2070951			Control	414	213	173	28			.37	27.7
ZBTB20	JPN_1st		Case	537	234	245	58	.494	.420	.61	33.6
rs9883949			Control	423	169	211	43			.05	35.1
ST6GAL2	JPN_1st	rs2241991	Case	533	224	246	63	.936	.814	.72	34.9
rs1448110		$r2 = 1$	Control	409	169	196	44			.25	34.7
PIP5K1B	JPN_1st		Case	536	173	275	88	.550	.764	.22	42.1
rs1414944			Control	420	145	208	67			.6	40.7
EPHA6	JPN_1st		Case	539	146	274	119	.877	.949	.65	47.5
rs727229			Control	419	110	217	92			.44	47.9
KCNH5	JPN_1st		Case	539	169	265	105	.224	.476	.95	44.1
rs10141458			Control	413	142	201	70			.94	41.3
AJAP1	JPN_1st	rs242056	Case	—	—	—	—	—	—	—	—
rs2071999		$r2 = .46$	Control	418	191	161	66			.0016	35.0

Bold numbers represent significant *p* value.

JPN_1st, first Japanese sample; JPN_2nd, second Japanese sample; MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium; M, major allele, m, minor allele; SNP, single nucleotide polymorphism; UK, United Kingdom sample.

[a]Based on one-tailed analysis.

antipsychotics have no realistic prospect of attaining the sorts of levels of significance suggested for genomewide significance (7.2×10^{-8} or 1×10^{-7}) (20,21). Although it is at least possible that the typical effects on gene expression of drugs may be much more substantial than that of SNPs on disease risk, broadly similar balances of power and type I error also apply to our genomewide expression study. Therefore, with the aim of prioritizing our findings, we attempted to cross-validate the top findings from our study using independent approaches as suggested (4). Our methods of prioritizing our findings were based on two hypotheses; one that the most highly significant sets of SNPs from our pharmacogenetic study of risperidone are likely to be enriched among genes whose expression is altered by that drug (and vice versa), the other that SNPs related to drug response may also be enriched among SNPs associated with disease. To what extent these hypotheses are correct is currently unknown.

From our data, we found 14 markers in genes that showed some degree of overlapping support in the pharmacogenomics

Table 5. Meta-Analysis of rs9389370 in PDE7B

Analysis	Sample	OR	95% CIs		*p* Value
			Lower Limit	Upper Limit	
	JPN_1st	1.26	1.03	1.54	**.0255**
	JPN_2nd	1.22	1.01	1.48	**.0214**[a]
	UK	1.11	.967	1.28	.0672[a]
Meta (Replication)	JPN_2nd+UK	1.15	1.03	1.29	**.0082**[a]
Meta (All)	JPN1st+JPN2nd+UK	1.17	1.06	1.30	**.0014**

Bold number represent significant *p* value.

CI, confidence interval; JPN_1st, first Japanese sample; JPN_2nd, second Japanese sample; OR, odds ratio; UK, United Kingdom Sample.

[a]Based on one-tailed analysis.

and mouse expression experiments. These top convergent candidate genes have no previous support for association with schizophrenia or risperidone response and are thus novel candidates for antipsychotic response. However, at present, they have no clinical utility in terms of predicting treatment response, and independent replication using other samples will be required. Moreover, even if replicated, the potential clinical utility for pharmacogenetics is questionable because the effect sizes in each case are small, although it is conceivable given the limited coverage of each gene that the true functional variants have much stronger effects.

Another method for prioritizing genes from genomewide data are to apply a gene ontology (GO) based approach to investigate whether sets of findings tend to converge on particular biological pathways or functions. Our previous experience of GO category analysis suggests that with respect to genetic data, these require large data sets (22). Nevertheless, in response to an anonymous reviewer's comments, for interested readers, we provide the results of our GO category analyses based on ALIGATOR (22) and David Bioinformatics Resources 2008 (http://david.abcc.ncifcrf.gov/) in Supplement 1. Although a number of categories were observed to be significant in each analysis (Tables S6 and S7 in Supplement 1), there is no overlap between the results of the two analytic approaches. Moreover, our favored approach based on ALIGATOR did not reveal any categories that were significant after correction for multiple testing, so it is likely that all of those findings represent chance positives.

Possible Predictor SNPs for Response to Risperidone

In this study, several genes were detected as possible novel predictors for treatment response to risperidone: *ATP2B2, HS3ST2, UNC5C, BAG3, PDE7B, PAICS, PTGFRN, NR3C2, ZBTB20, ST6GAL2, PIP5K1B, EPHA6, KCNH5,* and *AJAP1*. Because the multiple testing burden in SNPs is more severe, our primary analysis included selecting genes based on the more stringent thresholds in the pharmacogenomics data (Table 2) and were additionally shown to have altered expression in the mouse expression study. However, in response to review, we additionally provide data for much more weakly associated SNPs that have highly significant expression changes in the mouse brain (Table 3). Given the weak evidence for most of the latter group of SNPs, we think those are most likely to be chance positives but report the findings for others to test.

Among genes of particular interest in Table 2 is *ATP2B2*, which encodes one of four isoforms of the plasma membrane Ca^{2+} pumps of mammalian cells, showed both the strongest statistical association with treatment response ($p = 1.60 \times 10^{-5}$) and was among those genes that had the strongest association with differential expression because of exposure to risperidone ($p = .00071$). The product of this gene is thought to be involved in neurodevelopment (23) because of its influence on Ca^{2+} homeostasis and Ca^{2+} signaling. This in turn regulates multiple neuronal functions, including synaptic transmission, plasticity, and cell survival (24). Interestingly, several of the other genes with convergent evidence for a role in risperidone response might also be related to neurodevelopment via association with netrin (*UNC5C*) (25,26), interaction with heat shock proteins (*BAG3*) (27,28), cyclic adenosine monophosphate (cAMP) systems (*PDE7B*; details discussed later), glucocorticoids (*NR3C2*) (29), and ephirin (*EPHA6*) (30). Given the neurodevelopmental hypothesis of schizophrenia (31) and evidence that second-generation antipsychotics, including risperidone, have neurogenic actions in hippocampus and PFC (32), our findings suggest that genes involved in the regulation of neurodevelopment or neurogenesis are candidate genes for treatment response in schizophrenics, as well as for schizophrenia per se.

PDE7B Is Candidate Gene Either for Treatment Response and Susceptibility for Schizophrenia

We pursued the top findings from Study 3 to see whether the findings with best convergent evidence (human and mouse) for relevance to risperidone response might also influence susceptibility to schizophrenia. After correction for multiple testing, we found evidence for association between disease status and *PDE7B*, which was therefore the only gene supported across all study designs.

Phosphodiesterases (PDEs) are central in regulating degradation of cAMP and cyclic guanosine monophosphate (cGMP), which are important second messengers for many cellular functions (33). There are 21 known genes encoding PDEs in human, spread across 11 distinct PDE families (*PDE1* to *PDE11*). Among these, *PDE4B* has been reported as a candidate susceptibility gene for schizophrenia. This was on the basis of a translocation found in two affected members of a single pedigree and the observation that the protein interacts with Disrupted in Schizophrenia 1 (*DISC1*), itself another strong candidate gene for schizophrenia and affective disorders (34). Elevation of cellular cAMP leads to dissociation of *PDE4B* from DISC1 and an increase in *PDE4B* activity (34).

PDE7B degrades cAMP, but not cyclic guanosine monophosphate (cGMP), and is predominantly expressed in brain (33). To date, no direct evidence for association of *PDE7B* with schizophrenia has been reported; however, several findings provide some functional plausibility to our results. First, mRNA for *PDE7B* and dopamine D1, D2, and D3 receptors show a similar pattern of distribution, and it is thought that the dopamine D1 receptor activates *PDE7B* through the cAMP pathway (35). Second, *PDE7B* maps to 6q23-24, one of the most significant linkage regions for schizophrenia (OMIM %600511; SCZD3) (36). Lastly, association has recently been reported to the *Abelson Helper Integration Site 1* (AHI1) and *Family with sequence similarity 54 A* (*FAM54A*), which are respectively in the 5′ and 3′ regions of *PDE7B*. However, it should be noted that in those studies, SNPs in *PDE7B* were not associated with disease (37,38).

PDE inhibitors have recently emerged as being of interest as therapeutic agents for neuropsychiatric disorders, such as schizophrenia, depression, and dementia (33). Our results indicate that among these, drugs acting on *PDE7B* may be of particular value in schizophrenia, although particularly for clinical applications, our results should be treated with caution until independent replications have been reported.

Limitations and Conclusion

The major limitation in this study is that the sample sizes we used for the genomewide pharmacogenetics and gene expression studies are small. In particular, the phramacogenetics study is only highly powered to detect effects that are much larger than typical of common susceptibility alleles for diseases to date. This is less of a limitation with respect to one major goal of pharmacogenetics, namely, the identification of common markers with sufficiently large effects to be of value in guiding therapeutics. Our study suggests that in such large common effects may not exist, although being based on one of the earliest chips, the coverage of genes is incomplete, and it would be desirable to repeat this experiment with a denser set of SNPs. The extent to which clinical heterogeneity is likely to have an impact on treatment response, and therefore power to detect association to that response, is also currently

unknown. We presume it is likely to play some role, as is the possibility of imperfect adherence to treatment. More subtle effects are of potential value in informing about drug mechanisms relevant to therapeutic response, and here, power is limited. Given that limitation, we tried to minimize false negatives through the use of relaxed significance criteria but tried to control false positives by combining expression and genetic data. Nevertheless, replication of our findings are required. Our follow-up observation of association between *PDE7B*, a novel candidate gene, and schizophrenia does, however, suggest that the use of convergent data may have successfully enriched for findings of true relevance to schizophrenia and its response to treatment.

This work was supported in part by research grants from the Japan Ministry of Education, Culture, Sports, Science and Technology; the Ministry of Health, Labor and Welfare; from the Core Research for Evolutional Science and Technology; and from the Health Sciences Foundation (Research on Health Sciences focusing on Drug Innovation). Dr. Ikeda is a Japan Society for the Promotion of Science postdoctoral fellow for research abroad and is additionally supported by the Uehara Memorial Foundation and the Great Britain Sasakawa Foundation. We thank Ms. M. Miyata and Ms. S. Ishihara for their technical support.

The authors report no biomedical financial interests or potential conflicts of interest.

Supplementary material cited in this article is available online.

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Neuropharmacology 58 (2010) 1168–1173

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Association analysis of *SIGMAR1* with major depressive disorder and SSRI response

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ARTICLE INFO

Article history:
Received 21 November 2009
Received in revised form
11 February 2010
Accepted 12 February 2010

Keywords:
Major depressive disorder
Sigma1 non-opioid intracellular receptor 1 gene (*SIGMAR1*)
Functional SNP
Linkage disequilibrium
SSRIs response

SUMMARY

Background: Several investigations have suggested the possible involvement of sigma1 non-opioid intracellular receptor 1 (sigma1 receptor) in the pathophysiology of major depressive disorder (MDD). Sigma1 receptors are also one of the major pharmacological therapeutic targets of selective serotonin reuptake inhibitors (SSRIs). To evaluate the association of sigma1 receptor gene (*SIGMAR1*) and MDD and SSRIs therapeutic response in MDD, we conducted a case–control study of Japanese samples (466 MDD patients, 516 controls and 208 MDD patients treated by fluvoxamine or sertraline).
Method: We defined a clinical response as a decrease of more than 50% in baseline the Structured Interview Guide for Hamilton Rating Scale for Depression (SIGH-D) within 8 weeks, and clinical remission as an SIGH-D score of less than 7 at 8 weeks. Therefore, we selected rs1800866 in *SIGMAR1* for the following association analysis.
Results: In the logistic regression analysis, we detected an association of the phenotypes (MDD or controls) with rs1800866 genotype. However, we did not detect an association between rs1800866 and SSRI therapeutic response in Japanese MDD. In addition, remission with SSRI was not associated with rs1800866. Also, we did not detect a novel polymorphism in *SIGMAR1* when we performed a mutation search using MDD treated by SSRIs samples.
Conclusion: Our results suggest that rs1800866 in *SIGMAR1* may play a role in the pathophysiology of MDD in the Japanese population. Also, *SIGMAR1* does not play a role in the therapeutic response to SSRI in Japanese MDD patients. However, because our sample was small, a replication study using another population and larger sample will be required for conclusive results.

1. Introduction

Several investigations have suggested the possible involvement of sigma1 non-opioid intracellular receptor 1 (sigma1 receptor) in the pathophysiology of major depressive disorder (MDD) and the pharmacological therapeutic response in MDD (Bermack and Debonnel, 2005; Hashimoto and Ishiwata, 2006; Hayashi and Su, 2008; Ishikawa et al., 2007; Kulkarni and Dhir, 2009).

Preclinical studies suggest that fluvoxamine and sertraline, which are selective serotonin reuptake inhibitors (SSRIs), have effects on sigma1 receptors (Ishikawa et al., 2007; Narita et al., 1996). The sigma1 receptor is more abundant in the dentate gyrus of the hippocampal formation, facial nucleus and various thalamic and hypothalamic nuclei (Bouchard and Quirion, 1997). Sigma1 receptors regulate dopamine release in various areas of the brain (Gonzalez-Alvear et al., 1995), and regulate dopaminergic, N-methyl-D-aspartate (NMDA) and glutamatergic neurotransmission in limbic areas, including the nucleus accumbens and the ventral tegmental area (Ault and Werling, 1999; Gronier and Debonnel, 1999; Weatherspoon et al., 1996; Yamazaki et al., 2002). These mechanisms are known to be involved in the pathophysiology of MDD (Miller et al., 2009; Nestler and Carlezon, 2006). Neurodevelopmental and neurodegenerative models have been suggested to be involved in other aspects of the pathophysiology of

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0028-3908/$ – see front matter
doi:10.1016/j.neuropharm.2010.02.013

MDD (Ansorge et al., 2007). The hippocampal volume in MDD patients is significantly smaller than in control subjects (Kronmuller et al., 2009). Wang and Duncan reported that both neural survival and growth pathways are activated and the caspase-3-dependent apoptosis pathway is inactivated when sigma1 receptors are functionally active (Wang and Duncan, 2006). Nishimura and colleagues reported that sigma1 receptor agonist is involved in the mechanisms causing the drugs' potentiation of nerve growth factor (NGF)-induced neurite outgrowth in PC12 cells (Nishimura et al., 2008). Moreover, sigma1 receptors were shown to interact with inositol 1,4,5-triphosphate (IP3) receptors (Nishimura et al., 2008). Sigma1 receptor knockout mice also showed a depressive-like phenotype (Sabino et al., 2009). On the other hand, sigma1 receptors were reported to play an important role in the pathogenesis of anxiety and response to stress (Kamei et al., 1998; Lambert et al., 1998; Maurice et al., 1999), and depression (Akunne et al., 1997; Bermack and Debonnel, 2001).

From this evidence, we thought that the sigma1 receptor gene (*SIGMAR1*: OMIM *601978, 2 exons in this genomic region spanning 3.850 kb, and located on 9p13) was a good candidate gene for the pathophysiology of MDD. Several genome-wide association studies reported no association between *SIGMAR1* and MDD (Muglia et al., 2008; Sullivan et al., 2009) or clinical response to treatment with antidepressants in MDD (Garriock et al., 2010; Laje et al., 2009). However, we found no genetic association study that used Japanese samples. To evaluate the association of *SIGMAR1* and MDD and SSRIs therapeutic response in MDD, we conducted a case–control study of Japanese samples (466 MDD patients, 516 controls and 208 MDD patients treated by SSRIs).

2. Materials and methods

2.1. Subjects

The subjects in the association analysis were 466 MDD patients (218 males and 248 females; mean age ± standard deviation 48.7 ± 16.0 years) and 516 healthy controls (266 males and 250 females; 39.4 ± 17.1 years). The patients were diagnosed according to DSM-IV criteria with the consensus of at least two experienced psychiatrists on the basis of unstructured interviews and a review of medical records. 265 of the 466 MDD patients, who were treated with fluvoxamine (136 patients), sertraline (72 patients) or paroxetine (57 patients), were diagnosed according to DSM-IV criteria with the consensus of at least two experienced psychiatrists on the basis of a review of medical records and assessments with the Structured Interview Guide for Hamilton Rating Scale for Depression (SIGH-D) (Williams, 1988). Our subjects were hospital in-patients or outpatients, unrelated to each other and ethnically Japanese; cases were collected between January 2000 and December 2009. Almost patients were treated in the Department of Psychiatry, Fujita Health University Hospital, and University of Occupational and Environmental Health, Japan. The healthy controls were psychiatrically screened based on unstructured interviews. None had severe medical complications such as liver cirrhosis, renal failure, heart failure or other Axis-I disorders according to DSM-IV. No structured methods were used to assess psychiatric symptoms in the controls, which included hospital staff and medical students. The healthy controls were also unrelated to each other and ethnically Japanese, enrolled between January 2000 and December 2009. The study was described to subjects and written informed consent was obtained from each. This study was approved by the Ethics Committees at Fujita Health University, Nagoya University Graduate School of Medicine and the University of Occupational and Environmental Health.

2.2. Data collection

Fluvoxamine and sertraline affect on sigma1 receptors (Ishikawa et al., 2007; Narita et al., 1996). Therefore, we performed a pharmacogenic study using 208 MDD patients treated with fluvoxamine or sertraline. The 208 MDD patients in this study had scores of 12 or higher on the 17 items of the SIGH-D (Peveler and Kendrick, 2005). We defined a clinical response as a decrease of more than 50% in baseline SIGH-D within 8 weeks, and clinical remission as a SIGH-D score of less than 7 at 8 weeks. Fluvoxamine and sertraline were increased gradually to a maximum of 150 mg and 100 mg, respectively, depending on the patient's condition. Patients with insomnia and severe anxiety were prescribed benzodiazepine drugs, but no other psychotropic drugs were permitted during the study. Detailed information on data collection was described in a previous paper (Saito et al., 2006). The clinical characteristics of patients in this study, classified according to these definitions, can be seen in Table 1.

2.3. SNP selection and LD evaluation

We first consulted the HapMap database (release#24/phase II, Nov 2008, www.hapmap.org, population: Japanese Tokyo, minor allele frequencies (MAFs) of more than 0.05) and included one SNP (rs10814130) covering *SIGMAR1*. However, we thought that information of only one SNP on *SIGMAR1* from the HapMap database would be insufficient to reflect the detailed linkage disequilibrium (LD) background in the Japanese population. Rs1799729 and rs1800866 were reported to possibly be biologically functional SNPs (Prasad et al., 1998; Seth et al., 1997). Uchida and colleagues reported that LD from rs1800866 and rs1799729 was very tight ($r^2 = 1.00$) (Fig. 1) (Uchida et al., 2003). Therefore, we selected rs1800866 for LD evaluation. In order to reflect the LD background in the Japanese population, we genotyped rs1800866, which was reported to have a substitution from glutamine (CAG) to proline (CCG) that may perturb appropriate regulation of transportation from the endoplasmic reticulum to the plasma membrane of sigma1 receptors (Seth et al., 1997), and rs10814130 using our 40 of our own control samples, and made a more detailed LD evaluation of *SIGMAR1*. We determined the LD between rs1800866 and rs10814130 using HAPLOVIEW software (Barrett et al., 2005). Because LD from rs1800866 to rs10814130 was very tight in our control samples (r^2 more than 0.923) (Fig. 1), we selected rs1800866 for the following association analysis.

2.4. SNP genotyping

We used TaqMan assays (ABI: Applied Biosystems, Inc., Foster City, CA,) for rs1800866. One allelic probe was labeled with FAM dye and the other with fluorescent VIC dye. The plates were heated for 2 min at 50 °C and 95 °C for 10 min,

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

	N			Patients permitted with SSRIs, *n*[c]		Age, (mean ± SD)	Baseline SIGH-D, (avg ± SD)	SSRIs dose at eight weeks[d]	Number of previous episode, (avg ± SD)	Patients permitted with anxiolytics/hypnoticse, *n* (%)
	Total	Male	Female	FLV	STL					
Overall	208	100	108	136	72	47.8 ± 16.8	20.1 ± 5.48	119 ± 39.7	1.89 ± 0.818	69 (32.4)
Clinical response group[a]										
Responders	117	52	65	70	47	47.5 ± 15.9	21.1 ± 5.46	118 ± 37.9	1.83 ± 0.781	45 (21.1)
Nonresponders	91	48	43	66	25	48.4 ± 18.0	18.8 ± 5.11	122 ± 42.2	2.02 ± 0.871	24 (11.3)
P-value	0.249			0.0862		0.694	**0.00140**	0.462	0.179	0.103
Clinical remission group[b]										
Remitters	86	40	46	54	32	46.9 ± 15.8	19.7 ± 4.60	113 ± 39.6	1.94 ± 0.716	30 (14.1)
Nonremitters	122	60	62	82	40	48.6 ± 17.5	20.5 ± 5.98	124 ± 39.2	2.04 ± 0.878	39 (18.3)
P-value	0.814			0.397		0.499	0.291	**0.0462**	0.112	0.878

[a] Clinical response was defined as a 50% or greater decrease in the baseline SIGH-D score.
[b] Clinical remission was defined as a final SIGH-D score of less than 7.
[c] FLV: Fluvoxamine and STL: Sertraline.
[d] Imipramine-equivalent.

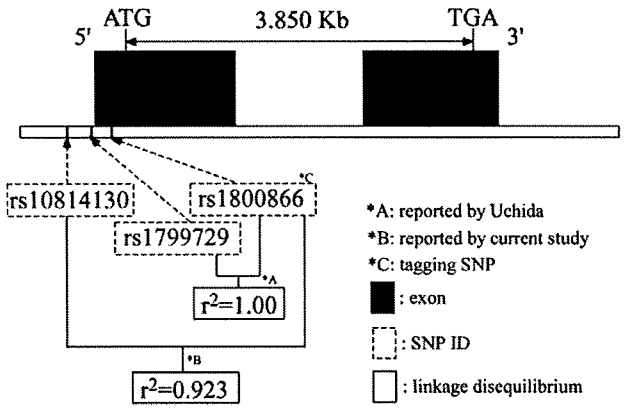

Fig. 1. LD evaluation in *SIGMAR1*. ATG is the start codon and TAG is the stop codon. Vertical bars represent exons. SNPs in *SIGMAR1*, which were already known, are represented by black boxes. The r^2 value is represented by broken line boxes. *A: reported by Uchida. *B: reported by current study. *C: tagging SNP.

followed by 45 cycles of 95 °C for 15 s and 58 °C for 1 min. Please refer to ABI for the primer sequence. We also used direct-sequence for rs10814130. Detailed information is available on request.

2.5. Mutation screening

We detected a significant association between *SIGMAR1* and MDD. In addition, therapeutic response and remission with fluvoxamine or sertraline was also marginally associated with rs1800866. Therefore, we performed mutation screening with *SIGMAR1* (promoter region, all exons including branch site) in 32 MDD treated by SSRIs patients (16 males and 16 females) using the primer extension method. We randomly selected 16 MDD patients of each sex after dividing by sex. The PCR method, including primer sequence and PCR condition, was adopted with reference to the study of Uchida and colleagues (Uchida et al., 2003). More detailed information about the primer extension method were published elsewhere (Suzuki et al., 2003).

2.6. Statistical analysis

Genotype deviation from the Hardy-Weinberg equilibrium (HWE) was evaluated by chi-square test (SAS/Genetics, release 8.2, SAS Japan Inc, Tokyo, Japan). The distribution of patient characteristics between MDD group and healthy control group was analyzed using a t test or a chi-square test. We found a significant difference in age among these groups ($P_{age} < 0.0001$), however, there was no difference in gender distribution ($P_{gender} = 0.339$). Although we investigated the difference in rs1800866' MAF by gender in the MDD cases, we did not detect a significant difference between MAF in males (0.257) and females (0.262). However, the number of male MDD patients was larger than the number of male healthy controls. We therefore performed a logistic regression analysis to compare the phenotype among rs1800866 genotypes to adjust possible confounding. The phenotype (MDD or controls) was the dependent variable, and gender, age at the time of recruitment and rs1800866 genotype were set as the independent variables. In the pharmacogenic study, individual *t* test and chi-square tests were used to compare means and categorical proportions (responders or nonresponders and remitters or nonremitters), respectively. Among the clinical characteristics of the patients in this study, a significant difference with total SIGH-D score was detected at the baseline in relation to SSRI therapeutic response (*P*-value = 0.00140) (Table 1). We also found another significant difference in SSRI dose at eight weeks between remitters and nonremitters (*P*-value = 0.0462) (Table 1). Therefore, we performed an analysis of the possible correlations between response or remission, SSRI treatment, and several clinical factors, using logistic regression. In these analyses, response classification was set as the dependent variable, and gender, age at the time of recruitment, SSRIs dose at eight weeks (imipramine-equivalent), SIGH-D total score at the baseline, and rs1800866 genotype were set as the independent variables. Polymorphisms were categorized into three genotypes, homozygous wild-type, heterozygous, and homozygous variant. Homozygosity for the more common allele was treated as the reference category. Tests for linear trend of odds ratio (additive model) were calculated using an ordered categorical variable by assigning scores to the genotypes: 0 (no variant allele), 1 (carrying one variant allele), and 2 (carrying two variant alleles). The statistical package JMP for Windows was used for logistic regression analysis (JMP 5.0. 1J, SAS Japan Inc, Tokyo, Japan).

Power calculation was performed using a Genetic Power Calculator (Purcell et al., 2003). We set each item in each value in the Genetic Power Calculator as follows: Prevalence: 0.2 in MDD and MDD treated with SSRI, respectively, User-defined: 0.05. The significance level for statistical tests was 0.05.

Table 2
Rs1800866 in *SIGMAR1* and major depressive disorder in the logistic regression analysis.

rs1800866	MDD, *n* (%)	Controls, *n* (%)	*P*-value[a]	OR[b]	95% CI[c]
AA	254 (54.5)	236 (45.7)			
AC	182 (39.1)	233 (45.2)	0.743	1.09	0.661–1.77
CC	30 (0.0644)	47 (9.11)	**0.0202**	0.563	0.346–0.913

Reference genotype is AA. Adjustment for age and gender.
[a] Bold numbers represent significant *P*-value.
[b] Odds ratio.
[c] 95% confidence interval.

3. Results

LD between rs1800866 and rs10814130 was very tight in our control samples (r^2 more than 0.9) (Fig. 1). Therefore, we selected rs1800866 for the association analysis.

Genotype frequencies of rs1800866 were in HWE. We performed a logistic regression analysis to compare the phenotype among rs1800866 genotypes to adjust possible confounding. In the logistic regression analysis, we detected an association of the phenotypes (MDD or controls) with rs1800866 genotype (Table 2).

Among the clinical characteristics of the patients in this study, a significant difference with total SIGH-D score was detected at the baseline in relation to SSRI therapeutic response (*P*-value = 0.00140) (Table 1). We also found another significant difference in SSRI dose at eight weeks between remitters and nonremitters (*P*-value = 0.0462) (Table 1). In addition to SSRI treatment in this cohort, one patient each was prescribed alprazolam, loflazepate, and bromazepam. Three patients each were prescribed estazoram, etizolam and quazepam. Sixteen patients each were prescribed triazolam and flunitrazepam. Twenty-five patients each were prescribed zolpidem. In the logistic regression analysis, we detected the significant correlation between SIGH-D total score at the baseline and SSRIs the therapeutic response (*P*-value: 0.00346). Also, SSRIs dose at eight weeks also did not correlate with remission (*P*-value: 0.486). However, response or remission with SSRI treatment, were not associated with rs1800866 (Table 3).

Although we performed mutation screening in *SIGMAR1* using MDD treated by SSRIs samples, we detected three SNPs (rs10814130, rs1800866 and rs1799729), which are already known (Fig. 1). LD among rs10814130, rs1800866 and rs1799729 was very tight (r^2 more than 0.9) (Fig. 1). LD from rs1799729 and rs1800866 in our controls was complete linkage disequilibrium, the same as in Uchida's study (Uchida et al., 2003). We did not find a novel SNP in *SIGMAR1*. Also, other previously reported SNPs were not detected in the Japanese MDD patients.

Table 3
Logistic regression analysis of rs1800866 in *SIGMAR1* in both definition groups.

rs1800866	Responder, *n* (%)	Nonresponder, *n* (%)	*P*-value[a]	OR[b]	95% CI[c]
AA	54 (46.2)	54 (59.3)			
AC	56 (47.9)	34 (37.4)	0.899	0.928	0.273–2.85
CC	7 (5.98)	3 (3.30)	0.273	2.88	0.476–23.9
rs1800866	Remitter, *n* (%)	Nonremitter, *n* (%)	*P*-value[a]	OR[b]	95% CI[c]
AA	39 (45.3)	69 (56.6)			
AC	40 (46.5)	50 (41.0)	0.484	0.667	0.199–2.00
CC	7 (8.14)	3 (2.46)	0.0769	5.49	0.915–45.4

Reference genotype is AA. Adjustment for age, gender, SIGH-D score at baseline, SSRI dose at 8 weeks and number of episode.
[a] Bold numbers represent significant *P*-value.
[b] Odds ratio.
[c] 95% confidence interval.

In the power analysis, we obtained more than 80% power for the detection of association when we set the genotype relative risk at 1.35 and 2.28 in MDD and SSRI response in MDD, respectively, for rs1800866 in *SIGMAR1* under a multiplicative model of inheritance.

4. Discussion

We first performed a gene-based association analysis between *SIGMAR1* and MDD in the Japanese population. We performed an analysis of the possible correlations between phenotype and rs1800866 genotype and several clinical factors using logistic regression. In the logistic regression analysis, we detected an association of the phenotypes (MDD or controls) with rs1800866 genotype. Our results suggest that *SIGMAR1* plays a role in the pathophysiology of MDD in the Japanese population. On the other hand, the significant difference in baseline SIGH-D scores between responders and nonresponders might have affected the results; the baseline SIGH-D scores could predict clinical response in the logistic regression analysis. We did not find an association between rs1800866 genotype and SSRI therapeutic response in the logistic regression analysis. Remission with fluvoxamine or sertraline was marginally associated with rs1800866 in the case-control study. We also found another significant difference in SSRI dose at eight weeks between remitters and nonremitters. Therefore, we also performed an analysis of the possible correlations between remission with SSRI treatment and several clinical factors using logistic regression. Rs1800866 genotype was not predictive of the therapeutic remission. Therefore, we suggest that rs1800866 in the *SIGMAR1* genotype was not a predictor of SSRI treatment remission in Japanese MDD.

There was a significantly lower percentage of the C allele among MDD patients (MAF: 26.0%) than among controls (MAF: 31.7%). On the other hand, there was the trend toward higher percentage of the C allele among patients (MAF in responders and remitters: 29.9% and 31.5%, respectively) than among nonresponders and nonremitters (MAF in nonresponders and nonremitters: 22.0% and 23.0%, respectively). Rs1800866 (also known as A61C) was reported to be a possible functional SNP in exon 1, leading to an amino acid change from glutamine (CAG) to proline (CCG) at position 2 of the receptor protein (Seth et al., 1997). It is considered that this SNP may be a biologically functional SNP (Seth et al., 1997). LD from rs1800866 and rs1799729 in our MDD samples was completed as in Uchida's study using a Japanese sample (Uchida et al., 2003).

Miyatake and colleagues reported functional polymorphisms (T-485A) in the promoter region of *SIGMAR1* (Miyatake et al., 2004). However, MAF of this SNP is less that 5% according to studies of Miyatake and colleagues (Miyatake et al., 2004) and Satoh and colleagues (Satoh et al., 2004). Therefore, we designed the study based on the common disease-common variants hypothesis (CD–CV hypothesis) (Chakravarti, 1999), and did not perform an analysis on this SNP. Also, although we performed mutation screening in *SIGMAR1*, we did not detect this SNP. We considered that this result might have been caused by a small sample size.

Recently, Ishikawa and colleagues reported that rs1799729 and T-485A in *SIGMAR1* were not associated with fluvoxamine binding potential (Ishikawa et al., 2007). These authors suggested that that rs1799729 and T-485A in *SIGMAR1* may not contribute to differences in sigma1 receptor in the human brain (Ishikawa et al., 2007). Moreover, they suggested that it will be necessary to conduct a replication study using larger samples, since T-485A, which is located in the promoter region and has a biologically functional effect, has very low MAF (less than 5% in the Japanese population) (Ishikawa et al., 2007).

Ishiguro and colleagues' haplotype analysis showed that SNPs in *SIGMAR1* (rs1799729 and rs1800866) were associated with schizophrenia (Ishiguro et al., 1998). These SNPs were also reported to be associated with Japanese alcoholism (Miyatake et al., 2004) and schizophrenia (Ishiguro et al., 1998). In addition, rs1800866 was reported to be associated with Alzheimer disease/Alzheimer type dementia (Uchida et al., 2005). On the other hand, rs1799729 and rs1800866 in *SIGMAR1* were not associated with Japanese methamphetamine use disorder (Inada et al., 2004). Ohmori and colleagues reported that rs1799729 was not associated with Japanese schizophrenia (Ohmori et al., 2000), and Satoh and colleagues reported that SNPs in *SIGMAR1* (examined SNP: rs1799729, rs1800866 and T-485A) were not associated with Japanese schizophrenia (Satoh et al., 2004). In addition, Uchida and colleagues reported no association between rs1800866 and Japanese schizophrenia in a case-control study and a meta-analysis (Uchida et al., 2003).

A few points of caution should be mentioned with respect to our results. Firstly, the association between *SIGMAR1* and MDD patients may be due to biased samples, such as small sample sizes or unmatched age. We therefore performed a logistic regression analysis to compare the phenotypes among rs1800866 genotypes, using several clinical factors as other independent variables to adjust possible confounding. In the power analysis, we obtained more than 80% power for the detection of association when we set the genotype relative risk at 1.35 and 2.28 in MDD and SSRI response in MDD, respectively, for rs1800866 in *SIGMAR1* under a multiplicative model of inheritance. Because our samples were small, statistical errors are possible in the results of these statistical association analyses. On average, the controls were much younger than the patients. This means that a number of young controls may go on to develop one of these disorders, most likely MDD, since the incidence of major depression is as high as 5% or more. Our subjects did not undergo structured interviews, and it is reported that MDD patients who are not diagnosed by structured interview may develop bipolar disorder in the future (Bowden, 2001; Kishi et al., 2008, 2009a–d). However, in this study patients were carefully diagnosed according to DSM-IV criteria with consensus of at least two experienced psychiatrists on the basis of a review of medical records. In addition, when we found a patient who had been misdiagnosed, we promptly excluded the misdiagnosed case to maintain the precision of our sample (Kishi et al., 2008, 2009a–d). Also, Yamaguchi-Kabata and colleagues reported that different proportions of individuals from different regions of Japan in case and control groups can lead to statistical error (Yamaguchi-Kabata et al., 2008). Therefore, the positive association with MDD could be due to type I error, possibly because of population stratification. Secondly, we performed a mutation scan of *SIGMAR1*. Thirdly, the analysis of epigenetic controls and other genetic variations such as CNV in *SIGMAR1* were not performed in our study. Fourthly, sixty-nine of the 208 fluvoxamine and sertraline treatment patients were prescribed anxiolytics/hypnotic. Since benzodiazepines are also effective antidepressant molecules, our results for these 69 MDD patients may have been influenced by the anxiolytics/hypnotic. Because we consider it to be difficult to evaluate the association of extremely rare variants from the viewpoint of statistical power, a replication study using a larger sample will be required for conclusive results.

Our results suggest that *SIGMAR1* plays a role in MDD in the Japanese population. Also, *SIGMAR1* does not play a role in the therapeutic response to SSRI in Japanese MDD patients. However, because our samples are small, it will be important to replicate and confirm these findings in other independent studies using larger samples.

Acknowledgements

We thank Ms M Miyata and Ms S Ishihara for their technical support. This work was supported in part by research grants from

the Ministry of Education, Culture, Sports, Science and Technology, the Ministry of Health, Labor and Welfare, and the Japan Health Sciences Foundation (Research on Health Sciences focusing on Drug Innovation).

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Progress in Neuro-Psychopharmacology & Biological Psychiatry 34 (2010) 401–405

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

QTc prolongation and antipsychotic medications in a sample of 1017 patients with schizophrenia

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ARTICLE INFO

Article history:
Received 8 September 2009
Received in revised form 11 January 2010
Accepted 11 January 2010
Available online 15 January 2010

Keywords:
Antipsychotics
QT prolongation
Schizophrenia
Sudden death

ABSTRACT

Many antipsychotic drugs cause QT prolongation, although the effect differs based on the particular drug. We sought to determine the potential for antipsychotic drugs to prolong the QTc interval (>470 ms in men and >480 ms in women) using the Bazett formula in a "real-world" setting by analyzing the electrocardiograms of 1017 patients suffering from schizophrenia. Using logistic regression analysis to calculate the adjusted relative risk (RR), we found that chlorpromazine (RR for 100 mg = 1.37, 95% confidence interval (CI) = 1.14 to 1.64; $p<.005$), intravenous haloperidol (RR for 2 mg = 1.29, 95% CI = 1.18 to 1.43; $p<.001$), and sultopride (RR for 200 mg = 1.45, 95% CI = 1.28 to 1.63; $p<.001$) were associated with an increased risk of QTc prolongation. Levomepromazine also significantly lengthened the QTc interval. The second-generation antipsychotic drugs (i.e., olanzapine, quetiapine, risperidone, and zotepine), mood stabilizers, benzodiazepines, and antiparkinsonian drugs did not prolong the QTc interval. Our results suggest that second-generation antipsychotic drugs are generally less likely than first-generation antipsychotic drugs to produce QTc interval prolongation, which may be of use in clinical decision making concerning the choice of antipsychotic medication.

1. Introduction

QTc interval prolongation is associated with presyncope, syncope, polymorphic ventricular tachycardia, the subtype torsade de pointes, and sudden cardiac death (Faber et al., 1994). Previous studies have indicated an increased risk of sudden cardiac death in patients treated with antipsychotics (Hennessy et al., 2002; Ray et al., 2001; Straus et al., 2004). A retrospective cohort study of 481,744 Tennessee Medicaid enrollees, of whom 1487 died from sudden cardiac death, found that current moderate-dose antipsychotic use (>100 mg of thioridazine equivalents) increased the rate of sudden cardiac death (multivariate risk ratio of 2.39), when compared with the nonuse of antipsychotics (Ray et al., 2001). A cohort study of three U.S. medical programs found that patients with treated schizophrenia had higher rates of cardiac arrest and ventricular arrhythmia than did controls (patients with glaucoma and those with psoriasis), with risk ratios ranging from 1.7 to 3.2 (Hennessy et al., 2002). A study of 554 sudden cardiac death subjects reported that the current use of antipsychotics was associated with a three-fold increased risk of cardiac death (Straus et al., 2004).

Although torsade de pointes and sudden death are rare, rate-corrected QT (QTc) prolongation serves as a risk factor for these conditions. In a study of 495 psychiatric patients receiving various psychotropic drugs and 101 healthy reference individuals, 8% of patients showed QTc prolongation (>456 ms) (Reilly et al., 2000). Advanced age (>65 years), as well as the use of tricyclic antidepressants, thioridazine, and droperidol were indicated as robust predictors of QTc lengthening (Reilly et al., 2000). High antipsychotic doses were also associated with QTc prolongation (Reilly et al., 2000). In a sample of 111 psychiatric inpatients receiving a median daily dose of more than 600 mg [chlorpromazine (CP) equivalent] of antipsychotics, 90% had schizophrenia or related psychoses, and 23% showed QTc interval of >420 ms, whereas only 2% of unmedicated controls did (Warner et al., 1996). However, there is little clinical data to aid in assessing the

Abbreviations: QTc, rate-corrected QT; 95% CI, 95% confidence interval; HPD, haloperidol; HPDiv, intravenous injection of haloperidol; RR, relative risk; ECG, electrocardiogram; SGAs, second-generation antipsychotics; FGAs, first-generation antipsychotics; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, 4th ed.; CP, chlorpromazine; LP, levomepromazine; OR, odds ratio.

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doi:10.1016/j.pnpbp.2010.01.008

risk of QTc prolongation for an individual antipsychotic in a dose-dependent manner, particularly for second-generation antipsychotics (SGAs). Some case reports have indicated that SGAs can induce QTc prolongation (Dineen et al., 2003; Vieweg, 2003). However, such anecdotal reports do not provide clear evidence of whether SGAs increase the risk of QTc prolongation, as in first-generation antipsychotics (FGAs), in a real-world setting. This study examined the risk of QTc prolongation of antipsychotic drugs in a large clinical sample from Japan. Japan is known to use higher doses of antipsychotics (Bitter et al., 2003), providing a unique opportunity to investigate the risk of QTc prolongation in a wide range of antipsychotic doses.

2. Methods

2.1. Patients

Clinical information, including data on QTc intervals, was collected from inpatients with schizophrenia who were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, 4th ed. (DSM-IV) in four independent hospitals. Approval from the ethics committee of each hospital was obtained. Data collection on all inpatients with schizophrenia was begun on the following dates in three psychiatric hospitals Biwako Hospital, Toyosato Hospital, and Minakuchi Hospital: February 2, 2007; February 3, 2007; and July 29, 2007, respectively. In the fourth hospital, the National Center of Neurology and Psychiatry Hospital, clinical records were collected for all patients who were admitted to its psychiatric wards between 1998 and 2007. A total of 1065 inpatients were included from the four hospitals, and all of them underwent ECG screening. Among them, 37 patients were excluded due to hypokalemia (serum potassium <3.5 mEq/L), which can induce QTc interval prolongation (Elming et al., 2003; Taylor, 2003). Two were excluded because of hypothyroidism, and nine because of cardiac disease (four patients with right bundle branch block, two with post-acute myocardial infarction, one with WPW syndrome, one with atrial–ventricular block, and one who underwent surgery for atrial septal defect). The remaining 1017 patients had a mean age of 42.6 years (S.D., 18.2) and were included in the analysis.

2.2. Procedure

A standard 12-lead ECG was recorded at 25 mm/s. Because the QTc interval is influenced by heart rate, it was corrected by Bazett's formula (QTc: $QTc = QT/RR^{1/2}$) (Bazett, 1920). An ECG recording showing the longest QTc interval was selected for each patient whose ECG was recorded two or more times. The QTc was measured automatically by a program on the ECG apparatus (MAC 5500 with 12SL algorithm by GE health care [Amersham Place, Little Chalfont, Buckinghamshire, UK]). For patients with a QTc > 430 ms, QTc and RR intervals were measured manually for the chest lead with the maximal T-wave amplitude, according to Charbit et al. (2006). The end of the T-wave was determined as the intersection between the tangent to the steepest downslope of the T-wave and the isoelectric line. QTc prolongation was defined as a QTc length of more than 470 ms in males and more than 480 ms in females, as 99% of "healthy" people can be excluded by this cut-off value (Taggart et al., 2007). One of the coauthors (M.H.), a cardiologist who specializes in arrhythmias, trained the authors on how to evaluate an ECG recording. Information on drugs administered within 24 h of the ECG recording was obtained. Table 1 shows the distribution of drugs that were administered in more than 3% of the patients and the prevalence of QTc prolongation for each medication. One hundred forty-two patients were drug free when the ECG was recorded, because they were given the test at admission before they had taken any drugs. Two hundred sixty-five patients were on monotherapy. Doses of antipsychotics, antiparkinsonian drugs, and benzodiazepines were converted into those of CP, biperiden, and diazepam equivalents, respectively (Inagaki and Inada, 2006). Subjects who were coadministered medical drugs (i.e., non psychotropic drugs) with an increased risk of producing torsade de pointes were excluded (Chan et al., 2007).

Table 1
Medication and rate of QTc prolongation in 1017 patients. Drugs which were administrated to more than 3% of patients are shown.

Administrated drugs	No. of Patients $n = 1017$ (100%)	Mean dose (SD), mg	No. of patients (%) with QTc prolongation (male: >470 ms, female >480 ms)
Equivalent dose			
CP eq.	875 (86)	963.0 (879.0)	23 (2.6)
Diazepam eq.	672 (66)	14.6 (14.6)	18 (2.7)
Biperiden eq.	645 (63)	3.8 (2.2)	19 (2.9)
Mood stabilizer			
CBZ	74 (7)	478.9 (201.8)	3 (4.1)
VPA	54 (5)	650.0 (334.1)	1 (1.9)
Lithium	47 (5)	587.2 (199.6)	4 (8.5)
Antipsychotics			
HPD	375 (37)	15.9 (12.6)	16 (4.3)
CP	299 (29)	190.5 (198.7)	9 (3.0)
LP	258 (25)	91.9 (94.5)	14 (5.4)
Risperidone	248 (24)	5.6 (3.7)	4 (1.6)
Zotepine	116 (11)	179.9 (124.9)	3 (2.6)
Olanzapine	104 (10)	15.6 (6.4)	0 (0.0)
Quetiapine	60 (6)	375.5 (258.5)	0 (0.0)
Bromperidol	49 (5)	10.7 (8.6)	0 (0.0)
Sultopride	49 (5)	1032.9 (810.2)	10 (20.4)
HPD iv	47 (5)	16.0 (10.5)	8 (17.0)

Abbreviations: eq = equivalent; HPD = haloperidol, CP = chlorpromazine; LP = levomepromazine, CBZ = carbamazepine, VPA = sodium valproate; No. = Number, SD = standard deviation.

2.3. Statistical analyses

First, logistic regression analysis was applied to examine risk factors for QTc prolongation. Age, sex, antipsychotic dose (CP equivalent), benzodiazepine dose (diazepam equivalent), and antiparkinsonian drug dose (biperiden equivalent) were included in the backward stepwise regression model. In the second analysis, age, sex, and individual antipsychotic doses were entered as independent variables in the logistic regression analysis. Then, the adjusted relative risks of important explanatory variables were calculated via the backward stepwise regression analysis. Drugs that were administrated in more than 3% of the patients were analyzed.

Linear regression analysis was used to determine which antipsychotics lengthened the QTc interval in a dose-dependent manner, as the antipsychotic dose was entered as a continuous variable. Then, the adjusted coefficients were calculated using the stepwise selection model. Age, sex, and individual antipsychotic doses were entered as independent variables.

The χ^2 test was used to examine the risk-increasing effect of excessive use of antipsychotics (cut-off points of 1000 or 1500 mg/day of CP equivalent). All statistical analyses were performed using the SPSS, version 13.0 (SPSS Japan, Inc., Tokyo, Japan). All p-values reported are two tailed. Statistical significance was considered when p-value was less than 0.05.

3. Results

The prevalence of QTc prolongation (>470 ms in male and >480 ms in female) was 2.5% (male: 3.7%; female: 1.0%). Logistic regression analysis showed that the antipsychotic dose was a significant risk factor for QTc prolongation (Table 2), whereas antiparkinsonian drugs, benzodiazepines, and mood stabilizers were not risk factors for QTc prolongation. Administration of antipsychotic doses greater than 1000 and 1500 mg/day of CP equivalent was found

Table 2
Result of logistic regression analysis on the risk of QTc prolongation for standardized doses.

	Unadjusted relative risk (95% CI)	Adjusted relative risk (95% CI)
Age	0.97 (0.94–0.99)	
Sex (risk of female)	0.33 (0.12–0.95)	
CP eq. (100 mg)	1.08 (1.05–1.12)*	1.07 (1.04–1.10)*
Diazepam eq. (1 mg)	1.01 (0.98–1.04)	
Biperiden eq. (1 mg)	0.87 (0.72–1.06)	
CBZ (100 mg)	1.00 (1.00–1.00)	
VPA (100 mg)	1.00 (0.99–1.00)	
Lithium (100 mg)	1.00 (1.00–1.01)	
	The Hosmer–Lemeshow Goodness-of-Fit Test $x^2=4.77$ df = 8 $p=0.85$	The Hosmer–Lemeshow Goodness-of-Fit Test $x^2=5.15$ df = 8 $p=0.74$

*$p<0.001$.
Abbreviations: eq = equivalent, CP = chlorpromazine, CBZ = carbamazepine; VPA = sodium valproate, CI = confidence interval.

to increase the risk of QTc prolongation 1.97 fold (95% CI, 1.48–2.59, $p<0.001$) and 2.76 fold (95% CI, 1.80–4.18, $p<0.001$), respectively, when compared to their counterparts. On examination of individual antipsychotics, haloperidol intravenous injection (HPDiv), CP, and sultopride were found to increase the risk of QTc prolongation (Table 3).

In the stepwise selection model of the multiple linear regression analysis, CP, HPDiv, levomepromazine (LP), and sultopride were found to lengthen the QTc interval. Age was also indicated as a risk factor for QTc lengthening. Adjusted coefficients for CP, HPDiv, LP, sultopride, and sex are shown in Table 4. Adding 100 mg of LP, for example, extended the QTc interval by 4.65 ms. Bromperidol, olanzapine, quetiapine, risperidone, and zotepine had no significant lengthening effect on the QTc interval.

Table 3
Result of logistic regression analysis on the risk of QTc prolongation for each antipsychotic drug.

	Unadjusted relative risk (95%CI)	Adjusted relative risk (95%CI)
Age	0.99 (0.96–1.03)	
Sex (risk of female)	0.38 (1.26–1.16)	
HPD (2 mg)	0.99 (0.92–1.06)	
CP (100 mg)	1.37 (1.13–1.67)*	1.37 (1.14–1.64)*
LP (100 mg)	1.55 (0.92–2.61)	
Risperidone (1 mg)	1.01 (0.84–1.12)	
Zotepine (66 mg)	0.91 (0.62–1.34)	
Olanzapine (2.5 mg)	0.00 (0.00 to >100)	
Quetiapine (66 mg)	0.00 (0.00 to >100)	
Bromperidol (2 mg)	0.00 (0.00 to >100)	
Sultopride (200 mg)	1.40 (1.23–1.60)**	1.45 (1.28–1.63)**
HPD iv (2 mg)	1.26 (1.13–1.40)**	1.29 (1.18–1.43)**
	The Hosmer–Lemeshow Goodness-of-Fit Test $x^2=5.04$ df = 8 $p=0.75$	The Hosmer–Lemeshow Goodness-of-Fit Test $x^2=17.81$ df = 8 $p=0.013$

*$p<0.005$.
**$p<0.001$.
Abbreviations: HPD = haloperidol, CP = chlorpromazine, LP = levomepromazine, iv = intravenous injection, CI = confidence interval.

4. Discussion

In a large clinical sample, we confirmed that a daily dose of antipsychotics (CP equivalents) was associated with a dose-dependent increased risk of QTc prolongation; however, the use of antiparkinsonian drugs, benzodiazepines, and mood stabilizers did not significantly increase this risk. With regard to individual antipsychotics, CP, HPDiv, and sultopride were shown to significantly increase the risk of QTc prolongation. CP, HPDiv, LP, and sultopride were found to significantly lengthen the QTc interval, whereas HPD, bromperidol, olanzapine, quetiapine, risperidone, and zotepine were not.

Our observation that a daily dose of antipsychotics was associated with a risk of QTc prolongation is consistent with previous studies (Reilly et al., 2000; Warner et al., 1996). In our sample, antipsychotic doses of more than 1000 and 1500 mg/day of CP equivalents were found to increase the risk of QTc prolongation by approximately 2.0 and 3.0 fold, respectively, when compared to their counterparts. Reilly et al. also reported that a high dose (1000 to 2000 mg/day) and a very high dose (>2000 mg/day) predicted QTc prolongation [odds ratio (OR), 5.3 and 8.2, respectively] (Reilly et al., 2000). Warner et al. reported an OR of 4.3 for doses higher than 2000 mg/day (Warner et al., 1996). In contrast to antipsychotics, mood stabilizers showed no significant risk-increasing effect. This is consistent with a previous finding, which showed that lithium or carbamazepine did not significantly increase the risk of QTc prolongation (Reilly et al., 2000). However, a recent study suggested that lithium increases the QTc interval significantly (18.6 ms; 95% CI, 4.8–32.4 ms) (van Noord et al., 2009). Furthermore, lithium is known to cause T-wave changes (Mitchell and Mackenzie, 1982; Reilly et al., 2000) that may lead to torsade de pointes when combined with a QTc-lengthening antipsychotic (Liberatore and Robinson, 1984). Thus, the use of lithium requires careful ECG monitoring. With respect to valproate, our study may be the first to investigate the risk of QTc prolongation for this drug in a clinical setting. With regard to coadministered benzodiazepine and antiparkinsonian drugs, our results suggest no significant effect on QTc prolongation. Although some patients taking diazepam and biperiden equivalent showed QTc interval prolongation (Table 1), the results of logistic regression analysis showed no significant risk-increasing effect of these drugs (Table 2). Therefore, these patients were also taking chlorpromazine equivalent and it was the chlorpromazine equivalent that explained the QTc interval prolongation. Indeed, to our knowledge, there has been no study reporting that these drugs cause QTc prolongation or torsade de pointes.

With respect to individual antipsychotics, previous studies have reported that thioridazine, intravenous droperidol, sertindole, and ziprasidone are associated with a strong risk-increasing effect on QTc prolongation (Czekalla et al., 2001a; Harrigan et al., 2004; Taylor,

Table 4
QTc prolongation effect of each antipsychotic by linear regression model.

	Forced entry model	Stepwise selection model
	Coefficient (95% CI)	Coefficient (95% CI)
Age	0.19 (0.10–0.28)*	0.20 (0.11–0.29)*
Sex (risk of female)	3.22 (−0.01–6.44)	
HPD (2 mg)	0.42 (0.09–0.76)	
CP (100 mg)	3.91 (2.69–5.13)*	3.82 (2.62–5.02)*
LP (100 mg)	4.87 (2.14–7.60)*	4.65 (1.94–7.37)*
Risperidone (1 mg)	0.07 (−0.47–0.61)	
Zotepine (66 mg)	−0.36 (−1.91–1.20)	
Olanzapine (2.5 mg)	0.30 (−0.47–1.08)	
Quetiapine (66 mg)	0.11 (−0.87–1.09)	
Bromperidol (2 mg)	0.08 (−1.00–1.16)	
Sultopride (200 mg)	3.65 (2.48–4.82)*	3.56 (2.41–4.72)*
HPD iv (2 mg)	3.16 (2.36–3.96)*	3.13 (2.34–3.93)*

*$p<0.001$.
Abbreviations: HPD = haloperidol, CP = chlorpromazine, LP = levomepromazine; iv = intravenous injection, CI = confidence interval.

2003). In Japan, commercial use of thioridazine ended in 2005; intravenous droperidol has not been used in psychiatric treatment; and sertindole and ziprasidone have not been introduced. Thus, we could not confirm the effect of these drugs. However, our results provide robust evidence that HPDiv increases the risk of QTc prolongation. This concurs with Hatta et al. who compared the differences in QTc length among psychiatric emergency patients who received intravenous flunitrazepam alone and those who received intravenous flunitrazepam and haloperidol and found that the latter group showed significantly longer QTc intervals than the former (Hatta et al., 2001). Vieweg et al. (2009) reviewed the literature and identified cases of patients aged ≥60 years who developed QTc interval prolongation, polymorphic ventricular tachycardia/torsade de pointes and/or sudden cardiac death while taking antipsychotic or antidepressant drugs or a combination of these medications. Among such cases, most frequently reported medication was HPDiv (14 out of 37 cases). These findings and ours support the recent alert of the U.S. Food and Drug Administration warning that HPDiv increases the risk of QTc prolongation and torsade de pointes based on at least 28 cases reported in the literature (U.S. Food and Drug Administration Cfdear, 2007). Oral HPD, in contrast, was found to have no statistically significant risk-increasing effect on QTc prolongation, although it had a significant QTc-lengthening effect. Previous findings have suggested that oral HPD at low or moderate doses had no clear effect on QTc, but that it is associated with QTc prolongation and torsade de pointes at higher clinical doses (>20 mg/day) (Czekalla et al., 2001a; Taylor, 2003). Taken together, excessively high blood levels of the drug after an intravenous injection or oral intake of high doses may be critical for the effect of HPD. Regarding bromperidol (oral use only), a chemically similar butyrophenone to HPD, we obtained no evidence for its effect on QTc prolongation or lengthening. To our knowledge, this is the first study to examine bromperidol for such effects. Further studies are warranted to confirm our results. With respect to CP, we detected significant effects on both QTc prolongation and QTc lengthening, which is consistent with previous findings, suggesting an intermediate effect of CP on QTc (i.e., a weaker effect than that of thioridazine, but stronger than oral HPD) (Czekalla et al., 2001a; Mehtonen et al., 1991; Witchel et al., 2003), although there have been some reports of no significant risk-increasing effect of CP (Reilly et al., 2000; Strachan et al., 2004). LP, another phenothiazine, was also found to lengthen the QTc interval in the multiple regression analysis. In the logistic regression, statistical significance was nearly achieved ($p=0.06$, Table 3). These results suggest that LP is likely to increase the risk of QTc prolongation. Although there have been little data on LP in relation to QTc in the literature, an association between sudden death and the use of phenothiazines is prominent, and LP might have been involved in such deaths (Mehtonen et al., 1991). Finally, sultopride, a benzamide derivative, was found to significantly increase the risk of QTc prolongation and QTc lengthening. To our knowledge, this is the first time that such evidence has been obtained for sultopride. Further studies are warranted to confirm our results.

Our results provide no evidence for the possible risk-increasing effect of the examined SGAs (olanzapine, quetiapine, risperidone, and zotepine) on QTc prolongation. Recently, Ray et al. (2009) reported that atypical antipsychotics double the risk of sudden cardiac death when compared with nonusers of antipsychotic drugs, a finding that contradicts our data. However, SGAs can induce weight gain, insulin resistance, and dyslipidemia (Tschoner et al., 2009), all of which are risk factors for ischemic heart diseases. Therefore, the increased sudden death observed by Ray et al. (2009) could be attributable to the increased risk of ischemic heart diseases rather than torsade de pointes due to QTc prolongation. The Pfizer 054 study (2000) reported that SGAs, such as risperidone, quetiapine, ziprasidone, and olanzapine, induced QTc interval prolongation. In the review of Czekalla et al. (2001a), it was suggested that risperidone and quetiapine could lengthen the QTc interval, although the effect observed was smaller than that of thioridazine and chlorpromazine. Olanzapine, in particular, was reported to have little effect on the QTc-interval length (Czekalla et al., 2001b). Dineen et al. (2003) reported the case of a patient who was treated with olanzapine and showed an abnormal QTc interval. Vieweg (2003) reviewed the literature and found nine cases in which QTc prolongation was associated with SGA administration (four cases of risperidone [one case was his original case], three cases of quetiapine, and two cases of ziprasidone). Taken together, although our results suggest that the SGAs (olanzapine, quetiapine, risperidone, and zotepine) are less likely to produce QTc interval prolongation than the FGAs examined herein, the SGAs can also cause QTc prolongation. Thus, further investigations with a more refined methodology are warranted. In particular, the current group-derived formula for correcting QT interval measurements to a heart rate of 60 beats per/min (QTc) are unsatisfactory (Malik, 2001), and, as pointed out by Vieweg (2003), determining the effect of drug-induced change amid the noise of random variation (regression to the mean) will require a new technology.

Female gender is known to be a risk factor for QTc prolongation (Taylor, 2003; Vieweg et al., 2009). However, we failed to detect female gender as a significant risk factor in our sample. Moreover, QTc prolongation was found more commonly in male patients than in female patients. One reason for these results was that the antipsychotic dose was substantially lower in female patients than in male patients (mean CP equivalent dose: 841 vs. 1066 mg/day; frequency of >1500 mg/day: 13.9% vs. 20.8%). In addition, because some previous studies in psychotic patients did not detect the gender difference (Chong et al., 2003; Hatta et al., 2000), such populations may have other factors that attenuate the gender difference.

There are several limitations to the study. First, we did not include medications other than psychotropic drugs in the analysis; however, the subjects included in the analysis were not coadministered other medical drugs that increased the risk for torsade de pointes (Chan et al., 2007). We also excluded patients suffering from cardiac diseases. Furthermore, psychotropic drugs that were administrated to 3% or fewer of the patients in the sample were not included in the analysis. The fact that nearly all patients received multiple drugs and a substantial proportion of participants (69%) were treated with antipsychotic polypharmacy may have made it difficult to obtain a clear result for each drug. However, there is great value in assessing the increased risk of QTc prolongation in such a practical setting. Our participants were all inpatients, and therefore individuals with severe symptomatology and those patients on high doses of antipsychotics were likely to be overrepresented. A recent study reported the possibility that an acute psychotic state itself may be a risk factor for QTc prolongation (Bar et al., 2007). Severe symptomatology might have biased the results toward an increased prevalence of the QTc interval in our subjects.

To screen QTc interval, we used an automated program, which may be fraught with errors. However, Charbit et al. (2006), for example, reported that patients with automatic QTc of <430 ms were at very low risk of having a prolonged QT interval where their definition of prolonged QTc interval was >450 ms in women and >440 ms in men. We measured QTc interval manually for patients with an automated QTc of >430 ms, although our definition of QTc prolongation was >480 ms in women and >470 ms in men. Thus, it was unlikely that we missed patients with QTc prolongation in our study. Furthermore, the reliability of the measurement algorithm of the ECG equipment (MAC 5500 with 12SL algorithm by GE health care [Amersham Place, Little Chalfont, Buckinghamshire, UK]) that we used was reported to be high. The data obtained by this algorithm was within 10 ms of the manual measurement in 95.9% of ECGs and within 15 ms in 99.3% of ECGs (Hnatkova et al., 2006). Thus, the possible effect of the use of the automated program is likely minimal. Another limitation might be that we used the chest lead with the maximal T-wave amplitude because clear T-wave leads are needed for precise

manual measurement. However, Bazett generally used limb lead II to determine his formula.

Despite these limitations, we obtained robust evidence among a large clinical sample in a real-world setting that suggested that a daily dose of antipsychotics is associated with a dose-dependent increased risk of QTc prolongation, whereas that of antiparkinsonian drugs, benzodiazepines, and mood stabilizers is not. With regard to individual antipsychotics, our results suggest that FGAs, such as HPDiv, CP, LP, and sultopride, have a risk-increasing effect on QTc prolongation and that SGAs, such as olanzapine, quetiapine, risperidone, and zotepine, are less likely to produce QTc prolongation than the FGAs. Such information may aid in clinical decision making concerning the choice of antipsychotic medication, particularly in patients who have an increased risk for arrhythmias.

5. Conclusions

We confirmed the statistical effect of chlorpromazine, levomepromazine, and HPDiv on QTc prolongation in a sample of 1017 patients with schizophrenia. Furthermore, statistical evidence for sultopride was obtained for the first time. Furthermore, in the range of the antipsychotic drugs that we examined, the data suggest that SGAs are less likely to produce QTc prolongation than FGAs, which may be useful in guiding the choice of antipsychotic drugs.

Acknowledgements

This study was supported by the Health and Labor Sciences Research Grants in Japan, Tokyo, Japan and the Dokkyo Medical University, Investigator-Initiated Research Grant (no. 2007-01-2), Mibu, Japan.

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Human Molecular Genetics, 2009, Vol. 18, No. 13 **2483–2494**
doi:10.1093/hmg/ddp166
Advance Access published on April 10, 2009

Involvement of *SMARCA2*/BRM in the SWI/SNF chromatin-remodeling complex in schizophrenia

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Received November 28, 2008; Revised March 26, 2009; Accepted April 2, 2009

Chromatin remodeling may play a role in the neurobiology of schizophrenia and the process, therefore, may be considered as a therapeutic target. The *SMARCA2* gene encodes BRM in the SWI/SNF chromatin-remodeling complex, and associations of single nucleotide polymorphisms (SNPs) to schizophrenia were found in two linkage disequilibrium blocks in the *SMARCA2* gene after screening of 11 883 SNPs (rs2296212; overall allelic $P = 5.8 \times 10^{-5}$) and subsequent screening of 22 genes involved in chromatin remodeling (rs3793490; overall allelic $P = 2.0 \times 10^{-6}$) in a Japanese population. A risk allele of a missense polymorphism (rs2296212) induced a lower nuclear localization efficiency of BRM, and risk alleles of intronic polymorphisms (rs3763627 and rs3793490) were associated with low *SMARCA2* expression levels in the postmortem prefrontal cortex. A significant correlation in the fold changes of gene expression from schizophrenic prefrontal cortex (from the Stanley Medical Research Institute online genomics database) was seen with suppression of *SMARCA2* in transfected human cells by specific siRNA, and of orthologous genes in the prefrontal cortex of *Smarca2* knockout mice. *Smarca2* knockout mice showed impaired social interaction and prepulse inhibition. Psychotogenic drugs lowered *Smarca2* expression while antipsychotic drugs increased it in the mouse brain. These findings support the existence of a role for BRM in the pathophysiology of schizophrenia.

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INTRODUCTION

Schizophrenia is a chronic, severe and disabling brain disorder that affects approximately 1% of the world's population. A large body of data consistently supports the involvement of complex genetic components causally linked to schizophrenia. Association studies including genome-wide scans have identified many risk alleles that have small effects (1–5). In addition to genetic studies, altered expression of many genes and proteins in schizophrenic brains has been documented (6) and epigenetic regulation in schizophrenia has also been studied (7). Among epigenetic mechanisms, the role of chromatin modification in psychiatric disorders and related fields has been reported for epilepsy (8), drug addiction (9,10), depression (11), autism (12), fear (13), learning and memory (13,14), social cognition (15) and stress-related behaviors (16,17). Histone modifications may contribute to the pathogenesis of prefrontal dysfunction in schizophrenia (18).

The *SMARCA2* gene encodes BRM, one of the earliest described chromatin remodeling multiprotein complexes in the yeast SWI/SNF complex (19–23), and highly conserved among eukaryotes (24). The SWI/SNF molecules are mutually exclusive within the complexes and harbor ATPase activity (25–27). This complex functions by destabilizing the interactions between DNA and histones in the nucleosome in an ATP-dependent reaction (28,29). Mammalian SWI/SNF complexes are present in biochemically diverse forms, indicating that they may have specialized nuclear functions (30). ATP-dependent remodeling complexes involved in chromatin opening or compaction are important in the regulation of transcriptional processes associated with development, cellular differentiation and oncogenesis (22,31–35).

BRM interacts with several transcription factors and other DNA-binding proteins and is involved in chromatin structural modification in the epigenetic regulation of gene expression (36). *SMARCA2* expression is induced to a high level during differentiation to neurons and astrocytes, suggesting an important role in neural cell differentiations (37). Because BRM potentially influences expression of many genes, it is hypothesized that functional changes to *SMARCA2*/BRM may contribute to gene expression changes reported to occur in schizophrenia.

RESULTS

SNPs in two linkage disequilibrium blocks within the SMARCA2 gene were associated with schizophrenia in the Japanese population

Initially, in 100 Japanese schizophrenic patients, 11 883 SNPs were screened for association with schizophrenia. These SNPs exist on the Illumina Human-1 BeadChip and are also deposited in a Japanese SNP (JSNP) database of 1480 Japanese control chromosomes (http://snp.ims.u-tokyo.ac.jp/index.html). The potential impact of population structure on this association study was evaluated by using the genome-wide χ^2 inflation factor, λ, as a genomic control (38,39). The estimated value of λ was 1.05, by which genome-wide association *P*-values were corrected. After correction for multiple testing of these 11 883 SNPs, no significant association was found between them and schizophrenia (Supplementary Material, Table S1).

In the replication 1 cohort, comprised of 576 Japanese schizophrenic patients and 576 Japanese control subjects, an attempt was made to replicate the association of the top 5 SNPs, as ranked by the association *P*-values, with schizophrenia. A potential association was found for only one, rs2296212 (one-sided allelic $P = 0.009$) (Supplementary Material, Table S1). Subsequent analysis in the replication 2 samples of 1344 Japanese schizophrenic patients and 1344 Japanese control subjects confirmed the association for this SNP (one-sided allelic $P = 0.04$). The *P*-value for the association was 5.8×10^{-5} when the initial genome-wide sample and the total replication 1 + 2 samples were combined (Table 1).

Because the *SMARCA2* gene encodes BRM in SWI/SNF chromatin remodeling, SNPs within genes that encode proteins potentially involved in chromatin remodeling were re-evaluated for association with schizophrenia. *CREBBP, DNMT1, DNMT2, DNMT3A, DNMT3B, HAT1, HDAC2, HDAC3, HDAC4, HDAC7, HDAC9, HNMT3A, MYST1, MYST2, MYST4, SIN3A, SIN3B, SMARCA3, SMARCA4, SMARCA5* and *SMARCC1* were selected in addition to the *SMARCA2* genes, with a less stringent criteria of association used (*P*-values of less than 0.05). For this screen, SNPs from the same 100 schizophrenia patients were screened with an Illumina HumanHap370 BeadChip and compared with those in 1868 chromosomes of Japanese volunteers listed in the JSNP database [deposited October 2007, (http://snp.ims.u-tokyo.ac.jp/index.html)]. Although potentially significant association was observed in the *SMARCC1* and *DNMT3B* genes, this did not exist after correction for multiple testing (Supplementary Material, Table S2). An attempt was made to replicate the associations of rs13063042 and rs17079785 in *SMARCC1* and rs2424932 in *DNMT3B* with schizophrenia in the replication 1 sample. However, no significant results were obtained (Supplementary Material, Table S2).

The *SMARCA2* gene is located at chromosome 9p24.3 and spans 178 282 bp comprising 34 exons. The SNP of rs2296212 was non-synonymous, D1546E, in exon 33. Subsequent genotyping of 34 tag SNPs in the *SMARCA2* gene in the replication 1 + 2 samples identified a significant association with schizophrenia for three SNPs [rs2066111 in intron 12 (allelic $P = 8.2 \times 10^{-5}$), rs3763627 in intron 12 (allelic $P = 1.2 \times 10^{-5}$) and rs3793490 in intron 19 (allelic $P = 3.0 \times 10^{-6}$), Fig. 1 and Table 1]. Resequencing of the coding region of *SMARCA2* in 24 Japanese patients with schizophrenia identified no non-synonymous mutations except for D1546E.

The distance between D1546E and rs3793490 was 95 351 bp and these two SNPs were not in linkage disequilibrium ($r^2 = 0$, $D' = 0$–0.16). The three intronic SNPs were in modest linkage disequilibrium with each other; the *r* squares were 0.59 between rs2066111 and rs3763627, 0.52 between rs206111 and rs3793490 and 0.85 between rs3763627 and rs3793490. Therefore, SNPs in two linkage disequilibrium blocks in the *SMARCA2* gene, one in the middle and one in the 3′ region of the gene, were found to be associated with schizophrenia in our Japanese population.

Table 1. Associations between SNPs in the SMARCA2 gene and schizophrenia in the Japanese populations

SNP ID	Population		Genotype count (frequency)			*P*	Allele count (frequency)		*P*	Odds ratio (95% CI)
rs2296212			CC	CG	GG		C	G		
	Affected (screening)	$n = 100$	59 (0.59)	32 (0.32)	9 (0.09)		150 (0.75)	50 (0.25)		1.90 (1.34–2.69)
	Controls (JSNP)[a]	$n = 750$					1276 (0.85)	224 (0.15)	0.0003	
	Affected (replication 1)	$n = 572$	370 (0.65)	168 (0.29)	34 (0.06)		908 (0.79)	236 (0.21)		1.29 (1.05–1.60)
	Controls (replication 1)	$n = 574$	398 (0.69)	160 (0.28)	16 (0.03)	0.02	956 (0.83)	192 (0.17)	0.009[b]	
	Affected (replication 2)	$n = 1343$	907 (0.68)	384 (0.29)	52 (0.04)		2198 (0.82)	488 (0.18)		1.14 (0.99–1.31)
	Controls (replication 2)	$n = 1338$	933 (0.70)	373 (0.28)	32 (0.02)	0.07	2239 (0.84)	437 (0.16)	0.04[b]	
	Affected (replication 1 + 2)	$n = 1915$	1277 (0.67)	552 (0.29)	86 (0.04)		3106 (0.81)	724 (0.19)		1.18 (1.05–1.33)
	Controls (replication 1 + 2)	$n = 1912$	1331 (0.70)	533 (0.28)	48 (0.03)	0.005	3195 (0.84)	629 (0.16)	0.0027[b]	
	Overall (screening+replicatinon)	$n = 2015$	1336 (0.66)	584 (0.29)	95 (0.05)		3256 (0.81)	74 (0.19)		1.25 (1.12–1.39)
	Overall (JSNP+replication)						4471 (0.84)	853 (0.16)	5.8×10^{-5}	
rs2066111			GG	AG	AA		G	A		
	Affected (replication 1 + 2)	$n = 1912$	974 (0.51)	776 (0.41)	162 (0.08)		2724 (0.71)	1100 (0.29)		1.22 (1.10–1.34)
	Controls (replication 1 + 2)	$n = 1907$	846 (0.44)	866 (0.45)	195 (0.10)	8.0×10^{-5}	2558 (0.67)	1256 (0.33)	8.2×10^{-5}	
rs3763627			AA	AT	TT		A	T		
	Affected (replication 1 + 2)	$n = 1905$	1116 (0.59)	690 (0.36)	99 (0.05)		2922 (0.77)	888 (0.23)		1.26 (1.14–1.40)
	Controls (replication 1 + 2)	$n = 1900$	1002 (0.53)	744 (0.39)	154 (0.08)	1.0×10^{-5}	2748 (0.72)	1052 (0.28)	1.2×10^{-5}	
rs3793490			GG	GT	TT		G	T		
	Affected (screening)	$n = 122$	9 (0.07)	37 (0.30)	76 (0.62)		55 (0.23)	189 (0.77)		1.29 (0.94–1.77)
	Controls (JSNP)	$n = 934$	83 (0.09)	344 (0.37)	507 (0.54)	0.13	510 (0.27)	1358 (0.73)	0.11	
	Affected (replication 1 + 2)	$n = 1895$	109 (0.06)	688 (0.36)	1098 (0.58)		906 (0.24)	2884 (0.76)		1.27 (1.15–1.41)
	Controls (replication 1 + 2)	$n = 1908$	162 (0.08)	767 (0.40)	979 (0.51)	4.0×10^{-6}	1091 (0.29)	2725 (0.71)	3.0×10^{-6}	
	Overall (screening+replicatinon)	$n = 2017$	118 (0.06)	725 (0.36)	1174 (0.58)		961 (0.24)	3073 (0.76)		1.25 (1.14–1.38)
	Overall (JSNP+replication)	$n = 2842$	245 (0.09)	1111 (0.39)	1486 (0.52)	2.0×10^{-6}	1601 (0.28)	4083 (0.72)	2.0×10^{-6}	

Allelic *P*-values were calculated by chi-square test (two-sided) and genotypic *P*-values were Cochran-Armitage test.
[a]http://snp.ims.u-tokyo.ac.jp/cgi-bin/SnpInfo.cgi?SNP_ID=IMS-JST050328.
[b]One-sided.