showed linkage of borderline significance to D22S1169 at 22q13.32 in the total sample when intersample heterogeneity was taken into account [Mowry et al., 2004].

A recent study [Severinsen et al., 2006] looked into this 22q13 region and reported that two single-nucleotide polymorphisms (SNPs) (rs4468 and rs138880) located within bromodomain-containing 1 gene (*BRD1*) were associated with schizophrenia in a single-marker association analysis. This gene, expressed in mammalian brain tissue, encodes a protein of unknown function that contains a bromodomain, a motif often found in transcriptional coactivators. The motif represents an evolutionarily conserved nucleotide sequence found in many chromatin-associated proteins and in nearly all known nuclear histone acetyltransferases. It is therefore thought that *BRD1* is related to transcriptional regulation [Zeng and Zhou, 2002].

BRD1 is an attractive candidate gene for schizophrenia for two reasons. First, *BRD1* as a putative transcriptional cofactor might have functional implications for susceptibility to schizophrenia. Second, it also maps to the 22q13.33 locus, the region with evidence for linkage to schizophrenia.

As mentioned, a single study has implicated genetic variants within *BRD1* locus as contributing factor to schizophrenia in a Scottish population [Severinsen et al., 2006]. To further investigate this possible association, we selected SNPs within the *BRD1* locus and carried out a case–control study in a Japanese population. In terms of understanding the relationship between *BRD1* and schizophrenia, our study brings additional information from a genetic point of view: a larger sample size, a different population, and better coverage (in terms of SNPs selected for analysis).

MATERIALS AND METHODS

Subjects

All subjects were of Japanese descent and recruited from the main island of Japan. For the association analysis, 626 patients with schizophrenia and 770 healthy controls were used (Supplementary Table I). For the expression analysis, 29 patients with schizophrenia and 30 healthy controls were used (Supplementary Table II). All patients were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, criteria based on the consensus of at least two experienced psychiatrists using an unstructured interview and review of medical records. All healthy controls were psychiatrically screened using an unstructured interview to exclude subjects with any brain disorder or psychotic disorder or who had first-degree relatives with psychotic disorders. The present study was approved by the Ethics Committees of Nagoya University, Fujita Health University. All subjects provided written informed consent after the study was described to them.

Tagging SNP Selection, SNP Genotyping, and Quality Control

The International Haplotype Mapping (HapMap) (www.hapmap.org) SNP database and ABI (Applied Biosystems) SNP browser were used to select tagging SNPs in the BRD1 locus. The screened region was extended 5 kb upstream of the annotated transcription start site and downstream at the end of the last *BRD1* exon [Neale and Sham, 2004]. The tagging SNP selection criteria were that polymorphisms had a minor allele frequency >5% in the Japanese population (release #21; phase II; July 2006). Then, we took advantage of observed linkage disequilibrium [Barrett et al., 2005] in the *BRD1* locus to exclude redundant SNPs from genotyping. In other words, if the correlation coefficient between two loci (r^2) was 0.9 or higher, then only one of the two loci was selected for the association study [Barrett et al., 2005]. Based on our criteria, 10 SNPs were selected for the analysis. The promoter SNP rs138880, which was one of the two SNPs associated with schizophrenia in the previous study [Severinsen et al., 2006], was included in these 10 SNPs. The 3′ UTR SNP rs4468, the other SNP associated with schizophrenia in the previous study, was also added to the tagging SNPs despite a lack of information on frequency of this polymorphism in a Japanese population in the HapMap database. Therefore, 11 SNPs made up the tagging set. All SNPs were genotyped by TaqMan assay (Applied Biosystems Japan Ltd, Tokyo, Japan). For quality control, three strategies were employed. First, we checked deviation from the Hardy–Weinberg equilibrium (HWE). Second, we genotyped 20 randomly selected samples for each SNP in duplicate in order to evaluate the genotype error rate. Third, we confirmed whether the minor allele frequency for each SNP genotyped in control samples was consistent with that in the Japanese population in the HapMap database.

Imputation of Ungenotyped SNPs

Because tagging SNPs was selected based on r^2, we included imputation as an exploratory method to compute genotypes of SNPs that were not selected for genotyping (untyped SNPs). The advantage of imputing untyped SNPs is that the coverage of common variants within the locus of interest can be enhanced, boosting the statistical power [Marchini et al., 2007]. The MACH program (http://www.sph.umich.edu/csg/abecasis/MACH/) was used to calculate the genotypic prediction of 20 untyped SNPs using directly typed SNP information (10 SNPs used in the screening scan) and the HapMap database (recombination map and haplotype data for the Japanese/Chinese population, release #23a; phase II; March 2008). The MACH program was recently reported to have similar imputation accuracy rates to IMPUTE and to outperform fastPHASE, PLINK, and Beagle [Pei et al., 2008]. The targeted region for imputation was limited to the *BRD1* locus as defined above.

Power Calculation

Power was calculated according to the methods described by Skol et al. [2006]. In brief, for a predefined alpha level, in the disease prevalence and inheritance model, statistical power of any given sample is a function of sample size and effect size. In other words, power is directly proportional to sample size on one side and minor allele frequency and genotype relative risk on the other side.

Statistical Methods for Association Study

Deviation from the HWE was tested by chi-square analysis. All single marker association analyses were done by calculating the

P-values for each SNP marker, and the significance was determined at the 5% level using the chi-square test, as implemented in SPSS v13 (SPSS, Inc., Chicago, IL). All *P*-values were two-sided. Multimarker analysis was carried out by log-likelihood ratio tests for assessing haplotype-wise associations between schizophrenia and a combination of tagging SNPs with a permutation test for calculating empirical significance levels for differences between haplotype frequencies in case and control subsets.

Meta-Analysis

We performed a meta-analysis for rs138880, one of the two SNPs associated with schizophrenia in the previous study [Severinsen et al., 2006]. The other SNP, rs4468, was excluded because it was not polymorphic in our sample. Thus far, only one study has been published regarding an association analysis of the *BRD1* locus [Severinsen et al., 2006]. We used data from Severinsen's study and our study. First, the Q statistic test was performed to assess the possible heterogeneity in the combined studies. Second, a fixed effects model meta-analysis was conducted. The significance of the overall odds ratio (OR) was determined by the Z-test. The analysis was carried out on Comprehensive Meta-Analysis software (Version 2.2.046, Biostat, Englewood, NJ).

Lymphoblastoid Cell Lines (LCLs)

Peripheral blood was drawn into 7-ml plastic tubes containing sodium heparin, and lymphocytes were separated by a standard protocol. The cells were cultured in RPMI-1460 medium containing 20% fetal bovine serum, penicillin, and streptomycin, and filtered supernatant of a B95-8 cell culture infected with Epstein–Barr virus. Cyclosporine A was added until colonies were observed. After colony formation, the cells were passaged three times per week, without the addition of 10% fetal bovine serum and cyclosporine A. The cells were frozen in liquid nitrogen until needed, at which time they were thawed, passaged at least three times, and used within 4 weeks. We paid special attention while establishing and maintaining cell lines to exclude environmental confounders as much as possible.

Real-Time Quantitative Polymerase Chain Reaction (PCR) and Statistical Analysis

Total RNA of LCLs was extracted using RNeasy Plus Mini kit (50) (Qiagen, Valencia, CA). RNA yield and quality were assessed by measuring absorbance at 260 and 280 nm. Integrity and overall quality of the total RNA preparation were determined by native agarose gel electrophoresis (inspection of the 28S and 18S bands). Total RNA was used for cDNA synthesis by High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems Japan Ltd). Real-time quantitative PCR using TaqMan gene expression assays (Applied Biosystems Japan Ltd) was performed with ABI PRISM 7900HT (Applied Biosystems Japan Ltd). Amplification efficiency for each gene-specific primer pair was calculated based on the dilution series method [Livak and Schmittgen, 2001]. In each experiment, the r^2 value of the curve was more than 0.99. Measurement of the cycle threshold was performed in triplicate. The relative expression of *BRD1* was calculated by the modified ΔΔ cycle threshold method as implemented in Relative Expression Software Tool 2008 (REST 2008) [Pfaffl et al., 2002]. The normalization factor was the geometric mean [Vandesompele et al., 2002] of the following genes: tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide (*YWHAZ*), beta-2-microglobulin (*B2M*), and ubiquitin C (*UBC*). These three genes were shown to have high expression stability in leukocytes [Vandesompele et al., 2002]. Bootstrapping techniques were used to provide 95% confidence intervals (CIs) for expression ratios without a normal or symmetrical distribution assumption.

RESULTS

Association Analysis

Regarding quality control, significant deviation from HWE was not observed. The genotypes of the duplicated samples showed complete concordance. Minor allele frequency for each tagging SNP in control samples generally showed a high concordance with that in HapMap database. Assuming a multiplicative model of inheritance and a disease prevalence of 1%, calculations showed that our sample had appropriate power (more than 80%) to detect gene-wide significant associations with genotype relative risk values from 1.24 to 1.55 (minor allele frequency values from 0.05 to 0.45). 3′ UTR SNP rs4468, which was associated with schizophrenia in a previous study, was not polymorphic in our Japanese sample, so we excluded rs4468 from subsequent analyses. Regarding the remaining 10 SNPs, no association was detected with schizophrenia in allele-/genotype-wise analyses or in the haplotype-wise analysis (two- to four-marker sliding window fashion; Table I). However, it should be noted that the rs138880 (associated SNP in previous article) showed the same trend in the Japanese population. In addition, haplotype showing the most significant association [Severinsen et al., 2006] was tested in the present study. We could not show a significant difference in the frequency of this haplotype between cases and controls (haplotype frequency in cases and controls: 0.0010 and 0.0010, respectively, $P = 0.99$).

Imputation of Ungenotyped SNPs

We used MACH to infer genotypes of 20 untyped SNPs. We provided genotypes for our own data (10 SNPs) as input together with haplotypes from the HapMap Japanese/Chinese population. The imputation method using MACH did not support an association between schizophrenia and the 20 SNPs in the *BRD1* locus (Table II).

Meta-Analysis

The SNP rs138880 that previously has been associated with schizophrenia showed the same trend in the Japanese population although it did not reach significance. The ORs for rs138880 reported in the Severinsen et al. [2006] and in this study were 1.73 and 1.14, respectively (Supplementary Table III). The pooled OR derived from the two studies (in total, 729 cases and 970 controls) was significant in the fixed model (pooled OR = 1.25, 95% CI = 1.03–1.52, $P = 0.02$; Supplementary Table III). Homogeneity analysis for the OR

TABLE I. Allele-Wise, Genotype-Wise, and Haplotype-Wise Analyses of 10 Tagging Single-Nucleotide Polymorphisms (SNPs)

	Allele frequency (proportion)				Single SNP		Haplotype wise		
	Case		Control						
dbSNP	M	m	M	m	Allele-wise	Genotype-wise	2-window[a]	3-window[a]	4-window[a]
rs138820	0.78	0.22	0.78	0.22	0.86	0.09			
							0.83		
rs4469	0.77	0.23	0.78	0.22	0.45	0.75		0.7	
							0.29		0.7
rs6009874	0.92	0.08	0.92	0.08	0.92	0.15		0.62	
							1.00		0.69
rs138840	0.92	0.08	0.92	0.08	0.82	0.92		1.00	
							1.00		0.47
rs138844	0.84	0.16	0.84	0.16	0.69	0.37		0.49	
							0.54		0.36
rs138850	0.59	0.41	0.59	0.41	0.82	0.25		0.46	
							0.52		0.44
rs138851	0.94	0.06	0.95	0.05	0.14	0.31		0.48	
							1.00		0.81
rs138863	0.94	0.06	0.95	0.05	0.15	0.38		0.71	
							0.68		1.00
rs2239848	0.85	0.15	0.86	0.14	0.79	0.72		1.00	
							1.00		
rs138880	0.86	0.14	0.87	0.13	0.22	0.09			

M, major allele; m, minor allele.
[a]Sliding window analysis, rare haplotype threshold 10%.

TABLE II. Allele-Wise Analysis of 20 Imputed Single-Nucleotide Polymorphisms

dbSNP	*P*-value	Quality[a]
rs138816	0.97	0.92
rs138821	0.91	0.96
rs2269626	0.72	0.95
rs138823	0.91	0.95
rs916418	0.96	0.99
rs916419	0.85	0.99
rs138827	0.82	0.99
rs138830	0.81	0.99
rs138834	0.87	0.99
rs138841	0.37	0.99
rs138843	0.86	1.00
rs138845	0.34	0.99
rs6009878	1.00	0.99
rs138853	0.14	1.00
rs138861	0.41	1.00
rs138866	0.27	0.99
rs138867	0.27	1.00
rs138870	0.27	0.99
rs138871	0.23	0.99
rs138884	0.23	1.00

[a]Quality is the average posterior probability for the most likely genotype.

revealed no significant evidence for heterogeneity of the OR ($Q = 2.98$, $df = 1$, $P = 0.084$).

Expression Analysis

The expression of *BRD1* mRNA was analyzed using LCLs from 29 cases and 30 controls. Cycle threshold values of *BRD1* and three internal controls (*B2M*, *UBC*, and *YWHAZ*) are shown in Supplementary Table IV. We could not detect any significant differences in *BRD1* mRNA levels between cases and controls ($P = 0.46$; Fig. 1).

DISCUSSION

The common disease–common variant hypothesis states that diseases that were evolutionarily neutral (i.e., had little or no effect on reproductive fitness), such as late-onset schizophrenia, during human history may be significantly influenced by common variants [Lander, 1996]. Therefore, if allelic variants at a disease susceptibility locus are responsible for the predisposition to a common complex disease, then allele-, genotype-, or haplotype-wise association tests will detect such variants (or tagging SNPs that are in linkage disequilibrium with the deleterious allele).

The first and only indication that the *BRD1*-related region harbors a variation that might influence susceptibility to schizophrenia was provided by Severinsen et al. [2006], who identified two fairly strong association signals between two SNPs (rs4468 and rs138880) and schizophrenia using a case–control sample from Scotland. The sample in this study consisted of 103 patients with

(i)

Gene	Type	95% C.I.	P(H1)
BRD1	Target	0.56 - 2.08	0.46

(ii)

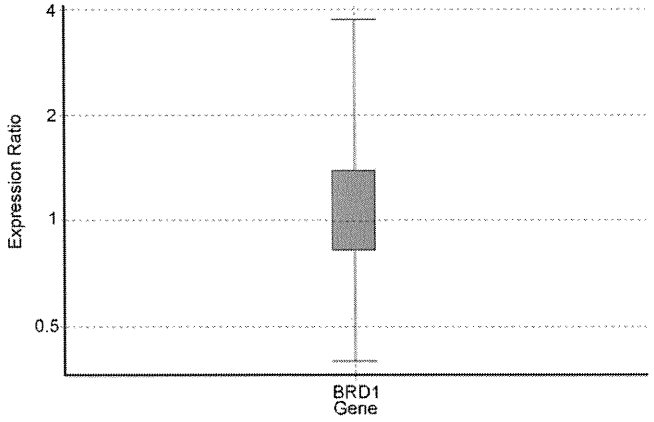

FIG. 1. Relative expression of bromodomain-containing 1 (BRD1) normalized to the geometric mean of three internal controls. i: The relative expression of BRD1 was normalized to the geometric mean of three internal controls (B2M, UBC, and YWHAZ). Bootstrapping techniques were used to provide 95% confidence intervals for expression ratios without normal or symmetric distribution assumption. The number of iterations was 10,000 in this analysis. *P* (H1) means the probability of the alternate hypothesis that the difference between sample and control groups is due only to chance. ii: Boxplot. Expression ratio is the relative expression of BRD1 in cases compared with controls (expression in control is equivalent to 1). Box represents the middle 50% of observations. The dotted line represents the median gene expression. Whiskers represent the minimum and maximum observations.

schizophrenia and 200 controls. Our study did not strongly support an association between schizophrenia and the *BRD1* locus although the only previously associated SNP included in our study (rs138880) showed the same trend, and the meta-analysis of this SNP using a fixed effects model was significant.

Psychiatric disorders are complex diseases that are characterized by the contribution of multiple susceptibility genes and environmental factors. Therefore, *BRD1* might be a population-specific factor for schizophrenia. However, this conclusion should be made only with the following considerations. First, it is possible that our study was still underpowered to reliably detect common low-risk variants. This may be related to etiological heterogeneity or inaccurate diagnoses in schizophrenia, which would attenuate the genetic relative risk. Second, only the hypothesis of an association with common SNPs of *BRD1* has been tested, both here and in the previous study; therefore, future studies using resequencing methods to detect rare variants in the *BRD1* locus will be needed for a complete understanding of relationship between this genetic locus and schizophrenia. Third, even though the Japanese population is relatively homogeneous [Haga et al., 2002], small population stratifications may have affected our findings. A recent analysis with the use of approximately 140,000 SNPs in 7003 Japanese individuals has shown that local regions within the main island of Japan are genetically differentiated in spite of frequent human migration within Japan in modern times [Yamaguchi-Kabata et al., 2008]. However, we believe that the impact of population stratification on our study is negligible, as our samples were collected in a relatively narrow region in the middle of the main island of Japan. Fourth, regarding the Japanese and the Caucasian populations, comparative linkage disequilibrium analysis of the HapMap data showed a different block structure around the *BRD1* locus [Gabriel et al., 2002]. Compared with the Caucasian population, linkage disequilibrium (LD) blocks in the Japanese population are shorter, and the block structure is coarser, having lower r^2 values. This might influence interpopulation transferability of tagging SNPs in the *BRD1* locus and result in a failure to detect an association with schizophrenia in the Japanese population. Interestingly, selective sweep analysis has provided evidence of recent positive selection on genes associated with schizophrenia, and *BRD1* gene was reported to have been affected by positive selection in Caucasian but not in Asian population [Crespi et al., 2007]. This indicates that the positive selection specific to the Caucasian population might produce the difference in LD structure in *BRD1* locus.

We could not detect significant differences in *BRD1* mRNA levels between cases and controls in the expression analysis. These results are consistent with the findings in the association study. However, there were several limitations in the expression assays. Using non-neuronal samples such as LCLs is based on the assumption that heritable mechanisms associated with the risk of schizophrenia have systemic effects and result in changes to gene expression in various tissues. To validate the use of gene expression data in a more accessible tissue as a surrogate for gene expression in the central nervous system, Sullivan et al. [2006] evaluated the comparability of transcriptional profiling of a variety of human tissues with Affymetrix U133A microarray augmented with a custom microarray. Their analyses suggested that careful use of peripheral gene expression may be a useful surrogate for gene expression in the central nervous system.

In conclusion, we could not strongly show that common SNPs in the *BRD1* gene account for a substantial proportion of the genetic risk for schizophrenia in the Japanese population, although small effects of population stratification or differences in LD structure could not be ruled out. Considering the significance in the meta-analysis for the only previously associated SNP included in our study, further investigations are needed for conclusive results.

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Serotonin 1A receptor gene is associated with Japanese methamphetamine-induced psychosis patients

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ABSTRACT

Background: Several investigations have reported associations the serotonin 1A (5-HT1A) receptor to schizophrenia and psychotic disorders, making 5-HT1A receptor gene (*HTR1A*) an adequate candidate gene for the pathophysiology of schizophrenia and methamphetamine (METH)-induced psychosis. Huang and colleagues reported that rs6295 in *HTR1A* was associated with schizophrenia. The symptoms of methamphetamine (METH)-induced psychosis are similar to those of paranoid type schizophrenia. It may indicate that METH-induced psychosis and schizophrenia have common susceptibility genes. In support of this hypothesis, we reported that the V-act murine thymoma viral oncogene homologue 1 (AKT1) gene was associated with METH-induced psychosis and schizophrenia in the Japanese population. Furthermore, we conducted an analysis of the association of *HTR1A* with METH-induced psychosis.

Method: Using one functional SNP (rs6295) and one tagging SNP (rs878567), we conducted a genetic association analysis of case-control samples (197 METH-induced psychosis patients and 337 controls) in the Japanese population. The age and sex of the control subjects did not differ from those of the methamphetamine dependence patients.

Results: Rs878567 was associated with METH-induced psychosis patients in the allele/genotype-wise analysis. Moreover, this significance remained after Bonferroni correction. In addition, we detected an association between rs6295 and rs878567 in *HTR1A* and METH-induced psychosis patients in the haplotype-wise analysis. Although we detected an association between rs6295 and METH-induced psychosis patients, this significance disappeared after Bonferroni correction.

Conclusion: *HTR1A* may play an important role in the pathophysiology of METH-induced psychosis in the Japanese population. However, because we did not perform a mutation scan of *HTR1A*, a replication study using a larger sample may be required for conclusive results.

1. Introduction

Altered serotonergic neural transmission is hypothesized to be a susceptibility factor for schizophrenia (Geyer and Vollenweider, 2008; Meltzer et al., 2003). Several postmortem studies reported increased serotonin 1A (5-HT1A) receptor in the prefrontal cortex

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of schizophrenic patients (Burnet et al., 1996; Hashimoto et al., 1993, 1991; Simpson et al., 1996; Sumiyoshi et al., 1996). Huang and colleagues reported that rs6295 in an SNP (C-1019G: rs6295) in the promoter region of the 5-HT1A receptor gene (*HTR1A*), which regulate HTR1A transcription (Le Francois et al., 2008; Lemonde et al., 2003), was associated with schizophrenia (Huang et al., 2004). These facts suggest a crucial relationship between the 5-HT1A receptor and schizophrenia, and that *HTR1A* is an adequate candidate for the etiology of schizophrenia. *HTR1A* (OMIM*109 760, 1 exon in this genomic region spanning 2.069 kb) is located on 5q11.

The symptoms of methamphetamine (METH)-induced psychosis are similar to those of paranoid type schizophrenia (Sato et al., 1992). It may indicate that METH-induced psychosis and schizophrenia have common susceptibility genes (Bousman et al., 2009). In support of this hypothesis, we reported that the V-act murine thymoma viral oncogene homologue 1 (AKT1) gene was associated with METH-induced psychosis (Ikeda et al., 2006) and schizophrenia (Ikeda et al., 2004) in the Japanese population. Furthermore, we conducted an analysis of the association of these genes with METH-induced psychosis, using the recently recommended strategy of 'gene-based' association analysis (Neale and Sham, 2004).

2. Materials and methods

2.1. Subjects

The subjects in the association analysis were 197 METH-induced psychosis patients (164 males: 83.2% and 33 females; mean age ± standard deviation (SD) 37.6 ± 12.2 years) and 337 healthy controls (271 males: 80.4% and 66 females; 37.6 ± 14.3 years). The age and sex of the control subjects did not differ from those of the methamphetamine dependence patients. All subjects were unrelated to each other, ethnically Japanese, and lived in the central area of Japan. The patients were diagnosed according to DSM-IV criteria with consensus of at least two experienced psychiatrists on the basis of unstructured interviews and a review of medical records. METH-induced psychosis patients were divided into two categories of psychosis prognosis, the transient type and the prolonged type, which showed remission of psychotic symptoms within 1 month and after more than 1 month, respectively, after the discontinuance of methamphetamine consumption and beginning of treatment with neuroleptics; 112 patients (56.9%) were the transient type, and 85 patients (43.1%) were the prolonged type. One hundred thirty-seven subjects with METH-induced psychosis also had dependence on drugs other than METH. Cannabinoids were the most frequency abused drugs (31.4%), followed by cocaine (9.09%), LSD (9.09%), opioids (7.69%), and hypnotics (7.69%). Subjects with METH-induced psychosis were excluded if they had a clinical diagnosis of psychotic disorder, mood disorder, anxiety disorder or eating disorder. More detailed characterizations of these subjects have been published elsewhere (Kishi et al., 2008b). All healthy controls were also 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.

The study was described to subjects and written informed consent was obtained from each. This study was approved by the Ethics Committee at Fujita Health University, Nagoya University School of Medicine and each participating member of the Institute of the Japanese Genetics Initiative for Drug Abuse (JGIDA).

2.2. SNPs selection and linkage disequilibrium (LD) evaluation

We first consulted the HapMap database (release#23.a.phase2, Mar 2008, www.hapmap.org, population: Japanese Tokyo: minor allele frequencies (MAFs) of more than 0.05) and included 3 SNPs (rs6449693, rs878567 and rs1423691) covering *HTR1A* (5′-flanking regions including about 1 kb from the initial exon and about 2 kb downstream (3′) from the last exon: HapMap database contig number chr5: 63287418…63291774). Then one tagging SNP was selected with the criteria of an r^2 threshold greater than 0.8 in 'pair-wise tagging only' mode using the 'Tagger' program (Paul de Bakker, http://www/broad.mit.edu/mpg/tagger) of the HAPLOVIEW software (Barrett et al., 2005).

HTR1A has also been reported to have one biologically functional SNP (C-1019G: rs6295) (Albert et al., 1996; Albert and Lemonde, 2004; Lemonde et al., 2003). Rs6295 (C-1019G) in the promoter region regulate *HTR1A* transcription (Le Francois et al., 2008; Lemonde et al., 2003). The C allele is a part of a 26 palindrome that connect transcription factors (Deaf-1, Hes1 and Hes5) by NUDR (nuclear deformed epidermal autoregulatory factor), whereas the G allele abolishes repression by NUDR (Le Francois et al., 2008; Lemonde et al., 2003). This would lead to elevated levels of 5-HT1A receptor in the presynaptic raphe nucleus in GG genotypes, compared with CC genotype (Le Francois et al., 2008; Lemonde et al., 2003). Since no information about rs6295 was shown in the HapMap database, we included this SNP. These two SNPs were then used for the following association analysis.

2.3. SNPs genotyping

We used TaqMan assays (Applied Biosystems, Inc., Foster City, CA) for both SNPs. Detailed information, including primer sequences and reaction conditions, is available on request.

2.4. 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).

Marker-trait association analysis was used to evaluate allele- and genotype-wise association with the chi-square test (SAS/Genetics, release 8.2, SAS Japan Inc., Tokyo, Japan), and haplotype-wise association analysis was conducted with a likelihood ratio test using the COCAPHASE2.403 program (Dudbridge, 2003). We used the permutation test option as provided in the haplotype-wise analysis to avoid spurious results and correct for multiple testing. Permutation test correction was performed using 1000 iterations (random permutations). In addition, Bonferroni's correction was used to control inflation of the type I error rate in the single marker association analysis and in the explorative analysis. For Bonferroni correction, we employed the following numbers for multiple testing: 2 for each sample set in allele- and genotype-wise analysis (2 examined SNPs). We had already performed a permutation test in the haplotype-wise analysis. Power calculation was performed using a genetic power calculator (Purcell et al., 2003).

The significance level for all statistical tests was 0.05.

3. Results

The LD from rs6449693, rs878567 and rs1423691 was tight in from the HapMap database samples ($r^2 = 1.00$). However, the LD structure of rs6295 (functional SNP) and rs878567 (tagging SNP) in our control samples was not tight ($r^2 = 0.160$). Genotype frequencies of all SNPs were in HWE (Table 1). Rs878567 was associated with METH-induced psychosis patients in the allele/genotype-wise analysis (P allele = 0.000122 and P genotype = 0.00103) (Table 1). Moreover, these significances remained after Bonferroni correction (P allele = 0.000244 and P genotype = 0.00203) (Table 1). In addition, we detected an association between rs6295 and rs878567 in *HTR1A* and METH-induced psychosis patients in the haplotype-wise analysis ($P = 0.0000643$) (Table 2). Although we detected an association between rs6295 and METH-induced psychosis patients (P allele = 0.0271), this significance disappeared after Bonferroni correction (P allele = 0.0542) (Table 1).

4. Discussion

We found associations between *HTR1A* and Japanese METH-induced psychosis patients. Therefore, we reasoned that *HTR1A* may play an important role in the pathophysiology of METH-induced psychosis in the Japanese population. However, our samples are small. Although Bonferroni's correction was used to control inflation of the type I error rate, we considered that there is a possibility of type I error in these results.

The 5-HT1A receptor is present in various regions of the brain, including the cortex, hippocampus, amygdala, hypothalamus and septum (Aznar et al., 2003; Barnes and Sharp, 1999; Le Francois et al., 2008; Varnas et al., 2004). Presynaptic 5-HT1A autoreceptors play an important role in the autoregulation of serotonergic neurons (Le Francois et al., 2008; Lemonde et al., 2003; Riad et al., 2000; Sotelo et al., 1990). The 5-HT1A receptor activation by serotonin induces the hyperpolarization of serotonergic neurons, decreasing their firing rate and consequently the release of serotonin in the brain (Le Francois et al., 2008; Lemonde et al., 2003; Riad et al., 2000; Sotelo et al., 1990). Also, the 5-HT1A receptor was associated hippocampal neurogenesis. The hippocampus is a part of the limbic system involved in cognitive function such as memory. Stimulation of 5-HT1A receptors has been known to reduce the

Table 1
Association analysis of *HTR1A* with methamphetamine-induced psychosis.

SNP[a]	Phenotype[b]	MAFs[c]	N	Genotype distribution[d]			*P*-value[f]			Corrected *P*-value[f,g]	
				M/M	M/m	m/m	HWE[e]	Genotype	Allele	Genotype	Allele
rs6295	Controls	0.254	336	192	117	27	0.132				
C > G	METH-induced psychosis	0.317	197	92	85	20	0.955	0.0657	**0.0271**		0.0542
rs878567	Controls	0.126	336	258	71	7	0.423				
C > T	METH-induced psychosis	0.216	197	124	61	12	0.233	**0.00103**	**0.000122**	**0.00203**	**0.000244**

[a] Major allele > minor allele.
[b] METH-induced psychosis: methamphetamine-induced psychosis.
[c] MAFs: minor allele frequencies.
[d] M: major allele, m: minor allele.
[e] Hardy–Weinberg equilibrium.
[f] Bold represents significant *P*-value.
[g] Calculated using Bonferroni's correction.

negative symptoms and cognitive dysfunction of schizophrenia (Meltzer et al., 2003; Meltzer and Sumiyoshi, 2008; Sumiyoshi et al., 2001, 2007). Mason and Reynolds (1992) reported that one of the major pharmacological therapeutic targets of clozapine is 5-HT1A receptors on cortical glutamatergic neurons. Several post-mortem studies reported increased 5-HT1A receptor in the prefrontal cortex of schizophrenic patients (Burnet et al., 1996; Hashimoto et al., 1993, 1991; Simpson et al., 1996; Sumiyoshi et al., 1996). NAN-190 (5-HT1A receptor antagonist) produced an inhibitory action on methamphetamine-induced hyperactivity (Ginawi et al., 2004; Millan and Colpaert, 1991). These facts suggest that altered serotonergic neural transmission caused by abnormalities in 5-HT1A receptor may be involved in the development of psychotic disorders such as schizophrenia and METH-induced psychosis (Geyer and Vollenweider, 2008; Meltzer et al., 2003).

Serretti et al. (2007) reported that rs878567 in *HTR1A* was associated with German and Italian suicidal attempters. Also, previous study have reported that rs878567 in *HTR1A* was found the interaction with childhood physical abuse in mood disorders (Brezo et al., 2009). These authors suggested rs878567 might influence hippocampus-mediated memory deficits in mood disorders (Brezo et al., 2009). The LD from rs6449693, rs878567 and rs1423691 was tight in from the HapMap database samples (r^2 = 1.00). As these results show, rs878567 covers a wide and important region including the exon and the promoter region in *HTR1A*. Because it is possible that rs878567 influences biological function in the brain, we suggest that functional analysis for rs878567 should be performed in future studies.

Rs6295 (C-1019G) in the promoter region regulate *HTR1A* transcription (Le Francois et al., 2008; Lemonde et al., 2003). The C allele is a part of a 26 palindrome that connect transcription factors (Deaf-1, Hes1 and Hes5) by NUDR (nuclear deformed epidermal autoregulatory factor), whereas the G allele abolishes repression by NUDR (Le Francois et al., 2008; Lemonde et al., 2003). This would lead to elevated levels of 5-HT1A receptor in the presynaptic raphe nucleus in GG genotyps, compared with CC genotype (Le Francois et al., 2008; Lemonde et al., 2003). This variant was associated with several studies, including major depressive disorder (Anttila et al., 2007; Kraus et al., 2007; Lemonde et al., 2003; Neff et al., 2009; Parsey et al., 2006) and panic disorder (Strobel et al., 2003) and antidepressant response in MDD (Arias et al., 2005; Hong et al., 2006; Lemonde et al., 2004; Parsey et al., 2006; Serretti et al., 2004; Yu et al., 2006). Huang et al. (2004) reported that rs6295 was associated with schizophrenia. Recent studies reported that rs6295 was associated with the improvement in negative symptoms from antipsychotics such as risperidone (Mossner et al., 2009; Reynolds et al., 2006; Wang et al., 2008) and that 5-HT1A receptor agonists such as tandospirone produced improvements in the cognitive impairment in schizophrenia (Meltzer and Sumiyoshi, 2008; Sumiyoshi et al., 2001, 2007).

A few points of caution should be mentioned with respect to our results. Firstly, the positive association may be due to small sample size. Ideal samples for this study are METH use disorder samples with and without psychosis. Because we had only a few METH use disorder samples without psychosis, and we wanted to avoid statistical error, we did not perform an association analysis with these samples. Secondly, we did not include a mutation scan to detect rare variants. We designed the study based on the common disease-common variants hypothesis (Chakravarti, 1999). However, Weickert et al. (2008) have shown associations between a common disease such as schizophrenia and rare variants. If the genetic background of METH-induced psychosis is described by the common disease-rare variants hypothesis, further investigation will be required, such as medical resequencing using larger samples. However, statistical power is needed to evaluate the association of rare variants. Lastly, our subjects did not undergo structured interviews. 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 (Kishi et al., 2008a,c, 2009). In addition, when we found misdiagnosis in a patient, we promptly excluded the misdiagnosed case to maintain the precision of our sample. To overcome these limitations, a replication study using larger samples or samples of other populations will be required for conclusive results.

In conclusion, our results suggest that *HTR1A* may play a major role in the pathophysiology of METH-induced psychosis in the Japanese population. However, because we did not perform a mutation scan of *HTR1A*, a replication study using a larger sample may be required for conclusive results.

Table 2
Haplotype-wise analysis of *HTR1A*.

Haplotype rs6295-rs878567	Phenotype[a]	Individual haplotype frequency	Individual *P*-value[b]	Phenotype[a]	Global *P*-value[b]
C–C	Control	0.811			
	METH-induced psychosis	0.694	**0.0000364**	METH-induced psychosis	**0.0000643**
G–C	Control	0.189			
	METH-induced psychosis	0.306	**0.0000364**		

[a] METH-induced psychosis: methamphetamine-induced psychosis.
[b] Bold numbers represent significant *P*-value.

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RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART B
Neuropsychiatric Genetics

Association Analyses Between Brain-Expressed Fatty-Acid Binding Protein (*FABP*) Genes and Schizophrenia and Bipolar Disorder

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Deficits in prepulse inhibition (PPI) are a biological marker for psychiatric illnesses such as schizophrenia and bipolar disorder. To unravel PPI-controlling mechanisms, we previously performed quantitative trait loci (QTL) analysis in mice, and identified *Fabp7*, that encodes a brain-type fatty acid binding protein (Fabp), as a causative gene. In that study, human *FABP7* showed genetic association with schizophrenia. FABPs constitute a gene family, of which members *FABP5* and *FABP3* are also expressed in the brain. These FABP proteins are molecular chaperons for polyunsaturated fatty acids (PUFAs) such as arachidonic and docosahexaenoic acids. Additionally, the involvement of PUFAs has been documented in the pathophysiology of schizophrenia and mood disorders. Therefore in this study, we examined the genetic roles of *FABP5* and *3* in

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schizophrenia (N = 1,900 in combination with controls) and *FABP7*, *5*, and *3* in bipolar disorder (N = 1,762 in the case–control set). Three single nucleotide polymorphisms (SNPs) from *FABP7* showed nominal association with bipolar disorder, and haplotypes of the same gene showed empirical associations with bipolar disorder even after correction of multiple testing. We could not perform association studies on *FABP5*, due to the lack of informative SNPs. *FABP3* displayed no association with either disease. Each *FABP* is relatively small and it is assumed that there are multiple regulatory elements that control gene expression. Therefore, future identification of unknown regulatory elements will be necessary to make a more detailed analysis of their genetic contribution to mental illnesses.

Key words: FABP7; FABP5; FABP3; polyunsaturated fatty acid; copy number polymorphism

INTRODUCTION

Despite entering the era of whole genome association analyses, the unequivocal identification of susceptibility genes for schizophrenia and bipolar disorder still warrants further work [Wellcome Trust Consortium, 2007; Baum et al., 2008; O'Donovan et al., 2008; Sklar et al., 2008; Hattori et al., 2009; Need et al., 2009]. One of the reasons for this may be that current diagnostic categorization is largely dependent on the subjective evaluation of patients' feelings and state of mood. This may result in etiologically (biologically) extremely heterogeneous disease states being categorized together [Need et al., 2009]. As an alternative approach, the analysis of biological traits associated with psychiatric illnesses called "endophenotypes" has gained importance. Although endophenotypes are an idealized concept, they are expected to assist in deconstructing complex diseases, allowing for easier genetic analyses [Gottesman and Gould, 2003; Gur et al., 2007].

As an example of an endophenotype, deficits in prepulse inhibition (PPI) have been well documented in psychiatric illnesses including schizophrenia and bipolar disorder [Braff et al., 2001; Giakoumaki et al., 2007]. The experimental advantage of PPI is that it is evaluable in animals. To identify the genes that control PPI, we performed quantitative trait loci analysis in mice, and detected a gene encoding Fabp7 (fatty acid binding protein 7, brain type) as a causative genetic substrate [Watanabe et al., 2007]. Furthermore, the human orthologue *FABP7* (located on chromosome 6q22.31) was associated with schizophrenia [Watanabe et al., 2007]. The *FABPs* constitute a gene family and at least 12 members have been reported [for review see Liu et al., 2008; Furuhashi and Hotamisligil, 2008]. Brain-expressed *FABPs* include *FABP5* (chromosome 8q21.13) and *FABP3* (chromosome 1p35.2), along with *FABP7* [Owada, 2008]. FABP proteins are lipid chaperons, and the ligands for the brain-expressed FABPs are thought to be polyunsaturated fatty acids (PUFAs) such as arachidonic (AA) and docosahexaenoic acid (DHA) [Furuhashi and Hotamisligil, 2008].

Accumulating evidence suggests roles for PUFAs in both schizophrenia and mood disorders [for review see Richardson, 2004]. Therefore in this study, we set out to expand our prior genetic association analysis (that is between *FABP7* and schizophrenia [Watanabe et al., 2007]), to between *FABPs 5* and *3* and schizophrenia and between *FABPs 7*, *5*, and *3* and bipolar disorder.

MATERIALS AND METHODS

Subjects

The set of schizophrenia and age-/sex-matched control samples consisted of 950 unrelated patients with schizophrenia (447 men, 503 women; mean age 47.0 ± 13.7 years) and controls (447 men, 503 women; mean age 46.9 ± 13.6 years). The sample panel for the bipolar study was the same as used in the COSMO consortium study [Ohnishi et al., 2007], which comprises 867 unrelated bipolar patients (425 men, 442 women; mean age 50.7 ± 14.2 years) and 895 age- and sex-matched controls (445 men, 450 women; mean age 49.9 ± 13.5 years). All samples are of Japanese origin. In our previous genome-wide analysis of a sample set consisting of subjects recruited at almost the same geographical locations as the bipolar case–control set in the current study, little effect of population stratification was detected by principal components analysis [Hattori et al., 2009] and this finding was consistent with another recent report [Yamaguchi-Kabata et al., 2008]. While bipolar case–control recruitment was spread over the Hondo area in Japan, schizophrenia case–control recruitment was restricted to the Kanto district, which includes Tokyo and its surrounding areas, and overlaps to a limited extent with Hondo. Therefore, population stratification should be negligible. All patients had a consensual diagnosis of schizophrenia or bipolar disorder according to DSM-IV criteria, from at least two experienced psychiatrists. Control subjects were recruited from hospital staff and volunteers who showed no present or past evidence of psychoses, during brief interviews by psychiatrists. The current study was approved by the Ethics Committees of all participating institutes. All participants provided written informed consent.

Re-Sequencing Analyses of FABP7 and FABP5

We previously performed a genetic association study between schizophrenia and *FABP7* (at chr6: 123142345–123146917 using the UCSC database: http://genome.ucsc.edu/cgi-bin/hgGateway?org=Human&db=hg18&hgsid=121236003), and reported nominal association of a missense polymorphism [rs2279381; 182C > T (Thr61Met) (F06 in Fig. 1)] and its spanning haplotype with schizophrenia [Watanabe et al., 2007]. Assuming the possibility of additional functional SNPs (to Thr61Met) we re-sequenced the entire gene region (spanning 908 bp upstream of exon 1 to 347 bp downstream of exon 4: total length 5,826 bp) using 10 randomly chosen patients with schizophrenia and 10 bipolar disorder samples. Information on the primer sets and PCR conditions for this analysis is available upon request. Sequencing was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and the ABI PRISM 3730 Genetic Analyzer (Applied Biosystems). Polymorphisms were detected by the SEQUENCHER program (Gene Codes Corporation, Ann Arbor, MI).

For analysis of *FABP5* (at chr8: 82355340–82359563 on the UCSC database), since there are no SNPs in the HapMap database for the Japanese population (rel #23a) (http://www.hapmap.org/

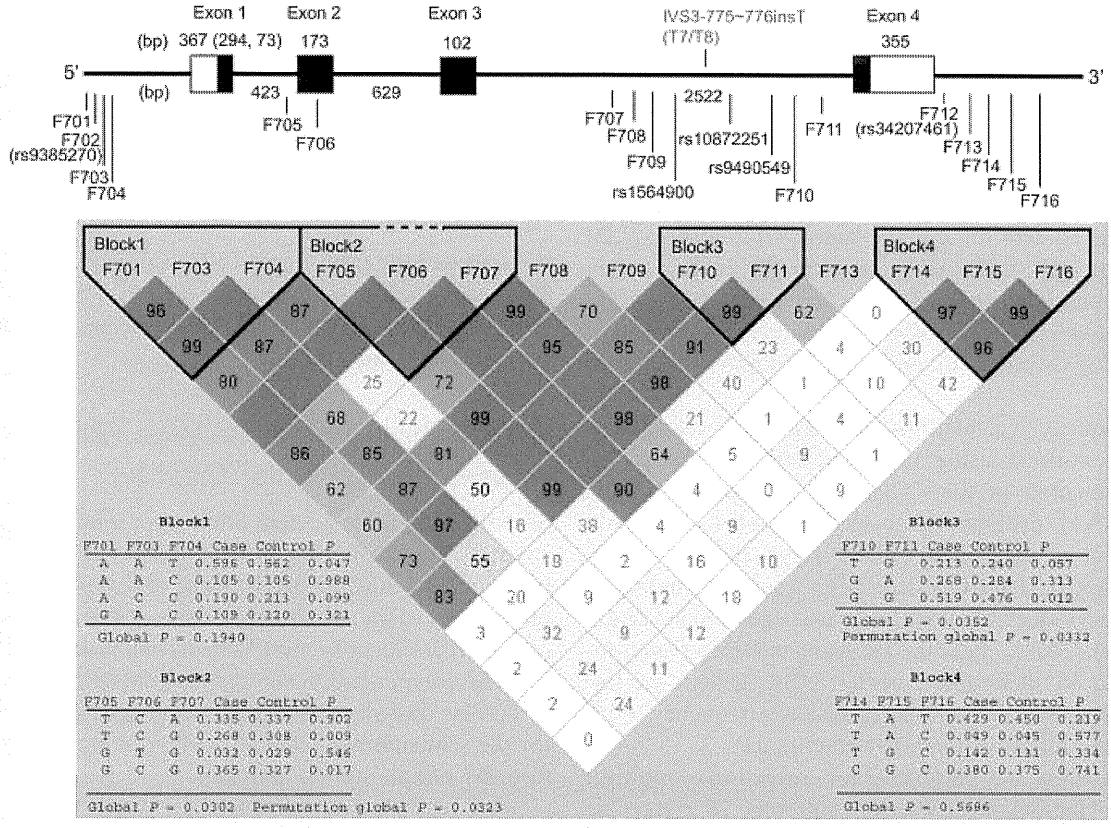

FIG. 1. Genomic structure, polymorphic sites and LD block structure of the *FABP7* gene. In the upper panel, exons are denoted as boxes, with coding regions in black and 5′-/3′-untranslated regions in white. The sizes of each exon and intron are also shown. In the lower panel, the number in each cell represents the LD parameter D' ($\times 100$), blank cells mean $D' = 1$. Each cell is painted in a graduated color relative to the strength of LD between markers, which is defined by both the D' value and confidence bounds on D'. The results of block-based haplotype analysis in bipolar disorder are also shown for LD blocks 1 through 4, along with haplotype frequencies and global *P* values.

ndex.html.ja), we re-sequenced the gene region (spanning 897 bp upstream of exon 1 to 447 bp downstream of exon 4: total length 5,568 bp) using the same 10 schizophrenic and 10 bipolar samples described previously.

This sample set used for the mutation screen will fail to detect a variant if all the cases with bipolar disorder and schizophrenia are either homozygous for a risk allele or for a non-risk allele. This is unlikely to be the case for common variations. The current sample set, which consists of 20 cases and no controls, provides a sensitivity of >0.99 for a risk allele, with a frequency range of 0.1–0.87. This is under the assumption of Hardy-Weinberg equilibrium in the general population and a multiplicative model with a genotype relative risk of 1.2.

Information on the primer sets and PCR conditions for this analysis is available upon request.

SNP Selection and Genotyping

For *FABP7*, we selected tag SNPs from all SNPs detected by re-sequencing, and from SNPs located from the 10 kb up- and down-stream regions of the gene [the HapMap data for the Japanese population (rel #23a)]. Tag SNPs were selected by Carlson's greedy algorithm, which is implemented in the LdSelect program [Carlson et al., 2004]. The minor allele frequency and the r^2 threshold were set to 0.1 and 0.85, respectively. The same tag SNP selection criteria were applied to *FABP3*.

SNP genotyping was performed using the TaqMan system (Applied Biosystems, Foster City, CA) according to the recommendations of the manufacturer. PCR was performed using an ABI 9700 thermocycler and fluorescent signals were analyzed using an ABI 7900 sequence detector single point measurement and SDS v2.3 software (Applied Biosystems).

Copy Number Polymorphism (CNP) Analysis of FABP3

Because the UCSC database (assembly March, 2006) showed a large CNP (cnp20; position: chr1: 31454968–32238918) spanning the entire *FABP3* region (at chr1: 31610687–31618510 on the UCSC database), we tested to confirm the existence of CNPs in Japanese subjects using genomic quantitative PCR. The amplicons were set at

both the 5′- and 3′-ends of the gene (detailed information is available on request).

Statistical Analyses

Deviations from Hardy–Weinberg equilibrium (HWE) were evaluated by the chi-square test (df = 1). Allele and genotype distributions between patients and controls were compared using Fisher's exact test. To determine the linkage disequilibrium (LD) block structure in each gene region, we used the genotype data from the schizophrenia (cases + controls: N = 1,900) and bipolar disorder sets (cases + controls: N = 1,762) and the Haploview program (http://www.broad.mit.edu/mpg/haploview/) [Barrett et al., 2005].

Haplotype frequency calculations and haplotypic association analyses were performed using the expectation–maximization algorithm implemented in the COCAPHASE program in the UNPHASED v3.0.11 program (http://www.mrc-bsu.cam.ac.uk/personal/frank/software/unphased/) [Dudbridge, 2003].

Statistical power for detecting association was calculated using the Genetic Power Calculator (GPC, http://statgen.iop.kcl.ac.uk/gpc/) [Purcell et al., 2003], under the following parameter assumptions with respect to allelic test statistics: GRR (genetic relative risk) = 1.2, prevalence of disease = 0.01, risk allele frequency = 0.3, $\alpha = 0.05$ and a multiplicative model of inheritance.

Permutation analysis was performed for correction of multiple testing, using the Haploview software (10,000 runs) [Barrett et al., 2005].

RESULTS

Association Results Between *FABP7* and Bipolar Disorder

By re-sequencing analysis of the entire gene region, we detected 12 SNPs (F705–F712, rs1564900, rs10872251, rs9490549, IVS3-775–776InsT in Fig. 1), of which IVS3-775–776insT (T7 or T8: T8 is a minor allele with a frequency of 0.025) was novel. However, there were no new variants that appeared to alter gene function(s). SNPs F01 to F16 (the additional four SNPs are from the HapMap database) were selected as tags, but SNPs F02 (rs9385270) and F712 (rs34207461) could not be typed using the TaqMan method. Accordingly, the remaining 14 SNPs were analyzed.

The allelic and genotypic distributions of each SNP in the bipolar patients and controls are summarized in Table I. All the SNPs were in HWE. SNPs F704 [T allele is over-represented in the bipolar group; OR (95% CI) = 1.15 (1.00–1.31)], F705 [G is over-represented in the bipolar group; OR (95% CI) = 1.20 (1.05–1.38)] and F709 [G is over-represented in the bipolar group; OR (95% CI) = 1.20 (1.05–1.38)] showed nominal associations ($P < 0.05$). However, after correction by permutation tests, none remained significant. The gene region consisted of four LD blocks (Fig. 1). In haplotype analysis, blocks 2 [T (F705)–C (F706)–G (F707) is over-represented in the control group; OR (95% CI) = 0.82 (0.71–0.95)] [G (F705)–C (F706)–G (F707) is over-represented in the disease group; OR (95% CI) = 1.19 (1.03–1.36)] and 3 [G (F710)–G (F711) is over-represented in the disease group; OR (95% CI) = 1.18 (1.04–1.35)] were associated with disease, even after correction for multiple testing by permutation tests (Fig. 1). The missense SNP F706, previously associated with schizophrenia [Watanabe et al., 2007], was located in block 2. Power analysis gave 72.2% power for the bipolar-control allelic test statistic.

Re-Sequencing Analysis of *FABP5*

We screened the gene region (5,568 bp) for polymorphisms using 20 disease samples, and detected a SNP, −36G/C. But the minor allele (C) frequency was 0.025. Therefore, we did not proceed with genetic association studies.

Association Results Between *FABP3* and Schizophrenia/Bipolar Disorder

As shown in Figure 2, eight SNPs were selected as tags. LD block analysis showed that SNPs F302–F308 constitute one LD block in both the schizophrenia-control and bipolar disorder-control sample sets (data not shown). None of the 8 SNPs showed association with schizophrenia (Table II) or bipolar disorder (Table III). Also, haplotype analysis showed no association with schizophrenia or bipolar disorder (Table SI). Power analysis gave 75.3% power for the schizophrenia-control allelic test statistic (for the bipolar disorder sample set, see above).

CNP of *FABP3*

Because CNP is frequently reported to be in LD with neighboring SNPs [Hinds et al., 2006], we selected 51 subjects who had different combinations of homozygous genotypes at F301 to F308 (i.e., all the SNP sites examined in the current study), to search for its existence (Table SII). However, none of them showed duplications or deletions of the *FABP3* genomic region, suggesting that if present, this CNP is rare in the Japanese population.

DISCUSSION

PUFAs are integral components of membrane phospholipids and they are found abundantly in the brain. PUFAs are thought to be involved in multiple functions including cognition and emotion [Antypa et al., 2008]. Because PUFAs are insoluble in the intracellular matrix, specific transporters are required to deliver PUFAs to appropriate organelles. FABPs are believed to play crucial roles as their cellular shuttles.

In this study, we analyzed the three *FABP* genes expressed in the brain and detected association signals between *FABP7* and bipolar disorder. A total of three SNPs (F704, F705, and F709) displayed allelic and genotypic associations with disease, although they were nominal. LD blocks 2 and 3 showed associations even after a gene-wide correction for multiple testing. Of the three SNPs, F05 is located in the associated LD block 2, but the other 2 SNPs were not in the associated LD blocks. This may be due to the differences in methods used to define tagging SNPs (r^2) and LD blocks (D') [Gabriel et al., 2002]. The three SNPs are in substantial LD to each other, especially in terms of D' (Table SIII). For instance, the SNP

TABLE I. Association Analysis of *FABP7* With Bipolar Disorder

				Allele			Genotype					
Our SNP ID and rs#		**HWE**	**N**	**A**	**G**	***P***	**A/A**	**A/G**	**G/G**	***P****	**MAF**	**Permutation *P****
F701	BP	0.2267	861	1532	190		678	176	7		11.0%	
rs4247671	CT	0.3312	894	1573	215	0.3693	695	183	16	0.2028	12.0%	0.9979

				Allele			Genotype					
Our SNP ID and rs#		**HWE**	**N**	**A**	**C**	***P***	**A/A**	**A/C**	**C/C**	***P****	**MAF**	**Permutation *P****
F703	BP	0.9501	865	1401	329		567	267	31		19.0%	
rs12662030	CT	0.9158	892	1404	380	0.0928	553	298	41	0.2393	21.3%	0.8168

				Allele			Genotype					
Our SNP ID and rs#		**HWE**	**N**	**T**	**C**	***P***	**T/T**	**T/C**	**C/C**	***P****	**MAF**	**Permutation *P****
F704	BP	0.1102	862	1026	698		294	438	130		40.5%	
rs9372716	CT	0.3170	893	1003	783	**0.0474**	289	425	179	**0.0236**	43.8%	0.5500

				Allele			Genotype					
Our SNP ID and rs#		**HWE**	**N**	**T**	**G**	***P***	**T/T**	**T/G**	**G/G**	***P****	**MAF**	**Permutation *P****
F705	BP	0.3365	861	1037	685		319	399	143		39.8%	
rs2279382	CT	0.4871	894	1153	635	**0.0099**	367	419	108	**0.0174**	35.5%	0.1544

				Allele			Genotype					
Our SNP ID and rs#		**HWE**	**N**	**T**	**C**	***P***	**T/T**	**T/C**	**C/C**	***P****	**MAF**	**Permutation *P****
F706 (T61M)	BP	0.3240	861	56	1666		0	56	805		3.3%	
rs2279381	CT	0.3803	895	51	1739	0.4937	0	51	844	0.4869	2.8%	0.9998

				Allele		***P***	Genotype					
Our SNP ID and rs#		**HWE**	**N**	**A**	**G**	**A/A**	**A/G**	**G/G**		***P****	**MAF**	**Permutation *P****
F707	BP	0.8253	862	577	1147		98	381	383		33.5%	
rs7752838	CT	0.2734	894	603	1185	0.8864	109	385	400	0.8239	33.7%	1.0000

				Allele			Genotype					
Our SNP ID and rs#		**HWE**	**N**	**T**	**C**	***P***	**T/T**	**T/C**	**C/C**	***P****	**MAF**	**Permutation *P****
F708	BP	0.3246	862	970	754		280	410	172		43.7%	
rs9401594	CT	0.3262	895	979	811	0.3594	275	429	191	0.6554	45.3%	0.9976

				Allele			Genotype					
Our SNP ID and rs#		**HWE**	**N**	**A**	**G**	***P***	**A/A**	**A/G**	**G/G**	***P****	**MAF**	**Permutation *P****
F709	BP	0.2443	857	1000	714		300	400	157		41.7%	
rs9401595	CT	0.3465	892	1120	664	**0.0077**	345	430	117	**0.0093**	37.2%	0.1165

TABLE I. (*Continued*)

Our SNP ID and rs#		HWE	N	Allele T	Allele G	P*	Genotype T/T	Genotype T/G	Genotype G/G	P*	MAF	Permutation P**
F710	BP	0.5759	858	367	1349		42	283	533		21.4%	
rs9490550	CT	0.7948	892	429	1355	0.0636	53	323	516	0.1713	24.0%	0.6244

Our SNP ID and rs#		HWE	N	Allele A	Allele G	P*	Genotype A/A	Genotype A/G	Genotype G/G	P*	MAF	Permutation P**
F711	BP	0.3970	859	462	1256		67	328	464		26.9%	
rs9401596	CT	0.5371	893	508	1278	0.3081	76	356	461	0.5911	28.4%	0.9955

Our SNP ID and rs#		HWE	N	Allele T	Allele C	P*	Genotype T/T	Genotype T/C	Genotype C/C	P*	MAF	Permutation P**
F713	BP	0.2383	859	1211	507		434	343	82		29.5%	
rs9482286	CT	0.2359	895	1283	507	0.4563	467	349	79	0.7505	28.3%	0.9996

Our SNP ID and rs#		HWE	N	Allele T	Allele C	P*	Genotype T/T	Genotype T/C	Genotype C/C	P*	MAF	Permutation P**
F714	BP	0.1232	858	1055	661		335	385	138		38.5%	
rs6899351	CT	0.1382	889	1107	671	0.6507	355	397	137	0.9025	37.7%	1.0000

Our SNP ID and rs#		HWE	N	Allele A	Allele G	P*	Genotype A/A	Genotype A/G	Genotype G/G	P*	MAF	Permutation P**
F15	BP	0.1649	856	821	891		207	407	242		48.0%	
rs6919681	CT	0.5725	893	882	904	0.4168	222	438	233	0.5940	49.4%	0.9992

Our SNP ID and rs#		HWE	N	Allele T	Allele C	P*	Genotype T/T	Genotype T/C	Genotype C/C	P*	MAF	Permutation P**
F716	BP	0.7784	864	746	982		159	428	277		43.2%	
rs6904500	CT	0.7331	894	810	978	0.2090	186	438	270	0.4068	45.3%	0.9648

BP, bipolar disorder; CT, control; HWE, Hardy–Weinberg equilibrium; MAF, minor allele, frequency.
Bold *P* values mean $P < 0.05$.
*Evaluated by Fisher's exact test.
**Permutation was run 10,000 times.

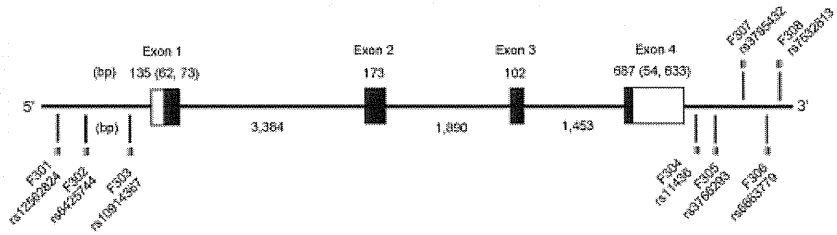

FIG. 2. Genomic structure and polymorphic sites in the *FABP3* gene. Exons are denoted as boxes, with coding regions in black and 5′-/3′-untranslated regions in white. The sizes of each exon and intron are also shown.

TABLE II. Association Analysis of *FABP3* with Schizophrenia

				Allele			Genotype				
Our SNP ID and rs#		**HWE**	**N**	**T**	**C**	***P****	**T/T**	**T/C**	**C/C**	**MAF**	***P****
F301	SZ	0.5897	942	625	1259		100	425	417	33.2%	
rs12562824	CT	0.5250	945	620	1270	0.8354	106	408	431	32.8%	0.6886
				Allele			**Genotype**				
Our SNP ID and rs#		**HWE**	**N**	**T**	**C**	***P****	**T/T**	**T/C**	**C/C**	**MAF**	***P****
F302	SZ	0.4533	944	975	913		246	483	215	48.4%	
rs6425744	CT	0.1292	949	965	933	0.6259	257	451	241	49.2%	0.2468
				Allele			**Genotype**				
Our SNP ID and rs#		**HWE**	**N**	**T**	**C**	***P****	**T/T**	**T/C**	**C/C**	**MAF**	***P****
F303	SZ	0.5241	944	1064	824		295	474	175	43.6%	
rs10914367	CT	0.5100	948	1079	817	0.7429	312	455	181	43.1%	0.6223
				Allele			**Genotype**				
Our SNP ID and rs#		**HWE**	**N**	**A**	**G**	***P****	**A/A**	**A/G**	**G/G**	**MAF**	***P****
F304	SZ	0.1950	942	1595	289		670	255	17	15.3%	
rs11436	CT	0.0321	949	1583	315	0.3073	651	281	17	16.6%	0.4655
				Allele			**Genotype**				
Our SNP ID and rs#		**HWE**	**N**	**A**	**C**	***P****	**A/A**	**A/C**	**C/C**	**MAF**	***P****
F305	SZ	0.3839	943	262	1624		15	232	696	13.9%	
rs3766293	CT	0.7071	950	224	1676	0.0580	12	200	738	11.8%	0.1390
				Allele			**Genotype**				
Our SNP ID and rs#		**HWE**	**N**	**A**	**G**	***P****	**A/A**	**A/G**	**G/G**	**MAF**	***P****
F306	SZ	0.9252	943	279	1607		21	237	685	14.8%	
rs6663779	CT	0.3626	948	285	1611	0.8552	25	235	688	15.0%	0.8512
				Allele			**Genotype**				
Our SNP ID and rs#		**HWE**	**N**	**A**	**G**	***P****	**A/A**	**A/G**	**G/G**	**MAF**	***P****
F307	SZ	0.9483	943	541	1345		78	385	480	28.7%	
rs3795432	CT	0.9833	947	508	1386	0.2038	68	372	507	26.8%	0.4391
				Allele			**Genotype**				
Our SNP ID and rs#		**HWE**	**N**	**G**	**C**	***P****	**G/G**	**G/C**	**C/C**	**MAF**	***P****
F308	SZ	0.5077	943	824	1062		175	474	294	43.7%	
rs7532813	CT	0.5005	947	814	1080	0.6697	180	454	313	43.0%	0.5818

SZ, schizophrenia; CT, control; HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency.
*Evaluated by Fisher's exact test.

TABLE III. Association Analysis of *FABP3* with Bipolar Disorder

				Allele			Genotype				
Our SNP ID and rs#		**HWE**	**N**	**T**	**C**	***P****	**T/T**	**T/C**	**C/C**	**MAF**	***P****
F301	BP	0.5503	860	572	1148		99	374	387	33.3%	
rs12562824	CT	0.9101	890	642	1138	0.0819	115	412	363	36.1%	0.1922

				Allele			Genotype				
Our SNP ID and rs#		**HWE**	**N**	**T**	**C**	***P****	**T/T**	**T/C**	**C/C**	**MAF**	***P****
F302	BP	0.4738	861	865	857		212	441	208	49.8%	
rs6425744	CT	0.7450	893	859	927	0.2114	209	441	243	51.9%	0.3406

				Allele			Genotype				
Our SNP ID and rs#		**HWE**	**N**	**T**	**C**	***P****	**T/T**	**T/C**	**C/C**	**MAF**	***P****
F303	BP	0.5951	861	961	761		272	417	172	44.2%	
rs10914367	CT	0.9812	895	978	812	0.4973	267	444	184	45.4%	0.7251

				Allele			Genotype				
Our SNP ID and rs#		**HWE**	**N**	**A**	**G**	***P****	**A/A**	**A/G**	**G/G**	**MAF**	***P****
F304	BP	0.3667	862	1432	292		591	250	21	16.9%	
rs11436	CT	0.3966	894	1492	296	0.7863	619	254	21	16.6%	0.9539

				Allele			Genotype				
Our SNP ID and rs#		**HWE**	**N**	**A**	**C**	***P****	**A/A**	**A/C**	**C/C**	**MAF**	***P****
F305	BP	0.0268	863	231	1495		23	185	655	13.4%	
rs3766293	CT	0.5909	893	267	1519	0.1916	22	223	648	14.9%	0.2176

				Allele			Genotype				
Our SNP ID and rs#		**HWE**	**N**	**A**	**G**	***P****	**A/A**	**A/G**	**G/G**	**MAF**	***P****
F306	BP	0.5076	862	253	1471		21	211	630	14.7%	
rs6663779	CT	0.5767	893	260	1526	0.9239	21	218	654	14.6%	1.0000

				Allele			Genotype				
Our SNP ID and rs#		**HWE**	**N**	**A**	**G**	***P****	**A/A**	**A/G**	**G/G**	**MAF**	***P****
F307	BP	0.1356	863	485	1241		77	331	455	28.1%	
rs3795432	CT	0.7422	893	528	1258	0.3517	76	376	441	29.6%	0.2734

				Allele			Genotype				
Our SNP ID and rs#		**HWE**	**N**	**G**	**C**	***P****	**G/G**	**G/C**	**C/C**	**MAF**	***P****
F308	BP	0.6382	861	762	960		172	418	271	44.3%	
rs7532813	CT	0.9270	893	810	976	0.5189	183	444	266	45.4%	0.7491

BP, bipolar disorder; CT, control; HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency.
*Evaluated by Fisher's exact test.

F709 did not constitute a haplotype block under Gabriel's model Gabriel et al., 2002] (Fig. 1). Since the extent of the haplotype block may delimit the range of a functional variant position, we reconstructed haplotype blocks using the solid spine model ($D' > 0.8$). Under this model, the marker F709 was located within a block consisting of SNPs F707, F708, F709, F710, and F711, and the haplotype G–T–G–G–G was significantly over-represented in the bipolar disorder group (frequency = 0.36) compared to the control group (frequency = 0.32) [$P = 0.014$, OR (95% CI) = 1.19 (1.04–1.38)].

We also tested for an association between SNP F706 and schizophrenia, using the current expanded panel (the previously used sample set consisting of 570 schizophrenics and 570 controls). The results were: allelic $P = 0.2352$ and genotypic $P = 0.2690$, thus failing to replicate the prior finding. Because the minor allele frequency of this SNP is low [2.4% in schizophrenia and 3.1% in controls in the current panel; 1.7% in schizophrenia and 3.1% in controls in the previous panel] and the crystallographic analysis points to a probable functional alteration by this SNP [Watanabe et al., 2007], analysis of a much larger sample will be needed to draw a definite conclusion. In any case, further studies are needed to confirm the true causative SNPs and/or combination of SNPs in schizophrenia and bipolar disorder.

In our previous study, we demonstrated schizophrenia-related phenotypes in *Fabp7* knockout mice, for example, reduced PPI and enhanced responses to repeated administration of MK-801 [Watanabe et al., 2007]. Based on these results, we are now examining emotion-related behavior in the gene-deficient mice. The results so far indicate elevated locomotor activity and enhanced anxiety traits in the knockout mice [unpublished data]. Therefore, although the human genetic data is modest, it may be possible that *FABP7* does have some role in the development of schizophrenia and bipolar disorder. It is interesting to note that Fabp7 shows abundant expression in neural progenitor cells during early developmental stages and augments neurogenesis [Arai et al., 2005; Watanabe et al., 2007; Owada, 2008]. The potential links between neurogenesis and mood disorder [see Eisch et al., 2008 for review] and schizophrenia [Reif et al., 2006] have been reported. Therefore if altered neurogenesis is a contributory mechanism to the pathogenesis of schizophrenia and bipolar disorder, *FABP7* may be a strong causative gene. Regarding the relationship between PUFAs and mood disorders, another line of evidence is also notable: administration of three mood stabilizers (lithium, valproate, and carbamazepine) at therapeutically relevant doses, selectively target the brain arachidonic acid cascade, and decrease turnover of arachidonic acid but not of docosahexaenoic acid in rat brain Rao et al., 2008].

The structure of each *FABP* gene has been conserved among all members of the family; they consist of four exons separated by three introns [Veerkamp and Zimmerman, 2001]. One of the impediments in genetic studies of *FABP* genes is the relatively small size of *FABP7* (=4.57 kb), *FABP5* (=4.22 kb), and *FABP3* (=7.82 kb). We could not obtain suitable SNPs for *FABP5*, even though we expanded the region of our search for polymorphisms to 10 kb-upstream and 10 kb-downstream from the first exon and last exon (re-sequencing analysis plus database search). Functionally, FABP5 shares similarities with FABP7, in terms of their ontogenic expression patterns [Owada, 2008] and roles in neurogenesis [unpublished data]. In contrast, the expression of *Fabp3* in the brain increases slowly in postnatal stages, reaching a plateau in adulthood [Owada, 2008]. Interestingly in relation to psychiatric illnesses, *Fabp3* co-localizes with dopamine receptor positive cells, and it interacts with the dopamine receptor D2L, and regulates the distribution of the D2L between the membrane and perinuclear cytoplasm [Takeuchi and Fukunaga, 2003].

Expression of each *FABP* gene is spatio-temporally regulated very tightly, using multiple regulatory elements in addition to the core promoter [Haunerland and Spener, 2004]. However, none of these regulatory genomic elements have been identified. For a more comprehensive evaluation of the genetic contribution of *FABP* genes to schizophrenia and bipolar disorder, future studies are needed to clarify such genomic elements and assess the roles of polymorphisms found in those regions.

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