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

Yoshimi Iwayama,[1] Eiji Hattori,[1] Motoko Maekawa,[1] Kazuo Yamada,[1] Tomoko Toyota,[1] Tetsuo Ohnishi,[1] Yasuhide Iwata,[2] Kenji J. Tsuchiya,[2] Genichi Sugihara,[2] Mitsuru Kikuchi,[3] Kenji Hashimoto,[4] Masaomi Iyo,[5] Toshiya Inada,[6] Hiroshi Kunugi,[7] Norio Ozaki,[8] Nakao Iwata,[9] Shinichiro Nanko,[10] Kazuya Iwamoto,[11] Yuji Okazaki,[12] Tadafumi Kato,[11] and Takeo Yoshikawa[1,13]*

[1]Laboratory for Molecular Psychiatry, RIKEN Brain Science Institute, Saitama, Japan
[2]Department of Psychiatry and Neurology, Hamamatsu University School of Medicine, Shizuoka, Japan
[3]Department of Psychiatry and Neurobiology, Kanazawa University Graduate School of Medical Science, Ishikawa, Japan
[4]Division of Clinical Neuroscience, Chiba University Center for Forensic Mental Health, Chiba, Japan
[5]Department of Psychiatry, Chiba University Graduate School of Medicine, Chiba, Japan
[6]Seiwa Hospital, Institute of Neuropsychiatry, Tokyo, Japan
[7]Department of Mental Disorder Research, National Institute of Neuroscience, Tokyo, Japan
[8]Department of Psychiatry, Graduate School of Medicine, Nagoya University, Aichi, Japan
[9]Department of Psychiatry, Fujita Health University School of Medicine, Aichi, Japan
[10]Department of Psychiatry and Genome Research Center, Teikyo University of Medicine, Tokyo, Japan
[11]Laboratory for Molecular Dynamics of Mental Disorders, RIKEN Brain Science Institute, Saitama, Japan
[12]Tokyo Metropolitan Matsuzawa Hospital, Tokyo, Japan
[13]CREST, Japanese Science and Technology Agency, Tokyo, Japan

Received 25 February 2009; Accepted 22 May 2009

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

How to Cite this Article:
Iwayama Y, Hattori E, Maekawa M, Yamada K, Toyota T, Ohnishi T, Iwata Y, Tsuchiya KJ, Sugihara G, Kikuchi M, Hashimoto K, Iyo M, Inada T, Kunugi H, Ozaki N, Iwata N, Nanko S, Iwamoto K, Okazaki Y, Kato T, Yoshikawa T. 2010. Association Analyses Between Brain-Expressed Fatty-Acid Binding Protein (*FABP*) Genes and Schizophrenia and Bipolar Disorder.
Am J Med Genet Part B 153B:484–493.

Additional Supporting Information may be found in the online version of this article.

Grant sponsor: RIKEN BSI Funds; Grant sponsor: Japan Science and Technology Agency (CREST Funds); Grant sponsor: MEXT of Japan.

*Correspondence to:
Dr. Takeo Yoshikawa, M.D., Ph.D., Laboratory for Molecular Psychiatry, RIKEN Brain Science Institute, 2-1 Hirosawa, Wako-city, Saitama 351-0198, Japan. E-mail: takeo@brain.riken.jp

Published online 24 June 2009 in Wiley InterScience (www.interscience.wiley.com)
DOI 10.1002/ajmg.b.31004

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/

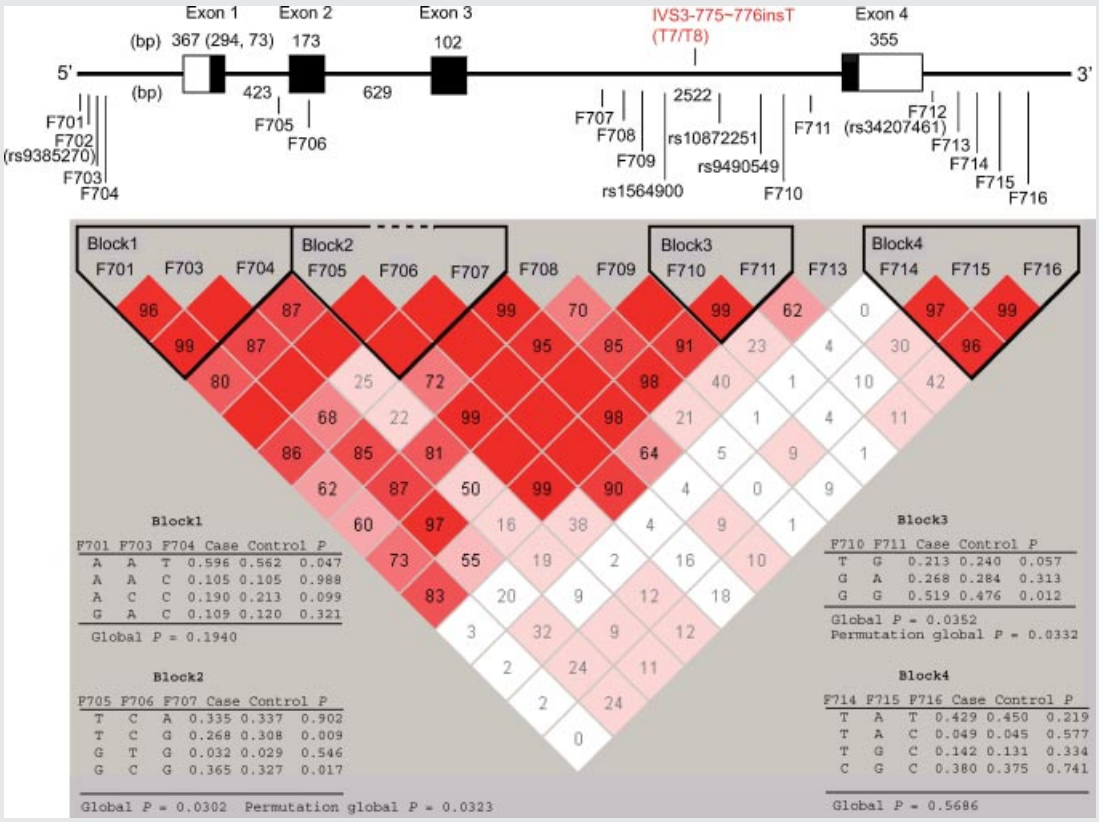

FIG. 1. Genomic structure, polymorphic sites and LD block structure of the *FABP7* gene. In the upper panel, exons are denoted as boxes, with coding regions in black and 5′-/3′-untranslated regions in white. The sizes of each exon and intron are also shown. In the lower panel, the number in each cell 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.

index.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.

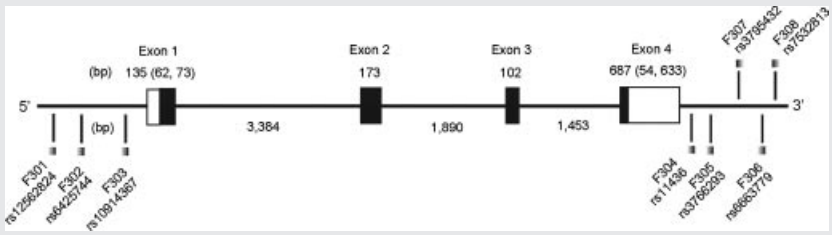

FIG. 2. Genomic structure and polymorphic sites in the *FABP3* gene. Exons are denoted as boxes, with coding regions in black and 5′-/3′-untranslated regions in white. The sizes of each exon and intron are also shown.

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 F11, 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.

ACKNOWLEDGMENTS

The authors would like to acknowledge all the subjects who participated in this study. This work was supported by RIKEN BSI Funds, CREST funds from the Japan Science and Technology Agency, and grants from MEXT of Japan. The authors report no involvement, financial or otherwise, that might potentially bias this work.

REFERENCES

Antypa N, Van der Does A, Smelt A, Rogers R. 2008. Omega-3 fatty acids (fish-oil) and depression-related cognition in healthy volunteers. J Psychopharmacol [Epub ahead of print].

Arai Y, Funatsu N, Numayama-Tsuruta K, Nomura T, Nakamura S, Osumi N. 2005. Role of Fabp7, a downstream gene of Pax6, in the maintenance of neuroepithelial cells during early embryonic development of the rat cortex. J Neurosci 25(42):9752–9761.

Barrett JC, Fry B, Maller J, Daly MJ. 2005. Haploview: Analysis and visualization of LD and haplotype maps. Bioinformatics 21(2):263–265.

Baum AE, Akula N, Cabanero M, Cardona I, Corona W, Klemens B, Schulze TG, Cichon S, Rietschel M, Nothen MM, et al. 2008. A genome-wide association study implicates diacylglycerol kinase eta (DGKH) and several other genes in the etiology of bipolar disorder. Mol Psychiatry 13(2):197–207.

Braff DL, Geyer MA, Swerdlow NR. 2001. Human studies of prepulse inhibition of startle: Normal subjects, patient groups, and pharmacological studies. Psychopharmacology (Berl) 156(2–3):234–258.

Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. 2004. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. Am J Hum Genet 74(1):106–120.

Dudbridge F. 2003. Pedigree disequilibrium tests for multilocus haplotypes. Genet Epidemiol 25(2):115–121.

Eisch AJ, Cameron HA, Encinas JM, Meltzer LA, Ming GL, Overstreet-Wadiche LS. 2008. Adult neurogenesis, mental health, and mental illness: Hope or hype? J Neurosci 28(46):11785–11791.

Furuhashi M, Hotamisligil GS. 2008. Fatty acid-binding proteins: Role in metabolic diseases and potential as drug targets. Nat Rev Drug Discov 7(6):489–503.

Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, et al. 2002. The structure of haplotype blocks in the human genome. Science 296:2225–2229.

Giakoumaki SG, Roussos P, Rogdaki M, Karli C, Bitsios P, Frangou S. 2007. Evidence of disrupted prepulse inhibition in unaffected siblings of bipolar disorder patients. Biol Psychiatry 62(12):1418–1422.

Gottesman II, Gould TD. 2003. The endophenotype concept in psychiatry: Etymology and strategic intentions. Am J Psychiatry 160(4):636–645.

Gur RE, Calkins ME, Gur RC, Horan WP, Nuechterlein KH, Seidman LJ, Stone WS. 2007. The Consortium on the Genetics of Schizophrenia: Neurocognitive endophenotypes. Schizophr Bull 33(1):49–68.

Hattori E, Toyota T, Ishitsuka Y, Iwayama Y, Yamada K, Ujike H, Morita Y, Kodama M, Nakata K, Minabe Y, et al. 2009. Preliminary genome-wide association study of bipolar disorder in the Japanese population. Neuropsychiatr Genet [Epub ahead of print].

Haunerland NH, Spener F. 2004. Fatty acid-binding proteins–insights from genetic manipulations. Prog Lipid Res 43(4):328–349.

Hinds DA, Kloek AP, Jen M, Chen X, Frazer KA. 2006. Common deletions and SNPs are in linkage disequilibrium in the human genome. Nat Genet 38(1):82–85.

Liu RZ, Li X, Godbout R. 2008. A novel fatty acid-binding protein (FABP) gene resulting from tandem gene duplication in mammals: Transcription in rat retina and testis. Genomics 92(6):436–445.

Need AC, Ge D, Weale ME, Maia J, Feng S, Heinzen EL, Shianna KV, Yoon W, Kasperaviciute D, Gennarelli M, et al. 2009. A genome-wide investigation of SNPs and CNVs in schizophrenia. PLoS Genet 5(2):e1000373.

O'Donovan MC, Craddock N, Norton N, Williams H, Peirce T, Moskvina V, Nikolov I, Hamshere M, Carroll L, Georgieva L, et al. 2008. Identification of loci associated with schizophrenia by genome-wide association and follow-up. Nat Genet 40(9):1053–1055.

Ohnishi T, Yamada K, Ohba H, Iwayama Y, Toyota T, Hattori E, Inada T, Kunugi H, Tatsumi M, Ozaki N, et al. 2007. A promoter haplotype of the inositol monophosphatase 2 gene (IMPA2) at 18p11.2 confers a possible risk for bipolar disorder by enhancing transcription. Neuropsychopharmacology 32(8):1727–1737.

Owada Y. 2008. Fatty acid binding protein: Localization and functional significance in the brain. Tohoku J Exp Med 214(3):213–220.

Purcell S, Cherny SS, Sham PC. 2003. Genetic power calculator: Design of linkage and association genetic mapping studies of complex traits. Bioinformatics 19(1):149–150.

Rao JS, Lee HJ, Rapoport SI, Bazinet RP. 2008. Mode of action of mood stabilizers: Is the arachidonic acid cascade a common target? Mol Psychiatry 13:585–596.

Reif A, Fritzen S, Finger M, Strobel A, Lauer M, Schmitt A, Lesch KP. 2006. Neural stem cell proliferation is decreased in schizophrenia, but not in depression. Mol Psychiatry 11(5):514–522.

Richardson AJ. 2004. Long-chain polyunsaturated fatty acids in childhood developmental and psychiatric disorders. Lipids 39(12): 1215–1222.

Sklar P, Smoller JW, Fan J, Ferreira MA, Perlis RH, Chambert K, Nimgaonkar VL, McQueen MB, Faraone SV, Kirby A, et al. 2008. Whole-genome association study of bipolar disorder. Mol Psychiatry 13(6):558–569.

Takeuchi Y, Fukunaga K. 2003. Differential subcellular localization of two dopamine D2 receptor isoforms in transfected NG108–15 cells. J Neurochem 85(4):1064–1074.

Veerkamp JH, Zimmerman AW. 2001. Fatty acid-binding proteins of nervous tissue. J Mol Neurosci 16(2–3):133–142.

Watanabe A, Toyota T, Owada Y, Hayashi T, Iwayama Y, Matsumata M, Ishitsuka Y, Nakaya A, Maekawa M, Ohnishi T, et al. 2007. Fabp7 maps to a quantitative trait locus for a schizophrenia endophenotype. PLoS Biol 5(11):e297.

Wellcome Trust Consortium. 2007. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 447(7145):661–678.

Yamaguchi-Kabata Y, Nakazono K, Takahashi A, Saito S, Hosono N, Kubo M, Nakamura Y, Kamatani N. 2008. Japanese population structure, based on SNP genotypes from 7003 individuals compared to other ethnic groups: Effects on population-based association studies. Am J Hum Genet 83(4):445–456.