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SHORT COMMUNICATION

Polymorphism screening of brain-expressed *FABP7, 5* and *3* genes and association studies in autism and schizophrenia in Japanese subjects

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Fatty acid-binding protein (FABP) **gene family encode fatty acid-binding proteins and consist of at least 12 members, of which *FABP7*, *5* and *3* are expressed in the brain. We previously showed that *FABP7* is associated with schizophrenia and bipolar disorder. Recently, genetic overlap between autism and schizophrenia has been reported. Therefore, in this study, we set out to examine the possible roles of brain-expressed *FABPs* in autism, focusing primarily on potentially functional polymorphisms (that is, missense polymorphisms). First, we resequenced the three genes using 285 autism samples. We identified 13 polymorphisms, of which 7 are novel. Of the novel single-nucleotide polymorphisms (SNPs), two are missense mutations, namely, 376G>C (Val126Leu) in *FABP7* and 340G>C (Gly114Arg) in *FABP5*. Second, we tested for the genetic association of four missense SNPs with autism and schizophrenia, but failed to detect significant results. Finally, as a web-based algorithm predicts that the 8A>G (Asp3Gly; rs17848124) in *FABP3* is 'probably damaging', we estimated the possible impact of this SNP, and found that the loss of charge and salt bridge, caused by the Asp3-to-Gly3, may affect stability of the FABP3 protein. Future searches for associated phenotypes with missense SNPs using larger samples are highly warranted.**
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Keywords: crystallographic analysis; fatty acid-binding protein; missense mutation

INTRODUCTION

Schizophrenia and autism are devastating psychiatric illnesses, with the former showing mainly adulthood onset and the latter childhood onset. Recent research proposes common genetic mechanisms between the two diseases.[1] As an additional environmental factor, the role of fatty acids has attracted attention in the pathophysiology of both diseases.[2] Our interest is in fatty acid-binding proteins (FABPs) as mediator molecules for linking fatty acids to brain function. *FABP*s constitute a gene family of at least 12 members.[3,4] Brain-expressed *FABP*s include *FABP7*, *FABP5* and *FABP3*.[5] We previously performed association studies between the above three *FABP* genes and schizophrenia and bipolar disorder, and detected modest associations between *FABP7* and schizophrenia and bipolar disorder.[6,7]

In this study, we set out to examine the possible genetic roles for *FABP7*, *FABP5* and *FABP3* in autism. First, we resequenced the three *FABP* genes using 285 autism samples. We then focused on the identified missense single-nucleotide polymorphisms (SNPs), undertaking a genetic evaluation of these SNPs in autism and schizophrenia because of the possible overlap in mechanisms between the two diseases. We also discussed the possible impact of a potentially important SNP in terms of its predicted structural and functional consequences.

MATERIALS AND METHODS

Subjects

A total of 285 autistic patients of Japanese descent (236 men, 49 women; aged between 3 and 32 years) were used for the resequencing analysis and association studies. The diagnosis of autism was made on the basis of the Autism Diagnostic Interview-Revised (ADI-R)[8] criteria. The detected missense polymorphisms were analyzed in association studies using the above mentioned autistic subjects, 1060 schizophrenics (diagnosed according to DSM-IV) (503 men, 557 women; mean age 48.0 ± 13.8 years) and 1060 controls who are free of mental disorders (503 men, 557 women; mean age 47.7 ± 13.6 years) during brief interviews by psychiatrists. Because we did not administer structural or semi-structural instruments for the recruitment of control subjects, it may be difficult to completely exclude the contamination of subjects with psychiatric

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problems, including Asperger syndrome. The study was approved by the ethics committees of the RIKEN and Hamamatsu University.

Polymorphism screening of *FABP* genes and genotyping

Protein-coding regions and exon/intron boundaries of *FABP7*, *FABP5* and *FABP3* were screened for polymorphisms by direct sequencing of PCR products (Supplementary Figure 1). The primers used for amplification and PCR conditions are listed in Supplementary Table 1. Custom TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA) were used to score the identified missense SNPs, along with ABI PRISM 7900 Sequence Detection System and SDS v2.3 software (Applied Biosystems). The insertion/deletion polymorphisms were scored by inspecting the sequencing electrophelograms.

RESULTS AND DISCUSSION

Identification of missense SNPs in the *FABP* genes and association studies

Our polymorphism screen detected 13 different variants in *FABP7*, *FABP5* and *FABP3*: 7 are novel and 6 were previously reported (Table 1). With respect to novel variants, the 376G>C (Val126Leu; SNP ID 'ss160853728' was obtained from NCBI) in *FABP7* and 340G>C (Gly114Arg; newly assigned as 'ss160853732') in *FABP5* were missense SNPs (Table 1). The total number of missense SNPs identified in this study is four. We tested these four missense SNPs for association with autism and schizophrenia, because of the potentially functional importance of missense polymorphisms. As autism is more prevalent in males than in females, the association analysis of autism samples was done separately in males and in females. However, none of them showed significant *P*-values, although the two new missense SNPs (Val126Leu in *FABP7* and Gly114Arg in *FABP5*) were not seen in over 1000 control samples (Table 2). When samples of both sexes were combined, the associations with autism were not significant at all the SNPs (data not shown). The low frequency of minor alleles from the examined SNPs, suggests that larger sample sizes are needed to exclude the possibility of type II errors, a limitation of the current study. The other caution of this study includes that age is not matched between autism and control samples.

Table 1 Identified polymorphisms in *FABP* genes from 285 autistic samples

Gene	*Polymorphism*	*dbSNP ID*[a]	*Minor allele frequency*		*Major allele*	*Predicted effect of minor allele*[b]
FABP7	−78T>A	New	1/570	0.2%	T	—
	IVS1−63T>G	rs2279382	219/568	38.6%	T	—
	182C>T (Thr61Met)	rs2279381	22/568	3.9%	C	Benign
	IVS2+39G>A	rs2243372	190/568	33.5%	G	—
	IVS3+21A>G	rs17848133	7/570	1.2%	A	—
	376G>C (Val126Leu)	ss160853728 (New)	1/568	0.2%	G	Benign
FABP5	IVS2+27C>T	New	1/566	0.2%	C	—
	331T>C (Leu11Leu)	New	1/570	0.2%	T	—
	340G>C (Gly114Arg)	ss160853732 (New)	1/570	0.2%	G	Benign
FABP3	−56G>A	New	1/568	0.2%	G	—
	8A>G (Asp3Gly)	rs17848124	5/568	0.9%	A	Probably damaging
	IVS2+16∼17Ins/DelAG>-	New	1/570	0.2%	Ins	—
	IVS2-85G>C	rs2271072	87/570	15.3%	G	—

Abbreviations: dbSNP, single nucleotide polymorphism database; FABP, fatty acid-binding protein.
[a]The NCBI database (http://www.ncbi.nlm.nih.gov/) was searched for known SNPs.
[b]Functional consequences of missense polymorphisms are evaluated using 'PolyPhen' (http://coot.embl.de/PolyPhen/).

Table 2 Results of association studies

Disease	*Sex*	*Gene*	*Missense SNP*	*dsSNP ID*	*Sample*	N	*Allele frequency*		P-*value (Fisher's exact test)*	*Genotype frequency*			P-*value (Fisher's exact test)*
Autism	Male	*FABP7*	Thr61Met	rs2279381			C	T		C/C	T/C	T/T	
					Control	502	975	29		473	29	0	
					Autism	236	453	19	0.2716	217	19	0	0.2636
		FABP7	Val126Leu	ss160853728 (New)			G	C		G/G	G/C	C/C	
					Control	499	998	0		499	0	0	
					Autism	235	469	1	0.3202	234	1	0	0.3202
		FABP5	Gly114Arg	ss160853732 (New)			G	C		G/G	G/C	C/C	
					Control	499	998	0		499	0	0	
					Autism	236	471	1	0.3211	235	1	0	0.3211
		FABP3	Asp3Gly	rs17848124			A	G		A/A	A/G	G/G	
					Control	498	989	7		491	7	0	
					Autism	235	466	4	0.7524	231	4	0	0.7517

Table 2 Continued

Disease	*Sex*	*Gene*	*Missense SNP*	*dsSNP ID*	*Sample*	N	*Allele frequency*		P-*value* (*Fisher's exact test*)	*Genotype frequency*			P-*value* (*Fisher's exact test*)
	Female	*FABP7*	Thr61Met	rs2279381			C	T		C/C	T/C	T/T	
					Control	558	1080	36		524	32	2	
					Autism	48	93	3	1.0000	45	3	0	0.7897
		FABP7	Val126Leu	ss160853728 (New)			G	C		G/G	G/C	C/C	
					Control	554	1108	0		554	0	0	
					Autism	49	98	0	—	49	0	0	—
		FABP5	Gly114Arg	ss160853732 (New)			G	C		G/G	G/C	C/C	
					Control	557	1114	0		557	0	0	
					Autism	49	98	0	—	49	0	0	—
		FABP3	Asp3Gly	rs17848124			A	G		A/A	A/G	G/G	
					Control	550	1087	13		537	13	0	
					Autism	49	97	1	1.0000	48	1	0	1.0000
Schizophrenia	Male/ female	*FABP7*	Thr61Met	rs2279381			C	T		C/C	T/C	T/T	
					Control	1060	2055	65		997	61	2	
					Schizophrenia	1053	2056	50	0.1857	1003	50	0	0.2127
		FABP7	Val126Leu	ss160853728 (New)			G	C		G/G	G/C	C/C	
					Control	1053	2106	0		1053	0	0	
					Schizophrenia	1055	2110	0	—	1055	0	0	—
		FABP5	Gly114Arg	ss160853732 (New)			G	C		G/G	G/C	C/C	
					Control	1056	2112	0		1056	0	0	
					Schizophrenia	1050	2098	2	0.2485	1048	2	0	0.2485
		FABP3	Asp3Gly	rs17848124			A	G		A/A	A/G	G/G	
					Control	1048	2076	20		1028	20	0	
					Schizophrenia	1053	2080	26	0.4590	1027	26	0	0.4565

Abbreviations: dbSNP, single nucleotide polymorphism database; FABP, fatty acid-binding protein.

The subject with *FABP7* Val126/Leu126 genotype is an 8-year-old male, whose diagnosis is autistic disorder. The intellectual examination using the Wechsler Intelligence Scale for Children-III (WISC-III) showed the following scores: FIQ (full scale IQ)=96, VIQ (verbal IQ)=94 and PIQ (performance IQ)=100. Regarding the ADI-R scores, domain A (social interaction)=26, B (communication and language)=14, C (restricted and repetitive behaviors)=3 and D (onset of disorder)=3. His parents are mentally healthy, and the 126Leu allele was transmitted from his mother (Supplementary Figure 2). The subject with *FABP5* Gly114/Arg1114 genotype is a 33-year-old male, whose diagnosis is PDDNOS (pervasive developmental disorder not otherwise specified including atypical autism). He does not have any family history of mental disorders. The Wechsler Adult Intelligence Scale III (WAIS-III) scores were FIQ=95, VIQ=94 and PIQ=97. The ADI-R scores were domain A=5, domain B=5, domain C=2 and domain D=0. The DNA samples of his parents were not available.

Possible impact of the missense SNPs on the structure and function of FABP proteins

A tool-website 'PolyPhen' can estimate the possible impact of an amino acid substitution on the structure and function of a

Figure 1 The site of the missense polymorphism, Asp3Gly, in the FABP3 protein structure (PDB ID, 1HMT; complex with stearic acid).[9] The salt bridge between Asp3 and Lys45 is shown as a dashed line, with its distance. This graphic was created using PyMOL (DeLano Scientific, Palo Alto, CA, USA).

protein (http://genetics.bwh.harvard.edu/pph/). According to this algorithm, only the Asp3Gly mutation in FABP3 is predicted to be 'probably damaging' among the four missense SNPs (Table 1). The possible impact of the Thr61Met mutation in FABP7 is discussed elsewhere.[6]

The crystal structure of FABP3 is known (Figure 1).[9] Replacing Asp by Gly causes loss of a negative charge and breakage of a salt bridge between Asp3 and Lys45, suggesting that this missense polymorphism may affect protein stability. As FABP3 binds and transports fatty acids, destabilization of FABP3 could affect multiple biological processes.

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1 Guilmatre, A., Dubourg, C., Mosca, A. L., Legallic, S., Goldenberg, A., Drouin-Garraud, V. *et al.* Recurrent rearrangements in synaptic and neurodevelopmental genes and shared biologic pathways in schizophrenia, autism, and mental retardation. *Arch. Gen. Psychiatry* **66,** 947–956 (2009).
2 Kidd, P. M. Omega-3 DHA and EPA for cognition, behavior, and mood: clinical findings and structural-functional synergies with cell membrane phospholipids. *Altern. Med. Rev.* **12,** 207–227 (2007).
3 Liu, R. Z., Li, X. & Godbout, R. A novel fatty acid-binding protein (FABP) gene resulting from tandem gene duplication in mammals: transcription in rat retina and testis. *Genomics* **92,** 436–445 (2008).
4 Furuhashi, M. & Hotamisligil, G. S. Fatty acid-binding proteins: role in metabolic diseases and potential as drug targets. *Nat. Rev. Drug Discov.* **7,** 489–503 (2008).
5 Owada, Y. Fatty acid binding protein: localization and functional significance in the brain. *Tohoku J. Exp. Med.* **214,** 213–220 (2008).
6 Watanabe, A., Toyota, T., Owada, Y., Hayashi, T., Iwayama, Y., Matsumata, M. *et al.* Fabp7 maps to a quantitative trait locus for a schizophrenia endophenotype. *PLoS Biol.* **5,** e297 (2007).
7 Iwayama, Y., Hattori, E., Maekawa, M., Yamada, K., Toyota, T., Ohnishi, T. *et al.* Association analyses between brain-expressed fatty-acid binding protein (FABP) genes and schizophrenia and bipolar disorder. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* (e-pub ahead of print 24 June 2009) (2009).
8 Lord, C., Rutter, M. & Le Couteur, A. Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *J. Autism Dev. Disord.* **24,** 659–685 (1994).
9 Young, A. C., Scapin, G., Kromminga, A., Patel, S. B., Veerkamp, J. H. & Sacchettini, J. C. Structural studies on human muscle fatty acid binding protein at 1.4 A resolution: binding interactions with three C18 fatty acids. *Structure* **2,** 523–534 (1994).

Supplementary Information accompanies the paper on Journal of Human Genetics website (http://www.nature.com/jhg)