Neuroscience Letters 438 (2008) 70–75

Contents lists available at ScienceDirect

Neuroscience Letters

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

A genetic association study of the FXYD domain containing ion transport regulator 6 (*FXYD6*) gene, encoding phosphohippolin, in susceptibility to schizophrenia in a Japanese population

Yoshihito Ito[a,*], Yukako Nakamura[a], Nagahide Takahashi[d,a], Shinichi Saito[a], Branko Aleksic[a,b], Nakao Iwata[b], Toshiya Inada[c], Norio Ozaki[a]

[a] *Department of Psychiatry, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan*
[b] *Department of Psychiatry, Fujita Health University School of Medicine, Toyoake, Aichi, Japan*
[c] *Department of Psychiatry, Seiwa Hospital, Institute of Neuropsychiatry, Tokyo, Japan*
[d] *Laboratory of Molecular Neuropsychiatry, Department of Psychiatry, Mount Sinai School of Medicine, New York, NY, USA*

ARTICLE INFO

Article history:
Received 26 January 2008
Received in revised form 31 March 2008
Accepted 4 April 2008

Keywords:
Association study
FXYD6
Schizophrenia
Single nucleotide polymorphism
Linkage disequilibrium

ABSTRACT

The FXYD domain containing ion transport regulator 6 (*FXYD6*) gene is located within a region of chromosome 11 (11q23.3) that has been shown by a number of genome scans to be one of the most well-established linkages to schizophrenia. *FXYD6* encodes the protein phosphohippolin, which is primarily expressed in the brain. Phosphohippolin modulates the kinetic activity of Na,K-ATPase and has long-term physiological importance in maintaining cation homeostasis. A recent study reported that *FXYD6* was associated with schizophrenia in the United Kingdom samples. Applying the gene-based association concept, we carried out an association study regarding *FXYD6* and schizophrenia in a Japanese population, with a sample consisting of 2026 subjects (906 schizophrenics and 1120 controls). After linkage disequilibrium analysis, 23 single nucleotide polymorphisms (SNPs) were genotyped using 5′-exonuclease allelic discrimination assay. We found a significant association of two SNPs (rs11216573; genotypic *P* value: 0.022 and rs555577; genotypic *P* value: 0.026, allelic *P* value: 0.011, uncorrected). Nominal *P* values did not survive correction for multiple testing (rs11216573; genotypic *P* value: 0.47 and rs555577; genotypic *P* value: 0.55, allelic *P* value: 0.24, after SNPSpD correction). No association was observed between schizophrenia patients and controls in allelic, genotypic and haplotypic analyses. Our findings suggest that *FXYD6* is unlikely to be related to the development of schizophrenia in a Japanese population.

Schizophrenia is a severe and debilitating mental illness that affects approximately 1% of the population worldwide [21]. Although family, twin and adoption studies have demonstrated a high heritability (a heritability score of approximately 0.8), the molecular basis of the disease remains unclear [10,21]. The hypothesis that schizophrenia is a developmental disorder of the nervous system with a late onset of characteristic symptoms has been gaining acceptance over the past years, and the involvement of several candidate predisposition genes, such as neuregulin-1 (*NRG1*), disrupted-in-schizophrenia 1 (*DISC1*), and dysbindin (*DTNBP1*), in the development of schizophrenia has been reported [13,20]. Likewise, a hypothetical model based on deficient glutamatergic neurotransmission in the prefrontal cortex has been examined in the aetiology of schizophrenia [15]. In the central nervous system (CNS), most of the excitatory neurotransmission uses L-glutamate as the principal neurotransmitter. Glutamate transport depends on Na^+, K^+ transmembrane gradients generated by Na,K-ATPase [24].

The FXYD domain containing ion transport regulator 6 (*FXYD6*; located on 11q23.3) gene is a member of a family of seven FXYD genes. The chromosome 11q22-24 region has been shown to be one of the most well-established linkages to schizophrenia by a meta-analysis of 20 genome scans and other studies of genome scans (locus SCZD; MIM #181500) [12,14,16]. The FXYD proteins share homology for a single common transmembrane domain [25]. Each FXYD protein is expressed in a tissue-specific manner and functions by altering the kinetic activity of Na,K-ATPase.

Phosphohippolin, one of the FXYD proteins, modulates the kinetic activity of Na,K-ATPase and has long-term physiological importance in maintaining cation homeostasis [8]. *FXYD6* encodes

* Corresponding author at: Department of Psychiatry, Nagoya University, Graduate School of Medicine, 65 Tsuruma-Cho, Showa-Ku, Nagoya 466-8550, Japan. Tel.: +81 52 744 2282; fax: +81 52 744 2293.
E-mail address: y-ito@med.nagoya-u.ac.jp (Y. Ito).

0304-3940/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2008.04.010

the protein phosphohippolin, which is primarily expressed in the brain and kidney [28]. In rats, phosphohippolin has been found to be expressed in the neuronal fibers of the medial part of lateral habenula nucleus, thalamus, hypothalamus, stria terminalis, zona incerta, amygdaloid body, cingulum, olfactory bulb, hippocampus, cerebral cortex, and cerebellum [11]. Expression studies of the brain during development show the greatest amount of phosphohippolin in the postnatal 3-week-old rat brain, with a substantial capacity for phosphohippolin still existing in the adult brain [11]. This suggests that phosphohippolin may play an important role in neuronal excitability of the CNS during postnatal development, as well as that in the adult brain [11]. In humans, the expression of *FXYD6* is primarily in the brain, with the highest level of expression found in the fetal brain, prefrontal cortex, amygdala, occipital lobe, and hypothalamus according to the Novartis gene-expression-atlas database (GNF Sym-Atlas; Human GeneAtlas GNF1H, gcRMA; http://symatlas.gnf.org/SymAtlas/) and the GeneNote database (http://www.genecards.org/). The notable levels of expression occur in regions of the brain thought to be involved in schizophrenia, as identified by brain-imaging abnormalities [26]. In a postmortem brain study of schizophrenia and bipolar disorder, the expression of *FXYD6* in the dorsolateral prefrontal cortex (Brodmann area 46) was tended to be decreased compared with healthy subjects (the Stanley Brain Collection, http://www.stanleyresearch.org/brain/). *FXYD6* is, therefore, a positional and functional candidate gene for schizophrenia.

Recently, a case–control association study between two genes located on 11q23.3 (*FXYD2* and *FXYD6*) and schizophrenia was carried out, and *FXYD6* was demonstrated to be associated with schizophrenia in United Kingdom samples (rs3168238: $P = 0.009$; odds ratio 1.64, rs1815774: $P = 0.049$; odds ratio 1.21, rs4938445: $P = 0.010$; odds ratio 1.31, rs4938446: $P = 0.025$; odds ratio 1.26 and rs497768: $P = 0.023$; odds ratio 1.24) [5]. However, although *FXYD6* is considered one of the plausible candidate genes for schizophrenia, a replication of this positive association is required to maintain that *FXYD6* is a true susceptibility gene for schizophrenia.

It is widely accepted that there are differences in allele frequency or variations of the linkage disequilibrium (LD) structure (population dependence) among each ethnicity. Therefore, a gene-based approach, rather than a single nucleotide polymorphism (SNP)-based or haplotype-based approach, is currently recommended [17]. In such studies, it is important to include both the gene as well as the gene flanking regions when testing for any association, and it is also important to select genetic variants that adequately reflect the LD background by the standardized disequilibrium coefficient (D') and squared correlation coefficient (r^2) in the targeted population (e.g., tagging SNPs).

By applying the aforementioned concept, we carried out a gene-based association study between *FXYD6* and schizophrenia in a Japanese population to try to replicate previous findings.

The total sample used in this research, comprising 2026 subjects (906 schizophrenics and 1120 controls). The cohorts consisted of 906 patients with schizophrenia (513 males and 393 females, mean age ± S.D. = 42.3 ± 17.5 years) and 1120 healthy control subjects (559 males and 561 females, mean age ± S.D. = 38.6 ± 15.4 years). All subjects were unrelated to each other and were of Japanese ethnicity. The patients were diagnosed according to the DSM-IV-TR (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision) criteria for schizophrenia with the consensus reached among at least two experienced psychiatrists on the basis of unstructured interviews as well as a review of the subjects' medical records. All healthy control subjects were also psychiatrically screened on the basis of brief diagnostic unstructured interviews. Subjects who had current or past contact with psychiatric services were excluded. After describing the study to each subject, written informed consent was obtained. This study was approved by the Ethics Committee of the Nagoya University Graduate School of Medicine and Fujita Health University School of Medicine.

We used a gene-based approach to analyze genetic associations. This method implies the inclusion of both the gene region and gene flanking regions in the association study [17]. *FXYD6* contains eight exons spanning approximately 39,700 base pairs (bp) and no validated splicing isoform has been annotated thus far (RefSeq NCBI). We first consulted the HapMap database (http://www.hapmap.org/; release#22; phase2, April 2007, population: Japanese in Tokyo). All common SNPs (minor allele frequency (MAF) > 0.05) in the entire gene region, as well as the 5000 bp upstream 5′ flanking region and 5000 bp downstream 3′ flanking region were listed.

Then we defined 22 tagging SNPs (Table 1) with the criterion of an $r^2 > 0.8$ in 'pair-wise tagging only' mode using the 'Tagger' program, implemented by Haploview software version 4.0 (http://www.broad.mit.edu/mpg/haploview/index.php) [2,7]. In other words, if r^2 calculated from HapMap data was more than 0.8, only one of the two loci was selected for the association study. Significant SNPs (rs1815774, rs4938445 and rs497768) in the previous study [5] were selected by 'Tagger' (Table 1). rs4938446 was not selected because its r^2 between rs4938445 was 1.0, but we included rs4938446 in our analysis. rs3168238 was not included in our analysis, because it was not polymorphic in the Japanese population (dbSNP NCBI, HapMap database). Overall, 23 SNPs were examined.

Venous blood was drawn from the subjects and genomic DNA was extracted from whole blood according to standard procedures. Genotyping of all tagging SNPs was carried out using TaqMan 5′-exonuclease allelic discrimination assay (Applied Biosystems Japan Ltd., Tokyo, Japan). TaqMan probes and Universal PCR Master Mix were obtained from Applied Biosystems. Allelic-specific fluorescence was measured using the ABI PRISM 7900 Sequence Detector System (Applied Biosystems). Details regarding reagents or reaction conditions are available upon request.

Genotypes were tested for Hardy–Weinberg equilibrium using the chi-square goodness-of-fit test. Single-marker allelic and genotypic associations were evaluated using the chi-square test or Fisher's exact test. Genotypic association of SNPs that deviated from HWE was analyzed using Cochran–Armitage trend test for a multiplicative model of inheritance [1]. Analysis of LD between markers (r^2 and D') was performed using Haploview software. For haplotype-wise analysis, LD blocks were initially defined in accordance with Gabriel's criteria using Haploview software. Haplotypic analyses within LD blocks were performed with Unphased software version 2.403 [9], which performs log-likelihood ratio tests under a log-linear model for global P values. Rare haplotypes found in less than 5% of both case and control subjects were excluded from the association analysis to provide greater sensitivity and accuracy when the effect was seen in common haplotypes, and the expectation-maximization algorithm was then used. We also used in a 2- to 5-marker sliding window fashion analysis. The significance level for all statistical tests was $P < 0.05$. Power calculations were performed using the web-based genetic statistical package Genetic Power Calculator [19] (http://pngu.mgh.harvard.edu/~purcell/gpc/). Power was estimated under a multiplicative model of inheritance, assuming the disease prevalence to be 1% and the population susceptibility allele frequencies to be the values observed in control samples. The number of effective independent SNPs assayed was estimated by the spectral decomposition method of Nyholt using SNPSpD software. This software is able to reflect the correlation of markers (LD) on corrected P values to control for inflation of the type I error rate [18] (http://gump.qimr.edu.au/general/daleN/SNPSpD/).

Table 1
Association analysis of 23 SNPs with *FXYD6*

No.	SNP ID		M/m	N		CON			SCZ			MAF		HWE	Genotypic			Allelic		LD	Haplotypic		
				CON	SCZ	M/M	M/m	m/m	M/M	M/m	m/m	CON	SCZ	*P* value	*P* value	[a,b,c]	[g]	*P* value	[d g]	Block [d]	global *P* value [e]		GRR [f]
1	rs11216573		G/A	1084	895	583	417	84	482	370	43	0.27	0.26	0.47	**0.022**	[a]	0.47	0.28		/			1.22
2	rs2282506		C/T	1092	892	958	131	3	762	124	6	0.06	0.08	0.74	0.19	[b]		0.09		/			1.40
3	rs520333		C/T	1079	893	607	396	76	469	349	75	0.25	0.28	0.33	0.21	[a]		0.07		I			1.22
4	rs564989		T/C	1078	892	469	485	124	414	395	83	0.34	0.31	1.00	0.20	[a]		0.09		I			1.21
5	rs10892185		G/C	1081	896	510	455	116	417	394	85	0.32	0.32	0.36	0.55	[a]		0.84		I	0.21		1.21
6	rs496371		C/T	1077	896	753	294	30	603	268	25	0.16	0.18	0.90	0.44	[a]		0.28		I			1.26
7	rs555577		G/A	1076	893	502	464	110	363	423	107	0.32	0.36	0.90	**0.026**	[a]	0.55	**0.011**	0.24	I			1.21
8	rs7121573	*	A/G	1091	892	630	396	65	512	307	73	0.24	0.25	0.84	0.14	[a]		0.35		II			1.23
9	rs1815774	**	C/G	1083	894	619	399	65	512	313	69	0.24	0.25	1.00	0.28	[a]		0.56		II			1.23
10	rs11216591		A/T	1078	894	587	413	78	479	354	61	0.26	0.27	0.69	0.82	[a]		0.87		II	0.69		1.22
11	rs11216594		A/G	1087	894	600	410	77	486	331	77	0.26	0.27	0.58	0.45	[a]		0.40		II			1.22
12	rs876797	*	C/A	1089	894	627	395	67	517	327	50	0.24	0.24	0.69	0.87	[a]		0.77		II			1.23
13	rs10790218	*	G/A	1088	894	341	521	226	283	434	177	0.45	0.44	0.32	0.87	[a]		0.68		III			1.20
14	rs4938445	**	G/A	1042	897	798	220	24	710	173	14	0.13	0.11	0.09	0.28	[a]		0.11		III	0.63		1.29
15	rs4938446	**	T/A	1080	895	822	238	20	705	175	15	0.13	0.12	0.64	0.37	[a]		0.18		III			1.29
16	rs7119090		G/C	1078	892	470	488	120	356	428	108	0.34	0.36	0.76	0.25	[a]		0.13		/			1.21
17	rs11216598		A/G	1077	890	464	489	124	414	376	100	0.34	0.32	0.84	0.30	[a]		0.22		/			1.21
18	rs631898		G/A	1085	896	627	397	61	513	321	62	0.24	0.25	0.94	0.49	[a]		0.50		/			1.23
19	rs11605223		C/G	1088	893	814	249	25	694	184	15	0.14	0.12	0.30	0.27	[a]		0.10		/			1.28
20	rs3809044		T/C	1093	896	942	143	8	773	119	4	0.07	0.07	0.42	0.76	[b]		0.82		IV			1.38
21	rs3809043		C/G	1094	895	872	201	21	716	170	9	0.11	0.11	**0.04**	0.55	[c]		0.54		IV	0.96		1.31
22	rs3809042		G/C	1092	896	692	353	47	555	310	31	0.21	0.21	0.87	0.40	[a]		0.82		IV			1.24
23	rs497768	**	G/C	1073	892	428	486	159	357	419	116	0.38	0.37	0.30	0.49	[a]		0.53		/			1.20

N, number; M, major allele; m, minor allele; CON, control; SCZ, schizophrenia; MAF, minor allele frequency; HWE, Hardy–Weinberg equilibrium; LD, linkage disequilibrium; GRR, genotype relative risk ($\alpha = 0.05$, $1 - \beta = 0.8$).
IDs with asterisk represent significant (**) or marginally significant (*) SNPs in Choudhury's report.
Genotypic *P*-value was calculated by the chi-square test (a), Fisher's exact test (b) and Cochran–Armitage trend test (c).
Allelic *P*-value was calculated and LD block (Gabriel's criteria) was defined using Haploview software (d).
Haplotypic global *P*-value was calculated using Unphased software (e).
GRR was calculated using Genetic power calculator (f).
Bold numbers represent significant *P*-values (uncorrected).
Corrected *P*-value was calculated by Nyholt's method (g).
Effective number of independent marker loci: 21.38
Experiment-wide significance threshold required to keep Type I error rate at 5%: 0.0023.

Table 2
Linkage disequilibrium analysis of *FXYD6*

No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	rs11216573		0.53	0.66	0.69	0.56	0.67	0.76	0.42	0.43	0.45	0.46	0.43	0.35	0.00	0.01	0.51	0.19	0.08	0.02	0.01	0.07	0.29	0.25
2	rs2282506	0.01		0.96	0.76	1.00	0.64	0.90	0.51	0.55	0.28	0.56	0.21	0.46	0.85	0.78	0.07	0.46	0.12	0.02	1.00	0.04	0.12	0.12
3	rs520333	0.05	0.18		0.96	0.99	0.99	0.97	0.53	0.54	0.21	0.52	0.24	0.35	0.48	0.55	0.12	0.37	0.10	0.45	0.06	0.42	0.01	0.14
4	rs564989	0.34	0.02	0.16		0.97	0.98	0.99	0.35	0.35	0.60	0.33	0.56	0.35	0.02	0.01	0.53	0.24	0.02	0.11	0.09	0.03	0.24	0.17
5	rs10892185	0.05	0.03	0.15	0.22		1.00	0.99	0.87	0.88	0.45	0.79	0.47	0.49	0.11	0.12	0.31	0.14	0.02	0.10	0.05	0.14	0.44	0.18
6	rs496371	0.03	0.01	0.56	0.10	0.09		1.00	0.45	0.48	0.13	0.49	0.25	0.27	0.16	0.29	0.34	0.36	0.25	0.56	0.10	0.53	0.23	0.38
7	rs555577	0.10	0.12	0.68	0.23	0.21	0.42		0.56	0.56	0.28	0.53	0.29	0.21	0.32	0.39	0.01	0.41	0.05	0.10	0.03	0.21	0.14	0.18
8	rs7121573*	0.02	0.06	0.27	0.02	0.11	0.13	0.21		0.98	0.97	0.97	0.97	0.88	0.60	0.60	0.70	0.23	0.16	0.14	0.17	0.19	0.04	0.00
9	rs1815774**	0.02	0.06	0.28	0.02	0.12	0.14	0.22	0.94		1.00	1.00	1.00	0.91	0.63	0.63	0.71	0.24	0.17	0.15	0.18	0.16	0.02	0.01
10	rs11216591	0.03	0.00	0.01	0.07	0.16	0.00	0.01	0.11	0.12		0.89	0.97	0.92	0.97	1.00	0.93	0.31	0.07	0.02	0.16	0.20	0.48	0.43
11	rs11216594	0.03	0.06	0.27	0.02	0.10	0.13	0.21	0.87	0.93	0.10		1.00	0.90	0.65	0.66	0.62	0.25	0.18	0.18	0.16	0.15	0.05	0.01
12	rs876797*	0.02	0.00	0.01	0.05	0.15	0.00	0.01	0.10	0.10	0.85	0.11		0.98	0.82	0.85	0.93	0.32	0.08	0.05	0.14	0.19	0.66	0.50
13	rs10790218*	0.04	0.01	0.03	0.05	0.14	0.01	0.02	0.20	0.22	0.38	0.23	0.38		0.94	0.94	0.79	0.28	0.08	0.03	0.03	0.03	0.43	0.22
14	rs4938445**	0.00	0.01	0.01	0.00	0.00	0.00	0.01	0.02	0.02	0.05	0.02	0.03	0.16		0.97	0.94	0.29	0.11	0.04	0.60	0.10	0.46	0.02
15	rs4938446**	0.00	0.01	0.02	0.00	0.00	0.00	0.01	0.02	0.02	0.05	0.02	0.04	0.16	0.91		0.98	0.33	0.09	0.04	0.57	0.11	0.51	0.00
16	rs7119090	0.05	0.00	0.00	0.07	0.09	0.01	0.00	0.08	0.08	0.60	0.07	0.55	0.39	0.07	0.07		0.33	0.03	0.20	0.07	0.40	0.46	0.40
17	rs11216598	0.03	0.01	0.03	0.06	0.01	0.01	0.04	0.01	0.01	0.02	0.01	0.02	0.03	0.01	0.01	0.03		0.31	0.03	0.02	0.17	0.29	0.18
18	rs631898	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.02		0.28	0.37	0.01	0.04	0.05
19	rs 11605223	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04		0.77	0.72	0.81	0.48
20	rs3809044	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01		1.00	1.00	0.84
21	rs3809043	0.00	0.00	0.01	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.41	0.01		0.93	0.58
22	rs3809042	0.06	0.00	0.00	0.03	0.02	0.00	0.00	0.00	0.00	0.02	0.00	0.04	0.04	0.01	0.01	0.03	0.04	0.00	0.03	0.02	0.03		0.91
23	rs497768**	0.04	0.00	0.00	0.03	0.01	0.02	0.01	0.00	0.00	0.04	0.00	0.05	0.02	0.00	0.00	0.05	0.03	0.00	0.06	0.03	0.07	0.36	

Values shown above the diagonal are D' and values shown below are r^2.

Table 3
Haplotypic analysis of *FXYD6*

No.	SNPID	Haplotypic global *P* value Window size			
		2	3	4	5
1	rs11216573				
2	rs2282506	0.27			
3	rs520333	0.54	0.36		
4	rs564989	0.66	0.35	0.32	
5	rs10892185	0.49	0.63	0.49	0.46
6	rs496371	0.90	0.53	0.67	0.56
7	rs555577	0.09	0.11	0.16	0.21
8	rs7121573*	0.70	0.36	0.40	0.47
9	rs1815774**	0.63	0.56	0.26	0.26
10	rs11216591	0.65	0.66	0.39	0.15
11	rs11216594	0.58	0.44	0.43	0.26
12	rs876797	0.55	0.69	0.66	0.66
13	rs10790218*	0.59	0.75	0.86	0.81
14	rs4938445**	0.32	0.57	0.78	0.79
15	rs4938446**	0.88	0.61	0.56	0.72
16	rs7119090	0.80	0.89	0.64	0.50
17	rs11216598	0.28	0.58	0.27	0.60
18	rs631898	0.97	0.49	0.89	0.62
19	rs 11605223	0.69	0.97	0.69	0.95
20	rs3809044	0.25	0.66	0.88	0.77
21	rs3809043	0.98	0.26	0.86	0.94
22	rs3809042	0.78	0.96	0.93	0.77
23	rs497768**	0.76	0.59	0.67	0.73

Haplotypic global *P* value was calculated using Unphased software.
IDs with asterisk represent significant (**) or marginally significant (*) SNPs in Choudhury's report.

The genotype and allele frequency of each SNP in schizophrenic patients and healthy control subjects are summarized in Table 1. The observed genotype frequency of rs3809043 deviated from HWE. The LD relationships between markers are shown in Table 2. The LD patterns observed in controls were nearly identical to those of the JPT HapMap samples, but obviously different from those of the CEU HapMap samples. The haplotypic analysis is provided in Tables 1 and 3. We found a significant association of two tagging SNPs (rs11216573; genotypic *P* value: 0.022 and rs555577; genotypic *P* value: 0.026, allelic *P* value: 0.011) before correction for multiple testing. To correct for multiple testing, we estimated the effective number of independent SNPs using the method of Nyholt [18]. The number of independent SNPs was estimated at 21.38. Thus the gene-wide corrected *P* value for significance was 0.002338. Nominal *P* values did not survive correction for multiple testing. No association was observed between schizophrenia patients and controls in allelic and genotypic analyses after correction for multiple testing (Table 1). More than 80% power in detecting an associa-

tion with schizophrenia was obtained when the genotype relative risk (GRR) was set at 1.20–1.40 under a multiplicative model of inheritance.

The "common disease-common variant" hypothesis postulates that LD should be detected by the haplotype association test if the risk haplotype is linked to causal variants for disease [4]. Regarding the Japanese population, therefore, the data presented in this article do not provide sufficient evidence for the involvement of *FXYD6* in conferring susceptibility for schizophrenia.

In this study, we found a significant association of two tagging SNPs (rs11216573 and rs555577) before correction for multiple testing. It is important to control for inflation of type I errors due to multiple testing. Bonferroni correction is typically used for solving multiple testing problems; however, because markers are not independent due to the existence of LD, Bonferroni correction is thought to be too conservative. We thus performed multiple testing correction by the spectral decomposition method of Nyholt using SNPSpD software. Nominal *P* values did not survive correction for multiple testing (rs11216573; genotypic *P* value: 0.47 and rs555577; genotypic *P* value: 0.55, allelic *P* value: 0.24, corrected by the spectral decomposition method of Nyholt). The significant association in our results was thought to be a false-positive finding due to multiple testing. Over time, we could not replicate a previous report [5], which revealed a significant association between *FXYD6* and schizophrenia in a Caucasian population. The discrepancy between Japanese and Caucasians may derive from ethnic differences in the etiology of schizophrenia. Although the sample size used here is smaller than the sample from the original study, we obtained more than 80% power in detecting any possible association; thus the possibility of a type II error is less likely. The GRR value predefined in our power analysis was appropriate when compared with Choudhury's report (odds ratio: 1.21–1.64) and other promising candidate genes for schizophrenia [22,23]. We also performed haplotypic analysis excluding rs4938446, because this SNP was not selected by Tagger. However, no significant results were obtained (data not shown).

Two additional limitations need to be addressed to discuss the present results. First, age-unmatched and male-to-female ratio unmatched cohorts were examined in the present study. The mean age of the controls was younger than that of patients. This means that a number of these younger controls, though likely not more than 12 subjects given a lifetime morbidity risk of 1%, may later develop schizophrenia. In order to address this issue, we also included an explorative analysis of gender effect. However, no associations were found in analysis subdivided by gender (supplementary Table 1).

Second, the definition of phenotypes is vital for a genetic association study. Therefore, endophenotypes (being more specific than phenotypes) or symptoms are also thought to be important in this field [3,6]. We did not take advantage of these analyses to test for a genetic association; however, those analyses might be useful in elucidating the potential role of *FXYD6* in schizophrenia. Additional endophenotypic approaches, such as cognitive function assessment, brain imaging, and other phenotypes that reveal *FXYD6* traits would further contribute to our understanding of schizophrenia. In this regard, a recent study reported that *DAOA/G30* influences susceptibility to the symptomatology of psychiatric disorders including schizophrenia and bipolar disorder, but not to the diagnosis itself [27]. The phenotypic definition should be considered in future genetic association studies.

In summary, the findings of this present study suggest that *FXYD6* is unlikely to be related to the development of schizophrenia in a Japanese population. Further replication studies incorporating supplemental populations are required for conclusive results.

Acknowledgments

We sincerely thank the patients and healthy volunteers for their participation in this study. We also thank Dr. R. Ishihara for her technical assistance.

This work was supported in part by research grants from the Ministry of Eductation, Culture, Sports, Science of Japan, and Technology, the Ministry of Health of Japan, Labour and Welfare, Grant-in-Aid for Scientific Research on Pathomechanisms of Brain Disorders from the Ministry of Education, Culture, Sports, Science and Technology of Japan, MEXT ACADEMIC FRONTEIER, the Japan Health Sciences Foundation (Research on Health Sciences focusing on Drug Innovation) and the Core Research for Evolutional Science and Technology.

Competing interests: None.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neulet.2008.04.010.

References

[1] D.J. Balding, A tutorial on statistical methods for population association studies, Nat. Rev. 7 (2006) 781–791.
[2] J.C. Barrett, B. Fry, J. Maller, M.J. Daly, Haploview: analysis and visualization of LD and haplotype maps, Bioinformatics 21 (2005) 263–265.
[3] D.L. Braff, R. Freedman, N.J. Schork, I.I. Gottesman, Deconstructing schizophrenia: an overview of the use of endophenotypes in order to understand a complex disorder, Schizophrenia Bull. 33 (2007) 21–32.
[4] A. Chakravarti, Population genetics—making sense out of sequence, Nat. Genet. 21 (1999) 56–60.
[5] K. Choudhury, A. McQuillin, V. Puri, J. Pimm, S. Datta, S. Thirumalai, R. Krasucki, J. Lawrence, N.J. Bass, D. Quested, C. Crombie, G. Fraser, N. Walker, H. Nadeem, S. Johnson, D. Curtis, D. St Clair, H.M. Gurling, A genetic association study of chromosome 11q22-24 in two different samples implicates the FXYD6 Gene, encoding phosphohippolin, in susceptibility to schizophrenia, Am. J. Hum. Genet. 80 (2007) 664–672.
[6] N. Craddock, M.C. O'Donovan, M.J. Owen, Genes for schizophrenia and bipolar disorder? Implications for psychiatric nosology, Schizophrenia Bull. 32 (2006) 9–16.
[7] P.I. de Bakker, R. Yelensky, I. Pe'er, S.B. Gabriel, M.J. Daly, D. Altshuler, Efficiency and power in genetic association studies, Nat. Genet. 37 (2005) 1217–1223.
[8] B. Delprat, D. Schaer, S. Roy, J. Wang, J.L. Puel, K. Geering, FXYD6 is a novel regulator of Na,K-ATPase expressed in the inner ear, J. Biol. Chem. 282 (2007) 7450–7456.
[9] F. Dudbridge, Pedigree disequilibrium tests for multilocus haplotypes, Genet. Epidemiol. 25 (2003) 115–121.
[10] P.J. Harrison, M.J. Owen, Genes for schizophrenia? Recent findings and their pathophysiological implications, Lancet 361 (2003) 417–419.
[11] K. Kadowaki, K. Sugimoto, F. Yamaguchi, T. Song, Y. Watanabe, K. Singh, M. Tokuda, Phosphohippolin expression in the rat central nervous system, Brain Res. Mol. Brain Res. 125 (2004) 105–112.
[12] C.M. Lewis, D.F. Levinson, L.H. Wise, L.E. DeLisi, R.E. Straub, I. Hovatta, N.M. Williams, S.G. Schwab, A.E. Pulver, S.V. Faraone, L.M. Brzustowicz, C.A. Kaufmann, D.L. Garver, H.M. Gurling, E. Lindholm, H. Coon, H.W. Moises, W. Byerley, S.H. Shaw, A. Mesen, R. Sherrington, F.A. O'Neill, D. Walsh, K.S. Kendler, J. Ekelund, T. Paunio, J. Lonnqvist, L. Peltonen, M.C. O'Donovan, M.J. Owen, D.B. Wildenauer, W. Maier, G. Nestadt, J.L. Blouin, S.E. Antonarakis, B.J. Mowry, J.M. Silverman, R.R. Crowe, C.R. Cloninger, M.T. Tsuang, D. Malaspina, J.M. Harkavy-Friedman, D.M. Svrakic, A.S. Bassett, J. Holcomb, G. Kalsi, A. McQuillin, J. Brynjolfson, T. Sigmundsson, H. Petursson, E. Jazin, T. Zoega, T. Helgason, Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: schizophrenia, Am. J. Hum. Genet. 73 (2003) 34–48.
[13] D.A. Lewis, P. Levitt, Schizophrenia as a disorder of neurodevelopment, Annu. Rev. Neurosci. 25 (2002) 409–432.
[14] M. Maziade, V. Raymond, D. Cliche, J.P. Fournier, C. Caron, Y. Garneau, L. Nicole, P. Marcotte, C. Couture, C. Simard, Linkage results on 11Q21-22 in Eastern Quebec pedigrees densely affected by schizophrenia, Am. J. Med. Genet. 60 (1995) 522–528.
[15] E.K. Nanitsos, K.T. Nguyen, F. St'astny, V.J. Balcar, Glutamatergic hypothesis of schizophrenia: involvement of Na^+/K^+-dependent glutamate transport, J. Biomed. Sci. 12 (2005) 975–984.
[16] S. Nanko, M. Gill, M. Owen, N. Takazawa, J. Moridaira, H. Kazamatsuri, Linkage study of schizophrenia with markers on chromosome 11 in two Japanese pedigrees, Jpn. J. Psychiatry Neurol. 46 (1992) 155–159.
[17] B.M. Neale, P.C. Sham, The future of association studies: gene-based analysis and replication, Am. J. Hum. Genet. 75 (2004) 353–362.

[18] D.R. Nyholt, A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other, Am. J. Hum. Genet. 74 (2004) 765–769.
[19] S. Purcell, S.S. Cherny, P.C. Sham, Genetic power calculator: design of linkage and association genetic mapping studies of complex traits, Bioinformatics 19 (2003) 149–150.
[20] J.L. Rapoport, A.M. Addington, S. Frangou, M.R. Psych, The neurodevelopmental model of schizophrenia: update 2005, Mol. Psychiatry 10 (2005) 434–449.
[21] C.A. Ross, R.L. Margolis, S.A. Reading, M. Pletnikov, J.T. Coyle, Neurobiology of schizophrenia, Neuron 52 (2006) 139–153.
[22] S.G. Schwab, M. Knapp, S. Mondabon, J. Hallmayer, M. Borrmann-Hassenbach, M. Albus, B. Lerer, M. Rietschel, M. Trixler, W. Maier, D.B. Wildenauer, Support for association of schizophrenia with genetic variation in the 6p22.3 gene, dysbindin, in sib-pair families with linkage and in an additional sample of triad families, Am. J. Human Genet. 72 (2003) 185–190.
[23] S. Shifman, M. Bronstein, M. Sternfeld, A. Pisante-Shalom, E. Lev-Lehman, A. Weizman, I. Reznik, B. Spivak, N. Grisaru, L. Karp, R. Schiffer, M. Kotler, R.D. Strous, M. Swartz-Vanetik, H.Y. Knobler, E. Shinar, J.S. Beckmann, B. Yakir, N. Risch, N.B. Zak, A. Darvasi, A highly significant association between a COMT haplotype and schizophrenia, Am. J. Human Genet. 71 (2002) 1296–1302.
[24] Y. Shigeri, R.P. Seal, K. Shimamoto, Molecular pharmacology of glutamate transporters, EAATs and VGLUTs, Brain Res. Brain Res. Rev. 45 (2004) 250–265.
[25] K.J. Sweadner, E. Rael, The FXYD gene family of small ion transport regulators or channels: cDNA sequence, protein signature sequence, and expression, Genomics 68 (2000) 41–56.
[26] J.A. Turner, P. Smyth, F. Macciardi, J.H. Fallon, J.L. Kennedy, S.G. Potkin, Imaging phenotypes and genotypes in schizophrenia, Neuroinformatics 4 (2006) 21–49.
[27] N.M. Williams, E.K. Green, S. Macgregor, S. Dwyer, N. Norton, H. Williams, R. Raybould, D. Grozeva, M. Hamshere, S. Zammit, L. Jones, A. Cardno, G. Kirov, I. Jones, M.C. O'Donovan, M.J. Owen, N. Craddock, Variation at the DAOA/G30 locus influences susceptibility to major mood episodes but not psychosis in schizophrenia and bipolar disorder, Arch. Gen. Psychiatry 63 (2006) 366–373.
[28] F. Yamaguchi, K. Yamaguchi, Y. Tai, K. Sugimoto, M. Tokuda, Molecular cloning and characterization of a novel phospholemman-like protein from rat hippocampus, Brain Res. Mol. Brain Res. 86 (2001) 189–192.