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A novel replicated association between *FXYD6* gene and schizophrenia

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ABSTRACT

FXYD6 gene is located in chromosome region 11q22–q24 where previous studies have shown an association with schizophrenia. However, the subsequent studies failed to replicate this finding. To investigate the relationship between *FXYD6* locus and schizophrenia in Chinese population, we genotyped six single-nucleotide polymorphisms (SNPs) in this region of *FXYD6* in 1142 *Han* Chinese subjects (576 cases and 566 controls), and performed an association analysis. Significant associations with schizophrenia and the marker rs11544201 ($P = 0.0028$) and the haplotype rs10790212–rs11544201 (global $P = 0.005$) were found. Our results support that *FXYD6* is a susceptibility gene of schizophrenia. Replication of larger samples and functional analysis of FXYD6 are needed.

1. Introduction

Schizophrenia (MIM 181500) is a complex disease that has a lifetime risk of approximately 1% and is characterized by delusions, hallucinations, altered cognition, emotional reactivity and disorganized behavior. Genetic factors account for more than 80% of the variance in susceptibility, and risk likely results from multiple loci of small effect [1].

Chromosome region 11q22–q24 has been shown to be one of the most well-established associations with schizophrenia. A rank-based genome scan meta-analysis (GSMA) applied to data from 20 schizophrenia genome scans showed that association was not by chance [2]. Recently, a genetic association study of chromosome 11q22–q24 in two different British samples demonstrated that *FXYD6* (located in chromosome region 11q23) was most likely associated with schizophrenia [3]. From a functional perspective, FXYD6 is a member of the FXYD protein family. All members of this family have been shown to modulate Na, K-ATPase and have long-term physiological importance in maintaining cation homeostasis [4]. More specifically, FXYD6 is a protein phosphohippolin, it modulates the transport properties of Na, K-ATPase in a tissue-specific way and is a novel regulator of Na, K-ATPase [5]. However, the subsequent studies failed to replicate this finding in an Asian population [6,7].

Considering the ethnic difference, we performed a case–control association study to explore the relationship between *FXYD6* and schizophrenia in the *Han* Chinese population.

2. Materials and methods

2.1. Subjects

The case–control sample was composed of 576 individuals diagnosed with schizophrenia (290 males, mean age = 35.3 ± 11.6; 286 females, mean age = 32.7 ± 13.4) and 566 healthy control subjects (308 males, mean age = 29.1 ± 13.6; 258 females, mean age = 29.4 ± 13.4). All patients were diagnosed by the Psychiatry Department of the Affiliated Hospital of Xi'an Jiaotong University School of Medicine according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria for schizophrenia. The diagnosis was checked and verified by two independent senior psychiatrists who reviewed the psychiatric case records. The controls were drawn from local volunteers and blood transfusion donors; subjects with a personal or family history of mental illness were excluded by psychiatric colleagues. All subjects were *Han* Chinese in origin. The study was approved by the local psychiatry research ethics committees and informed consent was obtained from all subjects.

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2.2. Genotyping

We genotyped six SNPs (rs10790212, rs3168238, rs555577, rs1815774, rs4938446 and rs497768) around the *FXYD6* gene locus that were shown to have a significant association with schizophrenia in previous studies [3,6]. Additionally we genotyped SNP rs11544201 which was a non-synonymous polymorphism (G to T) in *FXYD6* leading to a Leu to Met change in amino acid (www.ncbi.nlm.nih.gov) (Fig. 1). SNP rs3168238 was found not to be polymorphic (<5%) and was excluded from the study after being genotyped simultaneously with these SNPs. Genotyping was accomplished by allele-specific PCR, methods have been described elsewhere [8]. PCR primers used in this study were designed by a tetra-primer ARMS-PCR primer design program (http://cedar.genetics.soton.ac.uk/public_html/primer1.html). The primer sequences are listed in Table 1.

2.3. Statistical analysis

Hardy–Weinberg equilibrium (HWE) of all the SNPs was assessed using the software program Finetti (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl). Linkage disequilibrium (LD) was analyzed using the software program Haploview 4.1 [9]. The allele frequencies were analyzed using the software program PLINK version 2.05 [10]. Haplotype frequencies were estimated using the software program PHASE version 2.2 [11]. The distribution of global haplotype frequencies in cases and controls was compared using the software program Epi_Info (http://www.cdc.gov/epiinfo/). Bonferroni corrections were applied to all multiple statistical tests. The software program G*Power program [12] was used to determine statistical power of the case–control sample. Taking into account sample size, the case–control sample had >93% power to detect a significant association ($a < 0.05$), when an effect size index corresponding to a "weak" effect (0.2) was used.

3. Results

All SNPs were highly polymorphic in the case and the control samples. The genotype distribution of SNPs rs10790212, rs11544201, rs555577 and rs497768 were in HWE ($P > 0.05$) in both cases and controls. The genotype distribution of SNPs rs1815774 and rs4938446 departed weakly from HWE ($P = 0.021$ and $P = 0.031$, respectively) in cases.

Allele distribution and single marker analyses of six SNPs are shown in Table 2. The G allele of rs11544201 showed a significant difference ($X^2 = 8.92$, $P = 0.0028$) between cases and controls. Furthermore, the C allele of rs497768 showed a trend of the significant difference ($X^2 = 2.96$, $P = 0.085$). After correction for multiple testing, the difference observed for rs11544201 ($P = 0.017$) remained significant.

Recently, Zou et al. suggested that one should perform a trend test for the data deviating from HWE [13]. Therefore, we reanalyzed our data by using the trend test, and the marker rs11544201 was still found to be associated with schizophrenia ($X_A^2 = 8.72$, $P = 0.0031$). After Bonferroni correction, the difference observed for rs11544201 remained significant ($P = 0.019$).

No LD was observed in the linkage disequilibrium analysis ($D' < 0.8$, data not shown), and the results were consistent with the previous studies [3,6,7].

Haplotypes with a frequency of less than 3%, were excluded from the analysis (Table 3). Haplotype analyses of SNPs rs10790212 and rs11544201 suggested significant associations with schizophrenia in the current sample (global $P = 0.005$). A significant difference was found for the most common haplotype *CG* ($X^2 = 5.34$, $P = 0.021$) which was more prevalent in cases compared to controls (64.6% vs 60.0%). In addition, a strong significant difference was found for the haplotype *CT* ($X^2 = 11.4$, $P = 0.001$) which was less prevalent in cases compared to controls (6.5% vs 10.3%). After Bonferroni corrections (3 haplotypes and 6 allelic comparisons), the differences observed for the *CT* haplotype ($P = 0.009$) remained significant.

The three SNPs rs10790212, rs11544201, and rs555577 were also used in haplotype analysis, and they spanned approximately 24 kb of the *FXYD6* gene (this did not include the 5′ UTR region). In our sample, the global *P* value for the five common haplotypes was 0.042. When haplotypes were compared individually between cases and controls, a significant difference was found for the common haplotype *CTG* ($X^2 = 4.81$, $P = 0.028$), which was less prevalent in cases compared to controls (4.1% vs 6.1%).

4. Discussion

In this study, a case–control analysis found the direction of association rs11544201 and its related haplotype rs10790212–rs11544201, conferred a risk factor for schizophrenia susceptibility. Our analyses indicate an association with a common allele in the studied sample. The results are intriguing because they have not been previously tested in other studies. SNPs rs10790212, rs1815774, rs4938446 and rs497768 in our study were previously tested by Choudhury et al. [3] and the latter three SNPs showed association with schizophrenia. We failed to replicate the positive results of these SNPs in the study, which was not surprising because few association studies in the field of complex disorders can be replicated unequivocally. Although strong association in a particular variant of one gene may be found in one study, a different study may point to another risk allele of the same gene [14]. The difference in results may be due to different LD between different populations or from different allele frequencies. Though discordant, the studies support that *FXYD6* is associated with schizophrenia. The four SNPs rs10790212, rs555577, rs1815774 and rs497768 of our 6 SNPs were tested by Ito et al. in a Japanese sample [6] and their results did not show an association with

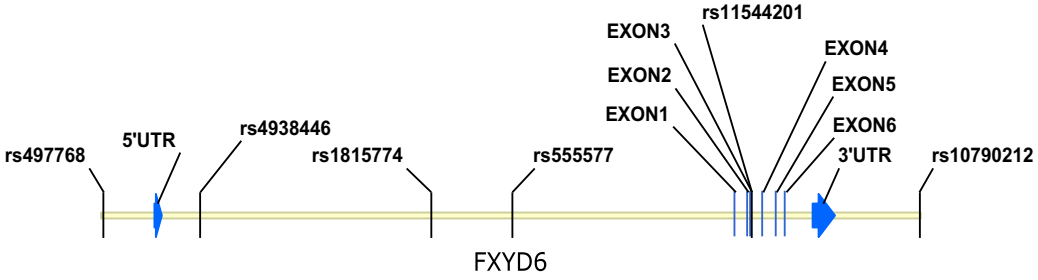

Fig. 1. Organization and position of selected SNPs of *FXYD6* gene.

Table 1
Markers and Primers used for allele-specific PCR.

Marker	Physical location[a]	Primer Sequence		Annealing temperature (°C)
		Forward[b]	Reverse	
rs10790212	117,702,690	5′-AATGGGGTTCTCCAGCTCAGC/T	5′-CAACACCAGGAGGAACTGATCT	56
rs11544201	117,712,569	5′-ACCAGTCCCCCAATCCTTAG/T	5′-TCTCACCCTTGGTTCCTTTG	58
rs555577	117,726,697	5′-CTCTAGGGTGTATTATGAGAACGAATTATA/G	5′-GAAAATTAACATGGAAGTGCTGCTT	58
rs1815774	117,731,439	5′-ATGATCAAGGTCAAGTGAGATAAAAC/G	5′-CTATTTAGAACAACACTTGGTATGGC	56
rs4938446	117,745,049	5′-CCCAAGACGTGTGGCTTCTATGGTAGA/T	5′-GTCTCCCTCCTCACCTTCATGTCCC	56
rs497768	117,750,740	5′-GGTTGGAGAGAGAGGAACGC/G	5′-TTTCCTAGCTCCTTGGCTGA	58

[a] UCSC Browser, Feb 2009; http://genome,ucsc.edu/cgi-bin/hgGateway.
[b] An additional mismatch was deliberately put at position −3 from the 3′ terminus of the allele-specific primer to confer the specificity of PCR amplification.

Table 2
The results of analysis for SNPs in the sample.

Marker	Allele	Allele distribution (%)		X^2	*P* value[b]	X_A^2	*P* value[b]
		Case[a]	Control[a]				
rs10790212	C	71.1 (819)	70.3 (796)	0.17	0.684		
	T	28.9 (333)	29.7 (336)				
rs11544201	G	91.8 (1057)	88.0 (996)	8.92	**0.0028**	8.72	**0.0031**
	T	8.2 (95)	12.0 (136)				
rs555577	G	62.2 (716)	63.0 (713)	0.17	0.681		
	A	37.8 (436)	37.0 (419)				
rs1815774	C	66.5 (766)	65.1 (737)	0.49	0.485		
	G	33.5 (386)	34.9 (395)				
rs4938446	T	80.4 (926)	80.7 (914)	0.05	0.828		
	A	19.6 (226)	19.3 (218)				
rs497768	G	65.9 (759)	69.3 (784)	2.96	0.085		
	C	34.1 (393)	30.7 (348)				

[a] Number of alleles for each SNP is given in parentheses.
[b] Significant *P* value (<0.05) are in boldface.

Table 3
Estimated frequency of haplotype and association significance.

Haplotype[a]	Distribution (%)		X^2	*P* value[b]
	Case[a]	Control[a]		
rs10790212–rs11544201			12.71	**0.005**
CG	64.6	60.0	5.34	**0.021**
CT	6.5	10.3	11.40	**0.001**
TG	27.1	28.0	0.24	0.623
rs10790212–rs11544201–rs555577			11.51	**0.042**
CGG	39.9	37.2	1.81	0.179
CGA	25.0	23.2	0.97	0.324
CTG	4.1	6.1	4.81	**0.028**
TGG	16.9	18.5	0.92	0.336
TGA	10.0	9.1	0.52	0.472

[a] It only lists *P* values of haplotype with a frequency >3%.
[b] Significant *P* values (<0.05) are in boldface.

schizophrenia. Furthermore, Zhang et al. tested the two SNPs rs10790212 and rs497768 of our 6 SNPs in a Chinese sample [7] and they failed to find a positive result. Interestingly, previous studies did not test the marker rs11544201, which is the only non-synonymous (Leu to Met) polymorphism located in exon 3 of *FXYD6* (NM 022003.1).

Although two SNPs (rs1815774 and rs4938446) showed weak deviations from HWE in cases, the significant association between marker rs11544201 and schizophrenia was likely not a false-positive result, because (1) the genotype frequency of rs11544201 was not deviation from HWE; (2) the marker allele frequency showed a significant difference between patients and control subjects ($P = 0.0028$); and (3) rs11544201 still showed significant differences in allele distributions ($P = 0.0031$) after a trend test corrected for the data deviating from HWE [13].

To obtain more statistical evidence for the association, we performed haplotype analysis, which uses additional information on linkage between the markers typed [15]. However, no LD was observed in adjacent SNPs, and this result is consistent with the studies in Asian population [6–7].

Haplotype analysis of the two SNPs rs10790212 and rs1154201 showed significant associations with schizophrenia (global $P = 0.005$). A significant difference were found for the common haplotype *CT* ($P = 0.001$). Haplotype analysis of the three SNPs rs10790212, rs11544201 and rs555577 showed a significant association with schizophrenia too (global $P = 0.042$), and a significant difference was found for the common haplotype *CTG* ($P = 0.028$). Both *CT* and *CTG* were significantly more frequent in controls than in cases. This may be explained by a protective effect of the haplotype [8].

Actually, FXYD6 is a protein phosphohippolin, it modulates the transport properties of Na, K-ATPase in a tissue-specific way and is a novel regulator of Na, K-ATPase [5]. Interestingly, Na, K-ATPases and glutamate (the major excitatory neurotransmitter in the mammalian brain) are part of the same macromolecular complex and operate as a functional unit to regulate glutamatergic neurotransmission [16]. Glutamate is also the neurotransmitter of the pyramidal cells which are located in brain regions associated with the pathophysiology of schizophrenia [17].

In rats, the protein phosphohippolin is expressed in the central nervous system with a unique distribution in the cerebellum. High levels of phosphohippolin were observed in the postnatal 3-week-old rat brain and significant amounts were also observed in the adult brain. This suggests that phosphohippolin may play an important role in the excitability of neurons in the central nervous system during postnatal development and in the adult brain [18]. In humans, the expression of *FXYD6* is also primarily in the brain (GNF Sym-Atlas). Moreover, *FXYD6* is prominently expressed in

regions of the brain that are coincident with brain-imaging abnormalities that have been observed in patients with schizophrenia [19]. In summary, *FXYD6* is a compelling candidate gene for schizophrenia from a developmental and functional perspective.

Although current analyses indicate an association between *FXYD6* gene and schizophrenia, our results should be interpreted with caution for at least three reasons. First, our sample size was relatively small compared to GWAS samples [1]. The statistical power to detect the moderate effect size for complex human diseases, such as schizophrenia, may be weak. Second, our positive study is mainly caused by the SNP rs11544201, therefore the results may not be considered very representative. A third concern is the lack of a clear function of this gene. What effect the non-synonymous mutation has on the FXYD6 protein is unknown. In addition, it has been found that FXYD6 appears to be expressed in excess over Na, K-ATPase in some structures may have other underlying functional roles than Na, K-ATPase regulation [20]. More studies on the function of *FXYD6* will help us to further understand its role in the pathogenic mechanism.

In summary, our results from the case–control analysis demonstrate that the G allele of rs11544201, located in the third exon of *FXYD6*, is a compelling risk factor for schizophrenia in *Han* Chinese, and provide further support for *FXYD6* in the etiology of schizophrenia. Further efforts to replicate this result clarify the transcription profile, and to study the function of *FXYD6* are warranted.

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