PICK1 is not a susceptibility gene for schizophrenia in a Japanese population: association study in a large case-control population

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Abstract

The protein interacting with C-kinase 1 (PICK1) has been implicated in the susceptibility to schizophrenia. PICK1 interacts with enzymes and receptors that play roles in the pathogenesis of schizophrenia via glutamatergic dysfunction. Recently, two studies reported associations between schizophrenia and two *PICK1* gene polymorphisms, rs3952 in Chinese and Japanese populations and rs2076369 in a Japanese population. We attempted to confirm these associations in a case-control study of 1765 Japanese patients with schizophrenia and 1851 Japanese control subjects. Neither polymorphism was associated with schizophrenia (rs3952, $p = 0.755$; rs2076369, $p = 0.997$). A haplotype block with these polymorphisms spanning the 5' region of the *PICK1* gene showed high linkage disequilibrium in the Japanese population ($D' = 0.98$, $r^2 = 0.34$); however, neither haplotype was significantly associated with schizophrenia. We conclude that the common haplotypes and polymorphisms of the *PICK1* gene identified thus far are unlikely to contribute to genetic susceptibility to schizophrenia in the Japanese population.

1. Objective of the study

The protein interacting with C-kinase 1 (PICK1) is also known as protein kinase C-alpha-binding protein (PRKCABP). PICK1 contains a PDZ domain and a conserved arfaptin homology (AH) domain. The PDZ domain of rat Pick1 interacts with splice variants of AMPA receptor subunits (Dev et al., 1999; Lu &Ziff, 2005; Sossa et al., 2006), and PICK1 appears to modulate synaptic transmission by regulating AMPA receptors in brain(Xia et al., 1999). AMPA receptors can improve cognitive function in patients with schizophrenia(Coyle et al., 2002). PICK1 also colocalizes and interacts with dopamine transporter (DAT) (Torres et al., 2001), and metabotropic glutamate receptor 7 (GRM7) (Boudin et al., 2000; Dev et al., 2001; Enz &Croci, 2003). More recently, Fujii *et al*. (2006) found that PICK1 interacts with serine racemase, which is a D-serine synthesizing enzyme that may play a role in the pathogenesis of schizophrenia. Taken together, the functional and structual features of PICK1 suggest that it may play a role in schizophrenia, because glutamatergic and dopaminergic dysfunction are thought to be involved in one of the most important neural pathways underlying vulnerability to the disease.

In situ hybridization analysis revealed that *PICK1* gene expression was decreased in dorsolateral prefrontal cortex (DLPFC) of patients with schizophrenia.(Beneyto &Meador-Woodruff, 2006) In contrast, Dracheva et al. (2005) could not find a significant change in *PICK1* gene expression in DLPFC. Therefore evaluation of possible effects of antipsychotics on *PICK1* expression or a transgenic animal study may be required to understand a role of the gene underlying schizophrenia.

Despite inconclusive results, genetic studies have yielded evidence that *PICK1* plays a role as a susceptibility gene in schizophrenia. *PICK1* contains 13 exons spanning approximately 18 kb and is located on chromosome 22q12.13-q13.2, where linkages for schizophrenia have been suggested (Gill et al., 1996; Kalsi et al., 1995). Further, two case-control studies of single nucleotide polymorphisms (SNPs), rs3952 and rs2076369 in *PICK1* indicated an association between *PICK1* and schizophrenia in Asian populations (Fujii et al., 2006; Hong et al., 2004). These SNPs are located in the 5' region (introns 3 and 4) of *PICK1* with 7054 bp distance in one haplotype block that covers most *PICK1* of the region both in Asian and European populations. There are less informative markers in the 3' region of *PICK1*, according to the HapMap database (http://www.hapmap.org/). The haplotype block contains the region encoding the functionally important PDZ domain of PICK1. In addition, because the haplotype block might contain the promoter region of *PICK1*, the association may explain reported differences in *PICK1* expression in schizophrenia (Beneyto &Meador-Woodruff, 2006).

The previously reported association studies composed 400 Japanese and 485 Chinese subjects. It should be noted that the minor allele of rs3952 was different one between those two populations. The NCBI database showed a similar genotype distribution of rs3952 in Japanese and European populations but provides no information for Chinese population. Larger population studies may solve a stratification problem in general Asian population. Because the most straightforward way to evaluate a genetic association is to perform analyses with sufficient statistical power to reveal the association, we attempted to confirm the association between *PICK1* and schizophrenia in a large Japanese population in the present study.

2. Materials and Methods

2.1. Subjects

All subjects were of Japanese descent and were recruited from the main island of Japan. A total of 1765 unrelated patients with schizophrenia (mean age ± SD, 49.0 ± 14.3 years; 944 men and 821 women) were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). Control subjects were 1851 mentally healthy unrelated subjects (mean age ± SD, 48.9 ± 14.5 years; 1020 men and 831 women) with no self-reported family history of mental illness within second-degree relatives. The present study was approved by the Ethics Committees of the University of Tsukuba, Niigata University, Fujita Health University, Nagoya University, Okayama University and Teikyo University, and all participants provided written informed consent.

2.2. Genotyping

DNA was extracted from blood samples. We genotyped two SNP markers, rs3952 in intron 3 and rs2076269 in intron 4, in *PICK1*. The SNPs were genotyped by TaqMan assay. Predesigned TaqMan SNP genotyping assays, C_2487464_10 for rs3952 and C_2487476_10 for rs2076369, were selected from the Applied Biosystems database (http://www.appliedbiosystems.com). The TaqMan reaction was performed in a final volume of 3 μl consisting of 2.5 ng genomic DNA and Universal Master Mix (Eurogentic, Seraing, Belgium), and genotying was performed with an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA).

2.3. Statistical analysis

Hardy-Weinberg equilibrium, linkage disequilibrium and allelic/haplotype frequencies, as well as an association between SNP or haplotype and schizophrenia, were evaluated with the Haploview software program (http://www.broad.mit.edu/mpg/haploview/). Permutation tests were also performed to calculate corrected *p* values for multiple testing with the Haploview software. Genotype-based association was tested with Cochran-Armitage test for trends. Statistical significance was accepted at $p < 0.05$.

3. Results

Genotypic/allelic distributions of the two *PICK1* SNPs among the subject groups are shown in Table 1 and Table 2. Distributions of the rs3952 and rs2076369 SNPs did not differ from Hardy-Weinberg equilibrium ($p = 0.15$ and 0.11, respectively). No genotype/allelic association with schizophrenia was detected for rs3952 ($p = 0.54$/$p = 0.48$ [corrected by multiple comparison: 0.76]), and rs2076369 ($p = 0.91$/$p = 0.90$ [corrected by multiple comparison: 1.00]). With respect to schizophrenia subtype, no association was found between either population with organized or disorganized schizophrenia *vs.* controls (Table 3). These SNPs were in linkage disequilibrium from each other in both groups of controls ($D' = 0.98$, $r^2 = 0.34$) and of the patients ($D' = 0.99$, $r^2 = 0.34$). Haplotype frequencies did not differ significantly between the schizophrenia and control groups (Table 4).

4. Discussion

The SNPs that have been reported to be associated with schizophrenia are included in one haplotype block that appears to have three major haplotypes in our Japanese population. A significant difference in the rs3952 genotype distribution between Japanese and Chinese populations have been observed repeatedly. However, the haplotype block extends from the 5' region towards most genetic region of the *PICK1* gene in Japanese and Caucasian populations according to the HapMap database (http://www.hapmap.org/cgi-perl/gbrowse/hapmap_B35/), although there are less informative markers with informative minor allele frequencies in the 3' region of *PICK1*. The results of the present study clearly exclude the 5' region of the *PICK1* gene as a factor contributing to susceptibility to schizophrenia. As noted, the haplotype block encodes the PDZ domain of PICK1, which interacts with several glutamatergic and dopaminergic molecules that have been suggested to play important roles in vulnerability to schizophrenia. Although only two markers were used in the present study, we could exclude the possibility genetic variation(s) in the 5' flanking region of *PICK1* involved in the strong LD haplotype block. However, because no study analyzed other markers in the *PICK1* gene, there still be a possibility of involvement of the 3' region of *PICK1* in pathogenesis of schizophrenia.

We calculated the power of our study on the basis of the genotype data. With a relative genetic risk of 1.2 and assuming an α value of 0.05 and a risk allele frequency of 0.4, the sample size of the present study had a power of 0.97. Therefore, the present data strongly indicate that an association between the rs3952 and rs2076369 and schizophrenia is not likely in the Japanese population. Although the Chinese patients with schizophrenia were limited of early 20s age-onset, general population of patients with schizophrenia has similar age-onset. Thus, age-onset distribution did not differ significantly between the present study and the previous of a Chinese population that reported an association between rs3952 and schizophrenia (Hong et al., 2004).

One limitation of this study is that we could examine an association between generalized schizophrenia and *PICK1*, but the number of samples for specific types of schizophrenia was limited (disorganized type, $n = 233$, Non-disorganized type, $n = 217$). Also, in a previous study of Japanese population, the association between the *PICK1* gene and schizophrenia was observed mainly for disorganized-type schizophrenia (rs3952, $p = 0.05$; rs2076369, $p = 0.012$; rs3952/rs2076369 haplotype, global $p = 0.011$, AT, $p = 0.015$, GG, $p = 0.05$), whereas no association was observed for organized-type schizophrenia (Fujii et al., 2006) (Tables 3 and 4). Because the minor allele frequency of the rs3952 SNP in our schizophrenic patients was similar to that of the total schizophrenic population in the previous study (Fujii et al., 2006), it is unlikely that most of our patients had organized-type schizophrenia. An observed difference in the allelic distribution of the rs2076369 SNP in schizophrenic patients between our subjects and those of Fujii et al. (2006) (Table 3) cannot be explained by a difference in the distribution of schizophrenia subtypes in these sample populations. In conclusion, the rs3952 and rs2076369 SNPs in the *PICK1* gene do not appear to play major roles in vulnerability to schizophrenia.

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Table 1. Distribution of the polymorphisms in the *PICK1* gene.

SNP marker	Controls				Schizophrenics			*p* value
rs3952	AA	AG	GG		AA	AG	GG	
(*n* = 1850)	46.3%	42.0%	11.7%	(*n* = 1765)	44.6%	43.8%	11.6%	0.299
rs2076369	TT	GT	GG		GG	GA	AA	
(*n* = 1851)	18.0%	47.4%	34.5%	(*n* = 1765)	17.7%	47.4%	34.9%	0.748

Table 2. Minor allele frequency of the polymorphisms in the *PICK1* gene.

SNP marker	Controls	Schizophrenics	χ^2	*p* value
rs3952				
Present study	32.7% (*n* = 1850)	33.9% (*n* = 1765)	1.08	0.299
Hong et al. (2004)	42.5% (*n* = 260)	35.3% (*n* = 255)	5.20	0.025
Fujii et al. (2006)	36.2% (*n* = 200)	32.0% (*n* = 200)	1.61	0.493
rs2076369				
Present study	41.8% (*n* = 1851)	41.4% (*n* = 1765)	0.10	0.748
Fujii et al. (2006)	38.2% (*n* = 200)	46.2% (*n* = 200)	5.25	0.080

* Minor allele is different in Chinese population according to Hong et al. (2004).

Table 3. Minor allele frequency of the polymorphisms in the PICK1 gene.

SNP marker	Controls		Schizophrenics *Disorganized*	*Non-disorganized*
rs3952				
Present study	32.7% (n = 1765)		32.8% (n = 238)	32.8% (n = 276)
		$\chi 2$	0.001	0.002
		p value	0.97	0.96
Fujii et al. (2006)	36.2% (n = 200)		26.1% (n = 90)	36.8% (n = 110)
		$\chi 2$	5.76	0.02
		p value	0.05	1.00
rs2076369				
Present study	41.8% (n = 1765)		40.3% (n = 237)	41.5% (n = 277)
		$\chi 2$	0.35	0.01
		p value	0.55	0.93
Fujii et al. (2006)	38.2% (n = 200)		48.9% (n = 90)	42.3% (n = 110)
		$\chi 2$	8.42	0.96
		p value	0.01	0.63

Table 3. Minor allele frequency of the polymorphisms in the PICK1 gene.

SNP marker	Controls		Schizophrenics	
			Disorganized	*Non-disorganized*
rs3952				
Present study	32.7%		32.8%	32.8%
	(n = 1765)		(n = 238)	(n = 276)
		$\chi 2$	0.001	0.002
		p value	0.97	0.96
Fujii et al. (2006)	36.2%		26.1%	36.8%
	(n = 200)		(n = 90)	(n = 110)
		$\chi 2$	5.76	0.02
		p value	0.05	1.00
rs2076369				
Present study	41.8%		40.3%	41.5%
	(n = 1765)		(n = 237)	(n = 277)
		$\chi 2$	0.35	0.01
		p value	0.55	0.93
Fujii et al. (2006)	38.2%		48.9%	42.3%
	(n = 200)		(n = 90)	(n = 110)
		$\chi 2$	8.42	0.96
		p value	0.01	0.63

Table 4. Haplotype frequencies of the *PICK1* gene

	Present study				Fujii et al. (2006)			
Haplotype	Control	Schizophrenics	χ^2	*p* value	Control	Schizophrenics	χ^2	*p* value
AT	0.416	0.412	0.15	0.695 (0.932)	0.382	0.460 (0.511)	4.93 (8.42)	0.062 (0.015)
GG	0.326	0.336	0.80	0.373 (0.650)	NA	NA		NS
AG	0.257	0.250	0.41	0.523 (0.817)	NA	NA		NS

Haplotype frequencies were compared between controls and schizophrenics; χ^2 and nominal (corrected) *p* values are shown.
Numbers and numbers in parantheses for Fujii et al. (2006) indicate the haplotype frequency and statistics, respectively, in patients with disorganiz
NS, not significant.

ed-type only.