

Association study between polymorphisms of the *PARD3* gene and schizophrenia

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Abstract. The aim of this study was to investigate whether par-3 partitioning defective 3 homolog (*C. elegans*) (*PARD3*) single nucleotide polymorphisms (SNPs) are associated with schizophrenia. A total of 204 Korean schizophrenic patients [117 male, 41.1±9.6 years (mean age ± SD); 87 female, 42.6±11.5] and 351 control subjects (170 male, 43.8±6.6 years; 181 female, 44.2±5.8) were enrolled. We genotyped nine SNPs of the *PARD3* gene [rs7075263 (intron), rs10827392 (intron), rs773970 (intron), rs2252655 (intron), rs10763984 (intron), rs3781128 (Ser889Ser), rs1936429 (intron), rs671228 (intron) and rs16935163 (intron)]. Genotypes of *PARD3* polymorphisms were evaluated by direct sequencing. We used SNPStats, SPSS 18.0 and Haploview 4.2 software for analysis of genetic data. Multiple logistic regression models were used to calculate the odds ratio (OR), 95% confidence interval (CI), and corresponding p-values (p), controlling for age and gender as covariables. Allele frequencies of the *PARD3* SNPs were significantly associated with schizophrenia (rs3781128, p=0.041; rs1936429, p=0.030; rs671228, p=0.028). Certain genotype frequencies of the *PARD3* SNPs also showed significant associations with schizophrenia (p<0.05, rs7075263, rs773970, rs2252655, rs10763984, rs3781128, rs1936429, rs16935163). To the best of our knowledge, this is the first report showing that *PARD3* is associated with susceptibility to schizophrenia in a Korean population. In conclusion, our findings suggest that *PARD3* may contribute to genetic susceptibility to schizophrenia.

Introduction

Schizophrenia is a severe, debilitating, psychiatric disorder. Although the exact etiology of schizophrenia is unknown, twin, family and adoption studies have provided consistent evidence that genetic factors play a major role in the pathogenesis of schizophrenia.

In a recent study, the data showed that polymorphisms of several genes affect gene expression or the function of the encoded protein in the human brain (1). Clinical, epidemiological, neuroimaging and postmortem data suggest that schizophrenia is a neurodevelopmental disorder, and that synaptic disturbances may play a critical role in the development of the disease (2). In addition, it is hypothesized that a loss of function of glial gap junctions may cause severe cognitive impairment in schizophrenia. Thus, several gap junction proteins may be capable of differentiating between the operation qualities of the cognate synapses defined by the neurotransmitter types (3). The major component for barrier-forming tight junctions may play a crucial role in response to the changing natural, physiological and pathological conditions of schizophrenia (4).

In this study, we investigated whether par-3 partitioning defective 3 homolog (*C. elegans*) (*PARD3*) is associated with schizophrenia in a Korean population.

Subjects and methods

Subjects. All subjects were ethnically Korean and unrelated to each other. A total of 204 Korean schizophrenic patients [117 male, 41.1±9.6 years (mean age ± SD); 87 female, 42.6±11.5] and 351 control subjects (170 male, 43.8±6.6 years; 181 female, 44.2±5.8) were enrolled in the study. All patients were diagnosed with schizophrenia by three psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders (4th edition) using all available information from interviews and clinical records. All patients were assessed using the Operational Criteria Checklist for Psychotic Illness (OPCRIT). The OPCRIT was designed as a best estimate diagnostic procedure, in which symptom and course features are coded by an experienced clinician, allowing integration of the relative prominence of clinical features over the entire course of the illness. The control subjects were recruited

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after they had been assessed as mentally healthy in a general health examination program. Written informed consent was obtained from all subjects involved in the study, and the study was approved by the Ethics Review Committee of the Medical Research Institute, Kyung Hee University Medical Center, Seoul, Republic of Korea. All studies were carried out according to the guidelines of the Declaration of Helsinki.

SNP selection and genotyping. We searched the SNPs of the *PARD3* gene in the SNP database of the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/SNP>, BUILD 132). We selected nine SNPs [rs7075263 (intron), rs10827392 (intron), rs773970 (intron), rs2252655 (intron), rs10763984 (intron), rs3781128 (Ser889Ser), rs1936429 (intron), rs671228 (intron) and rs16935163 (intron)] in the *PARD3* gene. SNP genotyping was conducted using direct sequencing. For direct sequencing, genomic DNA was amplified using polymerase chain reaction (PCR). The PCR products were sequenced by an ABI PRISM 3730XL analyzer (PE Applied Biosystems, Foster City, CA, USA), and sequence data were analyzed using SeqManII software (DNASTAR Inc., Madison, WI, USA).

Statistical analysis. The Chi-square test was used to assess Hardy-Weinberg equilibrium (HWE). Genetic data were analyzed using SNPStats (<http://bioinfo.iconcologia.net/index.php?module=Snpstats>), HelixTree (Golden Helix Inc., Bozeman, MT, USA) and SPSS 18.0 (SPSS Inc., Chicago, IL, USA). The analysis of SNPs was performed using multiple logistic models (codominant 1, codominant 2, dominant, recessive and log-additive models). The Haploview version 4.2 (5) was used for linkage disequilibrium (LD) and haplotype analysis between the nine SNPs.

Results

We selected nine SNPs of *PARD3*. The position of the *PARD3* SNPs is illustrated in Fig. 1A. Table I documents the allele frequencies of the nine examined SNPs in schizophrenia patients and control subjects. The HWEs of nine SNPs revealed no deviation in the control groups, respectively ($p > 0.05$, data not shown). Multiple logistic regression analysis with adjustment for age and gender was performed. Three SNPs were associated with schizophrenia (rs3781128, OR=0.76, 95% CI=0.59-0.99, $p=0.041$; rs1936429, OR=0.75, 95% CI=0.58-0.97, $p=0.030$; rs16935163, OR=1.69, 95% CI=1.06-2.68, $p=0.028$, respectively, Table I). These results suggest that the C allele of rs3781128, the A allele of rs1936429 and the C allele of rs16935163 may be risk factors for schizophrenia.

Table II shows genotype frequencies of the nine examined SNPs in schizophrenia patients and control subjects. The seven SNPs among the *PARD3* polymorphisms showed significant associations with schizophrenia [rs7075263, OR=6.00, 95% CI=1.21-26.98, $p=0.028$ in codominant 2 (T/T versus T/T), OR=6.06, 95% CI=1.23-29.87, $p=0.014$ in recessive (T/T+C/T versus C/C); rs773970, OR=0.52, 95% CI=0.30-0.89, $p=0.014$ in recessive (G/G+A/G versus A/A); rs2252655, OR=0.58, 95% CI=0.37-0.91, $p=0.016$ in recessive (A/A+A/G versus G/G); rs10763984, OR=0.53, 95% CI=0.34-0.84, $p=0.005$ in recessive (C/C+C/T versus T/T); rs3781128, OR=0.65, 95%

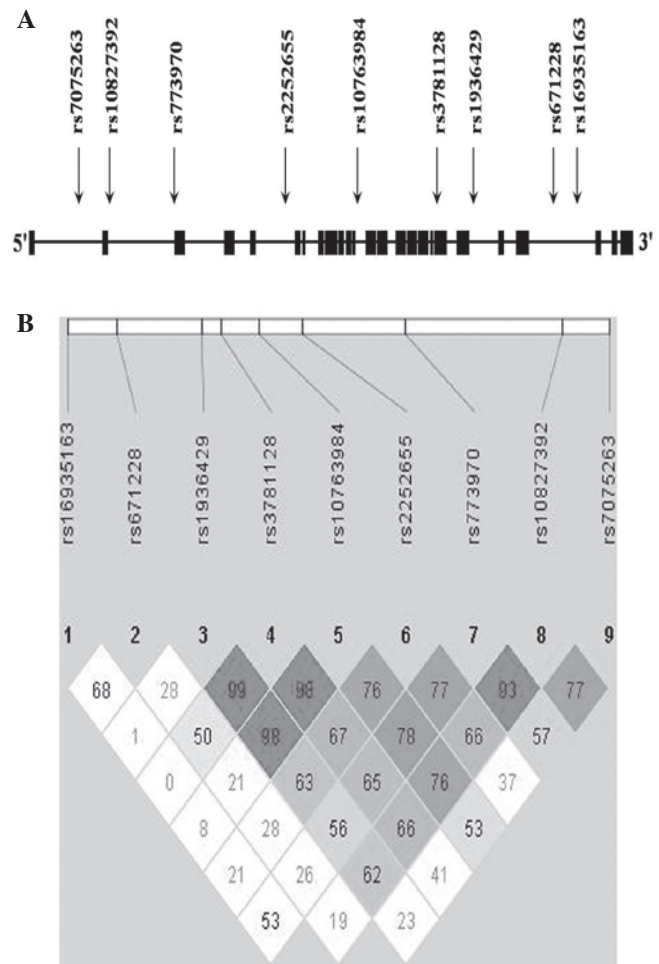


Figure 1. Gene map and linkage disequilibrium (LD) in the *PARD3* gene. (A) Gene map and single nucleotide polymorphisms (SNPs) in the *PARD3* gene on chromosome 10. Exons are marked with a box. The coding regions are black boxes. Arrow indicates the location of each SNP. (B) LD blocks among *PARD3* SNPs.

CI=0.45-0.95, $p=0.026$ in codominant 1 (C/C versus C/T), OR=0.66, 95% CI=0.46-0.94, $p=0.021$ in dominant (C/C versus C/T+T/T); rs1936429, OR=0.57, 95% CI=0.32-0.99, $p=0.047$ in codominant 2 (A/A versus G/G), OR=0.69, 95% CI=0.47-0.99, $p=0.045$ in dominant (A/A versus A/G+G/G), OR=0.75, 95% CI=0.57-0.97, $p=0.028$ in log-additive (A/A versus A/G versus G/G); and rs16935163, OR=1.68, 95% CI=1.02-2.76, $p=0.044$ in dominant (C/C versus A/C+A/A), OR=1.74, 95% CI=1.08-2.81, $p=0.024$ in log-additive (C/C versus A/C versus A/A) models, respectively]. The rs10827392 and rs671228 SNPs had no differences between the schizophrenia and control groups.

The LD block was estimated using Haploview version 4.2. The LD block did not be made among SNPs of *PARD3* (Fig. 1B, data not shown).

Discussion

In this study, we found that *PARD3* gene polymorphisms were associated with schizophrenia. The allele distributions of three SNPs (rs3781128, rs1936429 and rs16935163) and genotype frequencies of seven SNPs (rs7075263, rs773970,

Table I. Allele frequencies of polymorphisms of the *PARD3* gene in schizophrenia patients and control subjects.

SNP	Allele	Control		Schizophrenia		OR (95% CI)	P-value
		n	%	n	%		
rs7075263	T	623	88.7	354	86.8	1	
Intron	C	79	11.3	54	13.2	1.20 (0.83-1.74)	0.33
rs10827392	T	631	89.9	358	87.7	1	
Intron	C	71	10.1	50	12.3	1.24 (0.85-1.82)	0.27
rs773970	G	385	57.3	227	59.1	1	
Intron	A	287	42.7	157	40.9	0.93 (0.72-1.20)	0.56
rs2252655	A	343	50.7	216	53.5	1	
Intron	G	333	49.3	188	46.5	0.90 (0.70-1.15)	0.38
rs10763984	C	348	49.6	221	54.4	1	
Intron	T	354	50.4	185	45.6	0.82 (0.64-1.05)	0.12
rs3781128	C	435	62.5	272	68.7	1	
Ser889Ser	T	261	37.5	124	31.3	0.76 (0.59-0.99)	0.041
rs1936429	A	394	57.9	256	64.6	1	
Intron	G	286	42.1	140	35.4	0.75 (0.58-0.97)	0.030
rs671228	C	565	80.5	330	81.7	1	
Intron	T	137	19.5	74	18.3	0.93 (0.68-1.27)	0.63
rs16935163	C	658	94.3	361	90.7	1	
Intron	A	40	5.7	37	9.3	1.69 (1.06-2.68)	0.028

P-values were calculated from logistic regression analyses. Bold numbers indicate significant associations. SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; n, number of subjects.

rs2252655, rs10763984, rs3781128, rs1936429 and rs16935163) were significantly different between schizophrenia patients and control subjects.

PAR-3 is a scaffold-like PDZ-containing protein. PAR-3 forms a complex with PAR-6 and atypical protein kinase C (PAR-3-atypical protein kinase C-PAR-6 complex) and is associated with the establishment of cell polarization (6,7). Cell polarity has an important role in various cellular processes such as cell migration, asymmetric cell division, and axon and dendrite specification.

Chan *et al* reported that PAR-3 is directly associated and recruits the p75 neurotrophin receptor to the axon glial junction, forming a complex necessary for myelination (8). It was found that depletion of PAR-3 in mammalian epithelial cells profoundly disrupts tight junction assembly (9). Synapses of the junctions between nerve cells, through which the cells communicate, are formed by the coordinated assembly and tight attachment of presynaptic and postsynaptic specializations. Therefore, tight junction protein-related studies suggest that junctional protein polymorphisms are sufficient for a functional postsynaptic response change (10-12).

Tight junctions play a major role in cell polarity in vertebrate endothelial and epithelial cells. A protein complex consisting of the cell polarity proteins PAR-3, PAR-6 and the atypical protein kinase C localizes at tight junctions. It is crucial for tight junction formation. It has been shown that PAR-3 associates with

the junctional adhesion molecule (JAM). This suggests that the ternary complex is targeted to tight junctions of epithelial cells through PAR-3 binding to JAM (13).

In conclusion, this is the first report showing that *PARD3* is associated with the susceptibility to schizophrenia in a Korean population. Our results suggest that *PARD3* may contribute to a genetic susceptibility to schizophrenia.

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Table II. Genotype frequencies of polymorphisms of the *PAR3* gene in schizophrenia patients and control subjects.

SNP	Genotype	Control		Schizophrenia		Model	OR (95% CI)	P-value
		n	%	n	%			
rs7075263 intron	T/T	274	78.1	156	76.8	Codominant 1	0.96 (0.62-1.48)	0.85
	C/T	75	21.4	40	19.7	Codominant 2	6.00 (1.21-29.68)	0.028
	C/C	2	0.6	7	3.5	Dominant	1.09 (0.72-1.66)	0.68
						Recessive	6.06 (1.23-29.87)	0.014
						Log-additive	1.22 (0.84-1.77)	0.30
rs10827392 intron	T/T	281	80.1	157	77.3	Codominant 1	1.11 (0.72-1.72)	0.63
	C/T	69	19.7	42	20.7	Codominant 2	7.50 (0.82-68.89)	0.08
	C/C	1	0.3	4	2.0	Dominant	1.20 (0.78-1.84)	0.40
						Recessive	7.32 (0.80-67.16)	0.044
						Log-additive	1.28 (0.86-1.91)	0.22
rs773970 intron	G/G	108	32.1	55	28.8	Codominant 1	1.36 (0.91-2.04)	0.14
	A/G	169	50.3	116	60.7	Codominant 2	0.63 (0.34-1.16)	0.14
	A/A	59	17.6	20	10.5	Dominant	1.16 (0.79-1.72)	0.44
						Recessive	0.52 (0.30-0.89)	0.014
						Log-additive	0.90 (0.68-1.18)	0.44
rs2252655 intron	A/A	88	26.0	47	23.4	Codominant 1	1.34 (0.87-2.07)	0.18
	A/G	167	49.4	121	60.2	Codominant 2	0.71 (0.41-1.22)	0.22
	G/G	83	24.6	33	16.4	Dominant	1.13 (0.75-1.71)	0.56
						Recessive	0.58 (0.37-0.91)	0.016
						Log-additive	0.87 (0.67-1.13)	0.29
rs10763984 intron	C/C	87	24.8	49	24.3	Codominant 1	1.21 (0.79-1.86)	0.37
	C/T	174	49.6	122	60.4	Codominant 2	0.61 (0.35-1.04)	0.07
	T/T	90	25.6	31	15.3	Dominant	1.01 (0.67-1.52)	0.97
						Recessive	0.53 (0.34-0.84)	0.005
						Log-additive	0.80 (0.62-1.04)	0.10
rs3781128 Ser889Ser	C/C	130	37.4	94	47.7	Codominant 1	0.65 (0.45-0.95)	0.026
	C/T	175	50.3	82	41.6	Codominant 2	0.68 (0.37-1.22)	0.19
	T/T	43	12.4	21	10.7	Dominant	0.66 (0.46-0.94)	0.021
						Recessive	0.84 (0.48-1.48)	0.55
						Log-additive	0.76 (0.58-1.00)	0.045
rs1936429 intron	A/A	113	33.2	81	41.1	Codominant 1	0.73 (0.49-1.07)	0.11
	A/G	168	49.4	92	46.7	Codominant 2	0.57 (0.32-0.99)	0.047
	G/G	59	17.4	24	12.2	Dominant	0.69 (0.47-0.99)	0.045
						Recessive	0.68 (0.41-1.14)	0.14
						Log-additive	0.75 (0.57-0.97)	0.028
rs671228 intron	C/C	225	64.1	135	67.2	Codominant 1	0.83 (0.56-1.22)	0.35
	C/T	115	32.8	58	28.9	Codominant 2	1.17 (0.45-3.00)	0.75
	T/T	11	3.1	8	4.0	Dominant	0.86 (0.59-1.25)	0.43
						Recessive	1.24 (0.48-3.16)	0.66
						Log-additive	0.92 (0.67-1.26)	0.59
rs16935163 intron	C/C	309	88.5	163	82.3	Codominant 1	1.58 (0.95-2.62)	0.08
	A/C	40	11.5	33	16.7	Codominant 2	NA (0.00-NA)	NA
	A/A	0	0.0	2	1.0	Dominant	1.68 (1.02-2.76)	0.044
						Recessive	NA (0.00-NA)	0.040
						Log-additive	1.74 (1.08-2.81)	0.024

P-values were calculated from logistic regression analyses. Bold numbers indicated significant associations. SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; n, number of subjects; NA, not applicable.

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