

Association between promoter polymorphisms of the LIFR gene and schizophrenia with persecutory delusion in a Korean population

WON SUB KANG^{1*}, SU KANG KIM^{2*}, JIN KYUNG PARK¹, AH RANG CHO¹,
HAE JEONG PARK², JOO-HO CHUNG² and JONG WOO KIM¹

¹Department of Neuropsychiatry, School of Medicine, Kyung Hee University, Seoul 130-702;

²Kohwang Medical Research Institute, School of Medicine, Kyung Hee University,
Seoul 130-701, Republic of Korea

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Abstract. Schizophrenia has been associated with an imbalance in inflammatory cytokines. Leukemia inhibitory factor receptor α (LIFR) is an integral component of the glycoprotein 130-LIFR signaling complex, which participates in signal transduction by members of the interleukin (IL)-6 cytokine family. The aim of this study was to investigate whether genetic polymorphisms of the LIFR gene are associated with schizophrenia in the Korean population. We also explored possible associations between the polymorphisms and the clinical symptoms of schizophrenia. We selected three single nucleotide polymorphisms (SNPs) (rs2071237 -200C/G, rs2071236 -254C/T and rs6862038 -962A/G) on the promoter region of the LIFR gene and recruited 204 schizophrenia patients and 367 control subjects from the Korean population. All patients were evaluated according to the Operational Criteria Checklist for Psychotic Illness. SNP genotyping was performed by direct sequencing. An analysis of multiple logistic regression models (co-dominant 1, co-dominant 2, dominant, recessive and overdominant) was performed to evaluate odds ratios (ORs), 95% confidence intervals (CIs) and p-values. No significant difference was revealed between schizophrenia patients

and controls. However, a significant association was detected between the LIFR gene polymorphisms and schizophrenia patients with persecutory delusion (rs2071236, OR=2.18, 95% CI=1.20-3.94, P=0.009 in the overdominant model; rs6862038, OR=2.08, 95% CI=1.16-3.74, P=0.013 in the overdominant model). The present study suggests that the LIFR gene may be related to schizophrenia with persecutory delusion in the Korean population.

Introduction

Accumulating evidence indicates that schizophrenia is associated with an imbalance in T helper-1/T helper-2 (Th1/Th2) cytokines, with a shift towards the Th2 system (1), and that cytokines contribute to the pathogenesis of schizophrenia (2,3). Indeed, previous studies have revealed that the levels of interleukin-1 receptor antagonist (IL-1RA), soluble interleukin-2 receptor (IL-2R) and interleukin-6 (IL-6) were increased, and that the level of IL-2 secretion was decreased in schizophrenia patients (4). In addition, genetic studies revealed the association of polymorphisms and/or haplotypes of cytokine genes including IL-10 (5,6), IL-6 (6) and the IL-1 gene complex (IL-1 α , IL-1 β and IL-1RA) with schizophrenia (7).

Leukemia inhibitory factor (LIF) is a member of the IL-6 cytokine family comprising IL-6, oncostatin M, IL-11, ciliary neurotropic factor and cardiotropin-1; it signals via binding to a heterodimeric glycoprotein 130 (gp130)/LIF receptor (LIFR) complex (8). In the central nerve system, LIF was found to regulate the neuronal phenotype and coordinate astrocyte, oligodendrocyte, microglia and inflammatory cell responses through binding with the gp130/LIFR complex (8-10). In a previous study, single nucleotide polymorphisms (SNPs) and haplotypes of the LIF gene were found to be associated with schizophrenia and with clinical phenotypes of schizophrenia, suggesting the LIF gene as a candidate gene of schizophrenia (11). Moreover, in an experimental study, LIF-knockout mice displayed abnormal prepulse inhibition in the acoustic startle test during and following adolescence, which was considered as one of the intermediate phenotypes observed in schizophrenia patients (12).

Correspondence to: Dr Jong Woo Kim, Department of Neuropsychiatry, School of Medicine, Kyung Hee University, 1 Hoegi-dong, Dongdaemun-gu, Seoul 130-702, Republic of Korea
E-mail: psyjong@gmail.com

*Contributed equally

Abbreviations: LIFR, leukemia inhibitory factor receptor α ; LIF, leukemia inhibitory factor; IL, interleukin; gp130, glycoprotein 130; OPCRIT, Operation Criteria Checklist for Psychotic Illness; SNP, single nucleotide polymorphism; HWE, Hardy-Weinberg equilibrium; LD, linkage disequilibrium

Key words: leukemia inhibitory factor receptor α , cytokine, persecutory delusion, schizophrenia, single nucleotide polymorphism

In light of these findings, we speculated that LIF signaling may play a role in the pathophysiology of schizophrenia. In particular, considering that LIF signaling is mainly mediated through its receptor, LIFR may be related to schizophrenia. The LIFR gene is structured by six different domains encoding for a 1,097-amino acid transmembrane protein. The LIFR gene maps to the short arm of chromosome 5 in the p13-12 region (13). In this study, we investigated the genetic associations between schizophrenia and the LIFR gene in the Korean population through an analysis of the promoter SNPs of the LIFR gene.

Materials and methods

Subjects and clinical assessment. All participants were ethnically Korean and unrelated to each other. A total of 204 Korean schizophrenia patients [mean age \pm standard deviation (SD), 41.8 \pm 10.8 years] and 367 healthy control individuals (44.3 \pm 6.3 years) were evaluated. All patients were diagnosed with schizophrenia by well-trained psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders (4th edition) and were assessed according to the Operational Criteria (OPCRIT) checklist (14). The core and most common symptoms of schizophrenia are hallucinations and delusions. The most common delusions in schizophrenia patients are persecutory delusions, which are experienced by up to 80% of inpatients. Thus, we selected the persecutory delusion and hallucination criteria from the OPCRIT checklist to further analyze the association between the clinical symptoms of schizophrenia and the LIFR gene. For analysis by clinical symptoms, in particular, patients with item 54 on the OPCRIT checklist were classified as the persecutory delusion group and patients with any of the items 73-77 formed the hallucination group (15). In the present genotyped sample of schizophrenia patients, participants were subdivided into those who experienced persecutory delusion (n=124, 60.8%) and those who did not (n=80, 39.2%); and those who experienced hallucinations (n=101, 49.5%) and those who did not (n=103, 50.5%). The control group was recruited from subjects visiting the hospital for routine health checkups. Control subjects had no psychiatric disorders or history thereof. 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, Korea.

SNP selection and genotyping. SNPs for the LIFR gene were selected from the promoter regions within -1000 bp of the transcriptional start sites, using HapMap (www.hapmap.org/; genome build 36) and SNP databases (dbSNP) (www.ncbi.nlm.nih.gov/SNP/; build 132). The SNPs without genotype frequency data for the Asian population, and with a heterozygosity ≤ 0.05 or a minor allele frequency ≤ 0.05 were excluded. Finally, we selected three promoter SNPs as follows: rs2071237 (-200C/G), rs2071236 (-254C/T) and rs6862038 (-962A/G).

A DNA isolation kit for blood (Roche, Indianapolis, USA) was used to isolate DNA from a peripheral blood sample. SNP genotyping was conducted by direct sequencing using the following primers for each SNP: rs2071237 (sense, 5'-CCTTGAACCACAATCCAAGACAC-3' and antisense, 5'-GAAAGTCTGGGTAAGTACAGGTC-3'); rs2071236

(sense, 5'-GGATCACGAGGTCAGGAGATCGAG-3' and antisense, 5'-GTGCAGTTCTGCAGAGCGAAAGGG-3'); rs6862038 (sense, 5'-GTAAGGGCGATTTTACTGTCCG-3' and antisense, 5'-ATTCTTAGCCACGGAGGGAGCCTC-3'). The PCR products were sequenced using an ABI PRISM 3730XL analyzer (PE Applied Biosystems, Foster City, CA, USA). Sequence data were analyzed using SeqManII software (DNASTar Inc., Madison, WI, USA).

Statistical analysis. For the analysis of Hardy-Weinberg equilibrium (HWE) and genotype frequencies, SNPAnalyzer Pro (Istech Inc., Goyang, Korea) and SNPStats (<http://bioinfo.iconcologia.net/index.php>) were used. The associations between genotypes of SNPs and schizophrenia, as well as any associations between SNPs and schizophrenia subgroups were estimated by computing the odds ratios (ORs) and their 95% confidence intervals (CIs) with logistic regression analyses, controlling for age and gender as covariables. In the logistic regression analysis for each SNP, co-dominant 1 (major allele homozygotes versus heterozygotes), co-dominant 2 (major allele homozygotes versus minor allele homozygotes), dominant (major allele homozygotes versus minor allele homozygotes plus heterozygotes), recessive (major allele homozygotes plus heterozygotes versus minor allele homozygotes) or overdominant (heterozygotes versus homozygotes) models were used. To avoid chance findings due to multiple testing, the Bonferroni correction was applied by lowering the significance levels to $p=0.016$ ($p=0.05/3$) for the three SNPs.

Results

Genetic association of LIFR SNPs with schizophrenia patients. When the association between the promoter SNPs of the LIFR gene and schizophrenia was analyzed, differences in the genotype frequencies of one SNP were found between the schizophrenia patients and the controls (rs2071237, OR=0.66, 95% CI=0.45-0.97, $P=0.027$ in the co-dominant 1 model; OR=0.65, 95% CI=0.45-0.94, $P=0.023$ in the dominant model; Table I). The C allele frequency of rs2071237 was higher in the schizophrenia group than in the control group (OR=1.29, 95% CI=1.01-1.65, $P=0.045$; data not shown). However, these associations were not significant after applying the Bonferroni correction for multiple testing (Table I).

To further analyze the haplotype structure in our samples, we identified a linkage disequilibrium (LD) block between the three SNPs in the control subjects, using pairwise D' values ($D'=1$) by Gabriel's criteria (16). Three SNPs (rs2071237, rs2071236 and rs6862038) of the LIFR gene were analyzed for linkage disequilibrium (LD) and haplotypes using Haploview 4.2. The LD block was composed of rs2071237, rs2071236 and rs6862038 (data not shown). There were four haplotypes in the LD block (haplotype CCG, frequency=0.407; GTA, frequency=0.313; GCG, frequency=0.216; and GCA, frequency=0.063; Table II). Haplotype GCG in the LD block was found to be associated with schizophrenia (Chi square=4.468, $P=0.0345$; Table II). However, this association disappeared following the Bonferroni correction (Table II).

Clinical phenotypes in schizophrenia patients. An association between genotype and specific clinical symptoms

Table I. Genotype frequencies of promoter polymorphisms of LIFR in patients with schizophrenia and control subjects^a.

SNP location	Genotype	Schizophrenia		Control		Model	OR (95% CI)	P-value
		n	%	n	%			
rs2071237 -200	G/G	80	39.4	107	29.5	Co-dominant 1	0.66 (0.45-0.97)	0.027
	C/G	97	47.8	199	54.8	Co-dominant 2	0.63 (0.36-1.09)	0.077
	C/C	26	12.8	57	15.7	Dominant	0.65 (0.45-0.94)	0.023
						Recessive	0.80 (0.48-1.33)	0.390
						Overdominant	0.76 (0.54-1.08)	0.120
rs2071236 -254	C/C	97	47.8	160	44.1	Co-dominant 1	0.78 (0.54-1.12)	0.229
	C/T	86	42.4	177	48.8	Co-dominant 2	1.24 (0.65-2.37)	0.463
	T/T	20	9.8	26	7.2	Dominant	0.84 (0.59-1.19)	0.320
						Recessive	1.41 (0.76-2.62)	0.280
						Overdominant	0.75 (0.53-1.07)	0.110
rs6862038 -962	G/G	79	38.9	133	36.6	Co-dominant 1	0.79 (0.54-1.15)	0.337
	A/G	93	45.8	188	51.8	Co-dominant 2	1.19 (0.69-2.06)	0.431
	A/A	31	15.3	42	11.6	Dominant	0.86 (0.60-1.24)	0.420
						Recessive	1.36 (0.82-2.26)	0.240
						Overdominant	0.75 (0.53-1.07)	0.110

^aTo avoid chance findings due to multiple testing, $P < 0.016$ was considered statistically significant. SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; n, number of subjects.

Table II. Frequencies of haplotypes in schizophrenia and control subjects^a.

Haplotype	Frequency	Schizophrenia		Control		Chi square	P-value
		+	-	+	-		
CCG	0.407	150.9	257.1	309.9	414.1	3.641	0.0564
GTA	0.313	126.0	282.0	228.0	496.0	0.045	0.8319
GCG	0.216	102.1	305.9	142.1	581.9	4.468	0.0345
GCA	0.063	28.9	379.1	42.9	681.1	0.604	0.4369

^aTo avoid chance findings due to multiple testing, $P < 0.016$ was considered statistically significant. Haplotypes are comprised of rs2071237, rs2071236 and rs6862038.

of schizophrenia patients was elucidated. We also assessed the association of the three SNPs of the LIFR gene with the symptoms of persecutory delusion and hallucinations in the schizophrenia patients. Differences were found between those with and without symptoms of persecutory delusion (rs2071236, OR=2.06, 95% CI=1.11-3.81, $P=0.024$ in the co-dominant 1 model, OR=2.18, 95% CI=1.20-3.94, $P=0.009$ in the overdominant model; rs6862038, OR=2.06, 95% CI=1.09-3.87, $P=0.025$ in the co-dominant 1 model, OR=2.08, 95% CI=1.16-3.74, $P=0.013$ in the overdominant model; Table III). Following the Bonferroni correction, the significant association remained (rs2071236, $P=0.009$; rs6862038, $P=0.013$; Table III). The distribution of genotype and allele frequencies did not differ significantly between schizophrenia patients with and without hallucinations (data not shown). No haplotype association was found between the selected SNPs and hallucination criteria or persecutory delusion in the schizophrenia patient group (data not shown).

Discussion

In the present study, we explored the possibility of a genetic association between the LIFR gene and schizophrenia in the Korean population. However, no significant correlation was found between schizophrenia and the promoter region SNPs of the LIFR gene. An additional analysis was performed to explore the correlation between the three SNPs and clinical symptoms of schizophrenia, such as persecutory delusion and hallucinations. The results demonstrated that rs2071236 and rs6862038 were associated with persecutory delusion.

Previously, a proteomic study reported that the levels of glial fibrillary acidic protein (GFAP) expression, which is a cytoskeletal marker of astroglia, was reduced in the brains of individuals with schizophrenia (17). Another group demonstrated that the GFAP mRNA levels in the white matter of the anterior cingulate cortex were decreased in schizophrenia patients (18). These results indicate that astrocytes play a

Table III. Genotype frequencies of promoter polymorphisms of LIFR in schizophrenia patients with and without persecutory delusion^a.

SNP location	Genotype	Persecutory delusion				Model	OR (95% CI)	P-value
		With		Without				
		n	%	n	%			
rs2071237 -200	G/G	53	43.1	27	33.8	Co-dominant 1	0.74 (0.40-1.37)	0.377
	C/G	58	47.1	39	48.8	Co-dominant 2	0.44 (0.18-1.08)	0.071
	C/C	12	9.8	14	17.5	Dominant	0.66 (0.37-1.19)	0.160
						Recessive	0.52 (0.23-1.19)	0.120
						Overdominant	0.91 (0.52-1.61)	0.760
rs2071236 -254	C/C	53	43.1	44	55.0	Co-dominant 1	2.06 (1.11-3.81)	0.024
	C/T	61	49.6	25	31.2	Co-dominant 2	0.72 (0.27-1.91)	0.433
	T/T	9	7.3	11	13.8	Dominant	1.66 (0.94-2.94)	0.082
						Recessive	0.52 (0.20-1.32)	0.170
						Overdominant	2.18 (1.20-3.94)	0.009
rs6862038 -962	G/G	42	34.1	37	46.2	Co-dominant 1	2.06 (1.09-3.87)	0.025
	A/G	65	52.9	28	35.0	Co-dominant 2	0.96 (0.42-2.21)	0.883
	A/A	16	13.0	15	18.8	Dominant	1.67 (0.94-3.00)	0.082
						Recessive	0.66 (0.30-1.42)	0.290
						Overdominant	2.08 (1.16-3.74)	0.013

^aTo avoid chance findings due to multiple testing, $P < 0.016$ was considered statistically significant. Bold characters indicate statistical significance. SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; n, number of subjects.

significant role in the pathogenesis of schizophrenia. The functional implication of LIFR in neuronal and glial development has been studied both *in vivo* and *in vitro* (19). In addition, it has been established that LIFR is also implicated in the development of astrocytes (20,21). These studies revealed that LIFR mutant embryos completely lack GFAP expression in the spinal cord and hindbrain (21). In addition to the role of LIFR in the development of astrocytes, serum LIFR concentrations were significantly increased after starting treatment with antipsychotics in schizophrenia patients (22). Moreover, in our study, the LIFR gene was associated with persecutory delusion in schizophrenia. Taken together, LIFR may be implicated in the pathogenesis of schizophrenia.

To find whether the alleles of rs2071236 and rs6862038 relate to transcription factors, we used the online program 'AliBaba 2.1' (<http://www.gene-regulation.com/pub/programs/alibaba2>). At the rs2071236 SNP site, C-containing sequences are shown to act with ER and CREBP1 transcription factors, but CREBP1 disappears in T-containing sequences. At the rs2071236 SNP site, the A-containing sequence binds with HNF-1 and FTZ, and T-containing sequences do not bind with any transcription factor. Therefore, this SNP may regulate gene expression by controlling the binding of transcription factors.

For a clear understanding of the pathogenesis of schizophrenia, further research is required to investigate the correlation between other SNPs of the LIFR gene and schizophrenia. In addition, replication studies using larger samples need to be performed in several populations to confirm our initial findings. Nevertheless, even with these limitations, our

results suggest the possibility that polymorphisms of the LIFR gene may be related to persecutory delusion in schizophrenia.

In conclusion, a significant association was revealed between LIFR gene polymorphisms and symptoms of persecutory delusion in schizophrenia patients. In particular, rs2071236 and rs6862038 were associated with persecutory delusion. These results suggest that the LIFR gene may be related to the susceptibility to persecutory delusion in schizophrenia.

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