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Association between Interleukin 31 Receptor A Gene Polymorphism and Schizophrenia in Korean Population

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Recently, Sun et al (2008) reported that the IL6R polymorphism is associated with schizophrenia. Therefore, to detect the association between polymorphisms of interleukin 31 receptor A (IL31RA) and schizophrenia, we genotyped 9 SNPs [rs9292101 (intron 1), rs1009639 (exon 2, Pro43Pro), rs2161582 (intron 2), rs68761890 (intron 5), rs16884629 (intron 6), rs11956465 (intron 12), rs12153724 (intron 12), and rs16884641 (intron 14)] using the Golden Gate assay on Illumina BeadStation 500 GX. Two hundred eighteen patients with schizophrenia and 379 normal subjects were recruited. Patients with schizophrenia were diagnosed according to DSM-IV, and control subjects without history of psychiatric disorders were selected. We used SNPStats, Haploview, HapAnalyzer, SNPAnalyzer, and Helixtree programs for the evaluation of genetic data. Of nine polymorphisms, three SNPs (rs9292101, rs1009639, and rs11956465) were associated with schizophrenia. The rs9292101 and rs11956465 showed significant associations with the risk of schizophrenia in the codominant [rs9292101, odds ratio (OR)=0.74, 95% confidence interval (CI)=0.58～0.95, p=0.017] and recessive (rs11956465, OR=0.64, 95% CI=0.42～0.96, p=0.034) models, respectively. The rs1009639 also was statistically related to schizophrenia in both codominant (OR=0.76, 95% CI=0.60～0.97, p=0.025) and dominant (OR=0.66, 95% CI=0.44～0.98, p=0.035) models. Two linkage disequilibrium (LD) blocks were made. In the analysis of haplotypes, a haplotype (GCT) in block 1 and a haplotype (CCACAG) in block 2 showed significant associations between schizophrenia and control groups (haplotype GCT, frequency=0.509, chi square=4.199, p=0.040; haplotype CCACAG, frequency=0.289, chi square=5.691, p=0.017). The results suggest that IL31RA may be associated with risk of schizophrenia in Korean population.

Key Words: Interleukin 31 receptor A, Haplotype, Linkage disequilibrium, Schizophrenia, Single nucleotide polymorphism

INTRODUCTION

Despite vigorous investigations on the pathophysiology of schizophrenia for many years, the etiology of schizophrenia is still unknown. It has been reported that schizophrenia is associated with abnormalities in the immune system (Muller et al, 2000; Lang et al, 2007), and genetic factor of cytokine genes have been implicated, because of immune dysfunction in patients with schizophrenia. Therefore, several studies showed the association of interleukin (IL) 1 beta gene polymorphism with schizophrenia (Shirts et al, 2006; Papiol et al, 2007; Hanninen et al, 2008). IL3 (Chen et al, 2007; Chen and Kendler, 2008), and IL2 and IL4 gene polymorphisms (Schwarz et al, 2006) are related to schizophrenia. Recently, Sun et al (2008) reported the association between IL6R gene polymorphism and schizophrenia.

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IL31 receptor A (IL31RA; Aliases, CRL3, GLM-R, CRL, Glmr) is related to gp130, the common receptor subunit for IL6-type cytokines. IL31RA and oncostatin M receptor (OSMR) make a heterodimeric receptor on IL31 signal (http://www.ncbi.nlm.nih.gov/entrez). Expressions of IL31RA and OSMR mRNAs are induced in activated monocytes, and both mRNAs are constitutively expressed in epithelial cells (Dillon et al, 2004). IL31RA gene contains 15 exons and 732 amino acids. Amino acid residues from 20 to 519 comprise extracellular, 520 to 540 transmembrane, and 541 to 732 cytoplasmic domains (UniProt, http://beta.uniprot.org; SwissProt, http://www.expasy.org). The protein also contains a cleavable signal peptide domain comprising from 1 to 19 amino acid residues (MMWTWALWMLPSLCKFSLA). Diveu et al (2003) reported that the intron/exon organization of IL31RA is a pattern similar to IL6 signal transducer (IL6ST), its neighbor on chromosome 5q11.2. However, genetic association of IL31RA on schizophrenia is not yet known. In this study, we examined the association between

ABBREVIATIONS: CI, confidence interval; HWE, Hardy-Weinberg equilibrium; LD, linkage disequilibrium; IL31RA, interleukin 31 receptor A; OR, odds ratio; SNP, single nucleotide polymorphism.

IL31RA gene polymorphisms and schizophrenia.

METHODS

Study participants

Two hundred eighteen schizophrenia subjects (120 males, mean age 41.40 years; 98 females, 42.95 years) and 379 healthy individuals (191 males, 44.46 years; 188 females, 44.29 years) with no clinical evidence of any other disorder were recruited at Kyung Hee University Medical Center. Schizophrenic patients were subjected to medical history taking and clinical interviews. Symptoms of patients were rated according to DSM-IV criteria (American Psychiatric Association, 1994) by well-trained two psychiatrists. This study was approved by the Ethics Committee of the Medical Research Institute, College of Medicine, Kyung Hee University. Informed written consent was obtained from each subject. DNA was extracted using a commercially available Qiagen DNA Extraction kit (Qiagen, Tokyo, Japan), and stored at −20°C before use.

SNP selection and genotyping

In the IL31RA gene region, nine SNPs [rs9292101 (intron 1), rs1009639 (exon 2, Pro43Pro), rs2161582 (intron 2), rs68761890 (intron 5), rs16884629 (intron 6), rs11956465 (intron 12), rs12153724 (intron 12), and rs16884641 (intron 14)] were selected using human SNP websites (http://www.ensembl.org; www.ncbi.nlm.nih.gov/SNP). The SNPs with unknown heterozygosity and minor allele frequency (below 5%) were excluded. SNP genotyping was performed using the Golden Gate assay on an Illumina BeadStation 500 GX (Illumina Inc., San Diego, USA), according to the protocol supplied. Each oligonucleotide (bead type) represents a specific SNP locus. Each Golden Gate genotyping represents the mean intensity of 30 replicates, resulting in accuracy call rates (all >99%).

Statistical analysis

We analyzed Hardy-Weinberg equilibrium (HWE) and genotype frequencies by using SNPStats (http://bioinfo.iconcologia.net/index.php). A linkage disequilibrium (LD) block of polymorphisms was tested using Haploview 3.32. The haplotypes and their frequencies were calculated by the EM algorithm. We used SNPStats, SNPAnalyzer (ISTECH Inc., Goyang, Korea), and Helixtree (Golden Helix Inc., MT, USA) for analyzing odds ratio (OR), 95% confidence interval (CI), and p value (Jung et al, 2008a; 2008b). Logistic regression analysis controlling age and gender as covariables in three analysis models (codominant, dominant, and recessive models) were employed to show alternative effects of the variants. For all statistical tests, the significant level was set at 0.05.

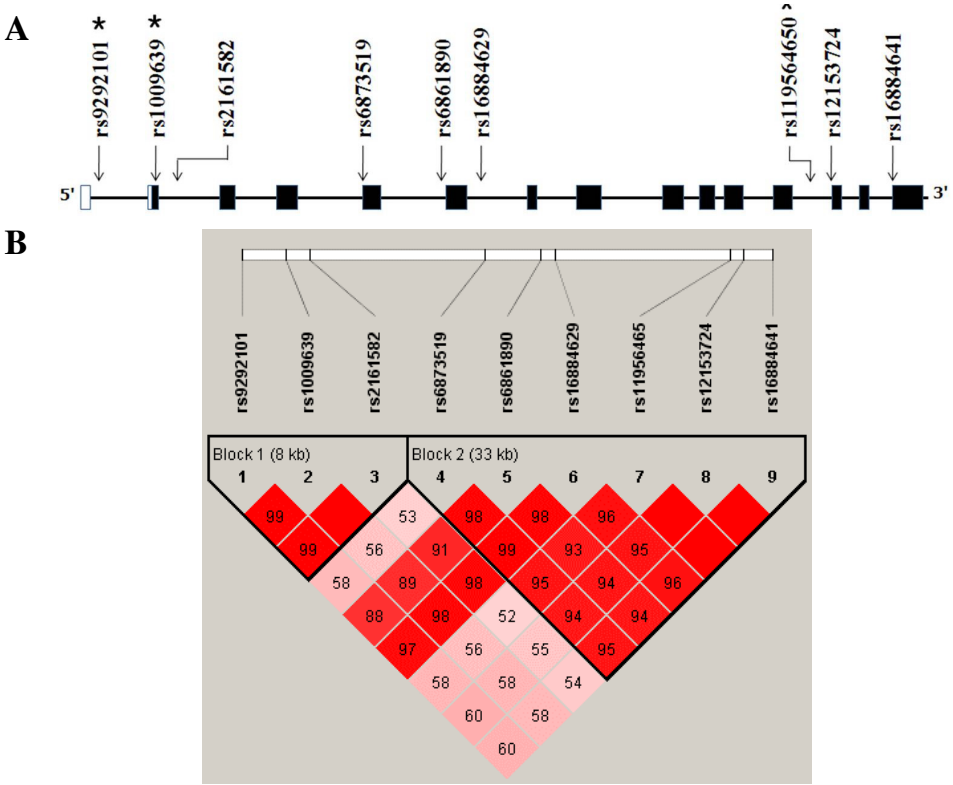

Fig. 1. Gene map and linkage disequilibrium (LD) in interleukin 31 receptor A (IL31RA) gene. (A) Gene map of single nucleotide polymorphisms (SNPs) in IL31RA. Exons are marked with box. The coding region is black-boxed and untranslated regions are white- boxed. Asterisk (*) indicates a significant SNP. Arrow indicates the location of each SNP. (B) LD coefficient (| D' |) and LD blocks among SNPs of IL31RA. Block 1 consists of rs9292101, rs1009639, and rs2161582. Block 2 comprises rs6873519, rs6861890, rs16884629, rs11956465, rs12153724, and rs16884641.

RESULTS

To evaluate whether IL31RA is associated with schizophrenia in Korean population, we genotyped the 9 SNPs selected. In the IL31RA gene containing various SNPs, the 9 SNPs selected included rs9292101 (intron 1), rs1009639 (exon 2, Pro43Pro), rs2161582 (intron 2), rs68761890 (intron 5), rs16884629 (intron 6), rs11956465 (intron 12), rs12153724 (intron 12), and rs16884641 (intron 14). Locations of 9 SNPs on the IL31RA gene region are shown in Fig. 1A. The logistic analysis of codominant, dominant, and recessive models show that there were significant differences between patients with schizophrenia and controls.

The genotype distributions of IL31RA gene polymorphisms in the schizophrenia and control groups are shown in Table 1. No deviation of HWE in genotype distributions of any polymorphisms was found, between schizophrenia and controls (data not shown). Of the nine SNPs, three pol-

Table 1. Logistic regression analysis and genotype frequency of interleukin 31 receptor A (IL31RA) polymorphisms in patients with schizophrenia and controls

SNP	Genotype	Schizophrenia	Control	Codominant	p	Dominant	p	Recessive	p
Locus		n=218 (%)	n=379 (%)	OR (95% CI)		OR (95% CI)		OR (95% CI)	
rs9292101	G/G	49 (23.2)	110 (30.1)	0.74 (0.58~0.95)	0.017	0.68 (0.46~1.01)	0.053	0.67 (0.45~1.00)	0.051
Intron 1	G/T	105 (49.8)	182 (49.7)						
	T/T	57 (27.0)	74 (20.2)						
rs1009639	C/C	47 (21.8)	110 (29.1)	0.76 (0.60~0.97)	0.025	0.66 (0.44~0.98)	0.035	0.74 (0.50~1.08)	0.120
Exon 2	C/T	108 (50.0)	182 (48.1)						
(Pro43Pro)	T/T	61 (28.2)	86 (22.8)						
rs2161582	T/T	56 (26.1)	108 (29.8)	0.82 (0.65~1.05)	0.120	0.81 (0.55~1.19)	0.290	0.73 (0.48~1.10)	0.140
Intron 2	C/T	107 (49.8)	185 (51.0)						
	C/C	52 (24.2)	70 (19.3)						
rs6873519	A/A	75 (34.6)	118 (31.1)	1.20 (0.94~1.53)	0.140	1.16 (0.81~1.65)	0.430	1.48 (0.94~2.35)	0.088
Intron 4	A/C	111 (51.1)	183 (48.3)						
	C/C	31 (14.3)	78 (20.6)						
rs6861890	C/C	73 (33.8)	154 (40.6)	0.80 (0.62~1.02)	0.076	0.74 (0.52~1.06)	0.095	0.75 (0.47~1.22)	0.250
Intron 5	C/T	109 (50.5)	176 (46.4)						
	T/T	34 (15.7)	49 (12.9)						
rs16884629	G/G	113 (53.3)	167 (47.4)	1.27 (0.95~1.70)	0.110	1.23 (0.87~1.73)	0.250	2.05 (0.86~4.86)	0.086
Intron 6	A/G	92 (43.4)	160 (45.5)						
	A/A	7 (3.3)	25 (7.1)						
rs11956465	C/C	46 (22.4)	96 (27.1)	0.78 (0.60~1.00)	0.052	0.81 (0.54~1.22)	0.300	0.64 (0.42~0.96)	0.034
Intron 12	C/T	105 (51.2)	192 (54.2)						
	T/T	54 (26.3)	66 (18.6)						
rs12153724	G/G	65 (30.5)	92 (25.6)	1.15 (0.89~1.47)	0.290	1.28 (0.88~1.88)	0.200	1.09 (0.71~1.67)	0.690
Intron 12	A/G	107 (50.2)	191 (53.1)						
	A/A	41 (19.2)	77 (21.4)						
rs16884641	T/T	63 (29.2)	92 (24.3)	1.20 (0.94~1.52)	0.140	1.28 (0.88~1.88)	0.200	1.26 (0.84~1.88)	0.270
Intron 14	G/T	108 (50.0)	190 (50.3)						
	G/G	45 (20.8)	96 (25.4)						

Genotype distributions are shown as number (%). Odds ratio (OR), 95% confidence interval (CI), and p-values were from logistic regression analysis with codominant, dominant, and recessive models controlling age and gender as covariates. Total number of each SNP is different, because genotypes of some SNPs are unreadable. SNP, single nucleotide polymorphism.

Table 2. Haplotype analysis of interleukin 31 receptor A (IL31RA) polymorphisms in patients with schizophrenia and controls

Block		Haplotype	Freq	Schizophrenia		Control		Chi square	p
				+	−	+	−		
Block 1	ht1	GCT	0.509	203.0	229.0	403.0	355.0	4.199	0.040
	ht2	TTC	0.451	210.9	221.1	325.9	432.1	3.773	0.052
Block 2	ht1	ATGTGT	0.368	172.3	261.7	265.9	490.1	2.423	0.120
	ht2	CCACAG	0.289	107.6	326.4	236.7	519.3	5.691	0.017
	ht3	ACGTGT	0.131	56.4	377.6	99.4	656.6	0.007	0.935
	ht4	CCGCAG	0.126	560.0	378.0	93.6	662.4	0.071	0.790

Each haplotype with a frequency of more than 0.1 is shown. p-values of haplotype association were calculated using Haploview 3.32. ht, haplotype; Freq, frequency.

ymorphic SNPs (rs9292101, OR=0.74, 95% CI=0.58～0.95, p=0.017 in the codominant model; rs1009639, OR=0.76, 95% CI=0.60～0.97 p=0.025 in the codominant model and OR=0.66, 95% CI=0.44～0.98, p=0.035 in the dominant model; rs11956465, OR=0.64, 95% CI=0.42～0.96, p=0.034 in the recessive model, respectively) were associated with the risk of schizophrenia. The frequencies of GG, GT, and TT genotypes for rs9292101 were 30.1%, 49.7%, and 20.2% in the control group, 23.2%, 49.5%, and 27.0% in schizophrenia groups, respectively. The frequencies of CC, CT, and TT genotypes for rs1009639 were 29.1%, 48.1%, and 22.8% in the control group, and 21.8%, 50.0%, and 28.2% in schizophrenia groups, respectively. The frequencies of CC, CT, and TT genotypes for rs11956465, were 27.1%, 54.2%, and 18.6% in the control group, whereas 22.4%, 51.2%, and 26.3% in schizophrenia groups, respectively. The rare allele of rs9292101 (rare allele, T), rs1009639 (rare allele, T), and rs11956465 (rare allele, T) increased the risk of schizophrenia. The rest of the SNPs (rs2161582, rs6873519, rs6861890, rs16884629, rs12153724, and rs16884641) were not statistically associated with schizophrenia (Table 1).

Pair-wise comparisons among the nine polymorphisms revealed strong LD. We identified two LD blocks by the Gabriel method (Fig. 1B). Block 1 consisted of rs9292101, rs1009639, and rs2161582, and block 2 comprised rs6873519, rs6861890, rs16884629, rs11956465, rs12153724, and rs16884641. Two haplotypes in block 1 and four haplotypes in block 2 had frequencies greater than 0.1, and all were used for haplotype association analysis. Analysis for the haplotype association was performed using Haploview 3.32. On the haplotype association test, the haplotype (GCT) in block 1 and haplotype (CCACAG) in block 2 had significant associations between schizophrenia and control groups (haplotype GCT, frequency=0.509, chi square=4.199, p=0.040; haplotype CCACAG, frequency=0.289, chi square=5.691, p=0.017) (Table 2).

Table 3. Genotype frequencies of interleukin 31 receptor A (IL31RA) polymorphisms in each population

SNP	Genotype	Korean		European	Chinese	Japanese
		Schizophrenia	Control			
rs9292101	G/G	0.232	0.301	0.085	0.326	0.209
	G/T	0.498	0.497	0.424	0.512	0.581
	T/T	0.27	0.202	0.492	0.163	0.209
rs1009639	C/C	0.218	0.291	0.083	0.311	0.2
	C/T	0.500	0.481	0.450	0.533	0.6
	T/T	0.282	0.228	0.467	0.156	0.2
rs2161582	T/T	0.261	0.298	0.417	0.111	0.182
	C/T	0.498	0.510	0.467	0.556	0.614
	C/C	0.242	0.193	0.117	0.333	0.205
rs6873519	A/A	0.346	0.311	0.567	0.4	0.356
	A/C	0.511	0.483	0.35	0.422	0.467
	C/C	0.143	0.206	0.083	0.178	0.178
rs6861890	C/C	0.338	0.406	0.317	0.444	0.25
	C/T	0.505	0.464	0.483	0.444	0.523
	T/T	0.157	0.129	0.200	0.111	0.227
rs16884629	G/G	0.533	0.474	0.000	0.093	0.095
	A/G	0.434	0.455	1.000	0.395	0.452
	A/A	0.033	0.071	0.000	0.512	0.452
rs11956465	C/C	0.224	0.271	0.050	0.178	0.156
	C/T	0.512	0.542	0.317	0.511	0.511
	T/T	0.263	0.186	0.633	0.311	0.333
rs12153724	G/G	0.305	0.256	0.051	0.195	0.146
	A/G	0.502	0.531	0.339	0.512	0.512
	A/A	0.192	0.214	0.61	0.293	0.341
rs16884641	T/T	0.292	0.243	0.05	0.178	0.156
	G/T	0.500	0.503	0.333	0.511	0.511
	G/G	0.208	0.254	0.617	0.311	0.333

From SNP database (http://www.ncbi.nlm.nih.gov/sites/entrez).

DISCUSSION

Schizophrenia is a complex genetic disorder and affects approximately 1% of the population around the world. However, etiology of schizophrenia is still unclear. Cytokines may be implicated in its etiology and pathology (Nawa et al, 2000). Genetic evidence has revealed the relationship between polymorphisms of ILs and schizophrenia. Especially, Sun et al (2008) reported that IL6R polymorphism (rs8192284, exon 9) was associated with schizophrenia in Chinese population. In this study, we investigated whether IL31RA gene polymorphisms are related to schizophrenia in Korean population., and found that three SNPs (rs9292101, rs1009639, and rs11956465) were significantly associated with schizophrenia, suggesting that IL31RA may be related to the development of schizophrenia. However, these results need to be replicated in other populations, because of its limited sample size,

The rs9292101 is located on intron 1. The GG, GT, and TT genotype frequencies were reported to be 0.085, 0.424, and 0.492 in European, 0.326, 0.512, and 0.163 in Chinese, and 0.209, 0.581, and 0.209 in Japanese, respectively (http://www.ncbi.nlm.nih.gov/SNP) (Table 3). The GG, GT, and TT genotype frequencies in Korean population were found to be 0.301, 0.497, and 0.202, which are similar to those in Chinese. The rs1009639 (Pro43Pro) is located at the coding of exon 2. The synonymous rs1009639 (Pro43Pro) is the SNP with known heterozygosity in the coding SNPs of IL31RA gene region. The CC, CT, and TT genotype frequencies are reported to be 0.083, 0.450, and 0.467 in European, 0.311, 0.533, and 0.156 in Chinese, and 0.200, 0.600, and 0.200 in Japanese, respectively. IL31RA protein (Q8NI17) consists of 732 amino acid residues and the molecular mass of IL31RA is 82,954 Da. Amino acid residues from 20 to 519 comprise extracellular, 520 to 540 transmembrane, and 541 to 732 cytoplasmic domain (UniProt, http://beta.uniprot.org; SwissProt, http://www.expasy.org). Extracellular domain also consisted of fibronectin type-Ⅲ 1 (22 to 116), 2 (121 to 222), 3 (223 to 315), 4 (319 to 416), and 5 (421 to 512) domains. The CC, CT, and TT genotype frequencies in Korean population were 0.291, 0.481, and 0.228, respectively. The rs11956465 is located on intron 12. The CC, CT, and TT genotype frequencies are reported to be 0.050, 0.317, and 0.633 in European, 0.178, 0.511, and 0.311 in Chinese, and 0.156, 0.511, and 0.333 in Japanese, respectively. The CC, CT, and TT genotype frequencies in Korean population were 0.271, 0.541, and 0.186, respectively. Therefore, ethnic differences are present in these SNPs.

In conclusion, the results suggest that IL31RA gene may be associated with susceptibility of schizophrenia in Korean population. Further biological and/or functional evidence is needed to confirm the suggested associations of IL31RA polymorphisms with the risk of schizophrenia in Korean

population.

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