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Association Analysis between Polymorphisms of NOTCH4 Gene and Schizophrenia in Korean Population

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Abstract

Notch signaling plays a crucial role in development of the nervous system. Neurodevelopmental hypothesis on etiology of schizophrenia has been implicated. The aim of this study is to determine whether single nucleotide polymorphisms (SNPs) of Notch homolog 4 (Drosophila) (NOTCH4) gene are associated with schizophrenia. This study included 283 schizophrenia patients diagnosed according to DSM-IV and 301 normal control subjects. Control subjects without history of psychiatric disorders were recruited. Four missense SNPs [rs915894 (exon 3, Lys117Gln), rs2071282 (exon 4, Pro204Leu), rs422951 (exon 6, Thr320Ala), and rs17604492 (exon 18, Gly942Arg)] of NOTCH4 gene were genotyped by the direct sequencing method. Multiple logistic regression models (codominant, dominant, and recessive models) were employed to evaluate odds ratio, 95% confidence interval, and p value. For analysis of genetic data, SNPStats, Haploview, HapAnalyzer, SNPAnalyzer, and Helixtree programs were also used. Of 4 SNPs, rs2071282 was weekly associated with schizophrenia in two alternative models (codominant model, P=0.049; dominant, P=0.041). However, these associations were not significant after Bonferroni correction. At 4 SNPs, one linkage disequilibrium (LD) block was made. This block consisted of rs915894 and rs2071282. In haplotype analysis, AC haplotype was weakly associated with schizophrenia (dominant, P=0.04). This association was disappeared after Bonferroni correction. Our result shows possibility that some SNPs of NOTCH4 gene may be weekly associated with development of schizophrenia in Korean population. However, replication result by other population will be needed.

Keywords: NOTCH4, Schizophrenia, Polymorphism, Haplotype, Association

Schizophrenia is a severe disease affecting approximately 1% of the general population[1,2]. Genetic component inferred from twin and family studies is thought to be important to etiology of schizophrenia[3-5]. Several lines of evidence also show that schizophrenia may be related to development of the central nervous system and signaling of multiple neurobiological pathways[6-8].

Notch homolog 4 (Drosophila) (NOTCH) signaling plays a crucial role in controlling cell-fate decisions, proliferation, and apoptosis throughout development of the nervous system[9,10]. The NOTCH4 gene is located at 6p21.3 known the centromeric end of major histocompatibility complex (MHC) class III region[11]. Chromosome 6 is suggested to be a candidate site for schizophrenia[12]. Since Wei & Hemmings (2000) reported that the NOTCH4 locus is associated with susceptibility to schizophrenia[13], several genetic association studies of NOTCH4 have been investigated[14-16]. Liu *et al.* (2007) reported association the evidence of schizophrenia with distal genomic region of NOTCH4 in Taiwanese families[17]. Glatt *et al.* (2005) reported that five NOTCH4 polymorphisms showed weak evidence for association with schizophrenia[18]. Ivo *et al.* (2006) reported no association between NOTCH4 and schizophrenia in a large family-based and case-control analysis[19]. However, previous studies have been shown controversial results on risk of schizophrenia. In this

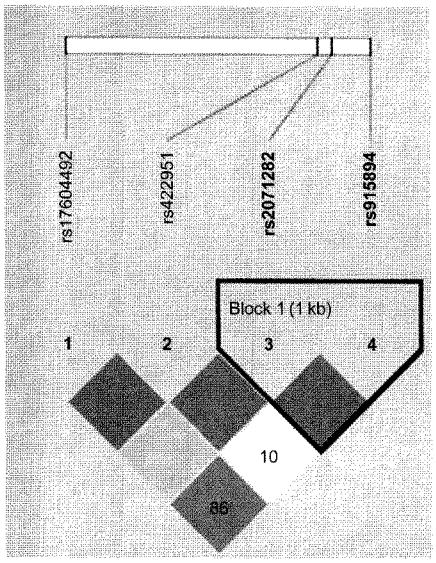

Figure 1. Linkage disequilibrium (LD) coefficient (|D'|) and LD block among 4 missense single nucleotide polymorphism (SNPs) of NOTCH4. Block 1 consists of rs2071282 and rs915894.

study, we investigated an association study between single nucleotide polymorphisms (SNPs) of NOTCH4 gene and schizophrenia in Korean population.

Genetic Association of NOTCH4 SNPs in Koreans with Schizophrenia

To investigate whether NOTCH4 is associated with schizophrenia in Korean population, missense 4 SNPs selected [rs915894 (exon3, Lys117Gln), rs2071282 (exon4, Pro204Leu), rs422951 (exon6, Thr320Ala), and rs17604492 (exon18, Gly942Arg)] were genotyped. Genotype distributions of each SNP were no deviation in HWE (data not shown). Locations of missense 4 SNPs on the NOTCH4 gene region are shown in Figure 1. In the logistic regression analysis (codominant, dominant, and recessive models), genotype distributions of each SNP in the schizophrenia and control groups are shown in Table 1. Of four SNPs, the rs2071282 SNP were weekly associated with risk of schizophrenia (OR=0.57, 95% CI=0.35-0.95, P=0.049 in the codominant model, and OR=0.60, 95% CI=0.36-0.95, P=0.041 in the dominant model). After Bonferroni correction, these associations were disappeared (Table 1). Frequencies of GG, GA, and AA genotypes for rs2071282 were 90%, 9%, and 0% in the control group, 85%, 15%, and 0% in the schizophrenia group, respectively. The rest SNPs (rs915894, rs422951, and rs17604492) were not statistically associated with schizophrenia (Table 1).

Pair-wise comparisons among 4 SNPs revealed LD. One LD block by the Gabriel method was made. Block 1 consisted of rs2071282 and rs915894 (Figure 1). For the analysis of haplotype association, Haploview 3.32 was used. Three haplotypes in Block 1 had frequencies greater than 0.1. The AC haplotype was weakly associated with schizophrenia (OR=1.68, 95% CI =1.02-2.27, P=0.04 in the dominant model). However, corrected p value of AC haplotype after Bonferroni correction was not significant (Table 2).

Discussion

It is well-known that genetic factor involves in the etiology of schizophrenia. It is also known that NOTCH4 is a candidate gene for schizophrenia. In this study, we investigated four missense SNPs (rs915894, rs2071282, rs422951, and rs17604492) of NOTCH4 in Korean schizophrenia patients. Several studies showed evidences for an association between polymorphisms of NOTCH4 and schizophrenia. In Taiwanese schizophrenia families, Liu *et al.* (2007) reported that the T allele of rs2071285 (P=0.035) and the G allele of rs204993 (P=0.0097) were significantly preferentially transmitted to the affected individuals in the single-locus association analysis[17]. Shibata *et al.* (2006) showed that the rs2071282 SNP was weakly associated with schizophrenia in Japanese population (P=0.04)[20]. In present study, rs2071282 among four missense SNPs was only found to be different between schizophrenia and controls (codominant, P=0.049; dominant, P=0.041). Our study supports previous results that rs2071282 was weakly associated with schizophrenia. After Bonferroni correction, however, these associations were disappeared (Table 1). Zhang *et al.* (2004) reported that the rs367398, rs915894, and rs422951 SNPs were not associated with schizophrenia in Han Chinese schizophrenia family[21], while a SNP (rs520692, P=0.017) was associated with schizophrenia[21]. Shibata *et al.* (2006) also showed that the rs367398 and 422951 SNPs were not associated with schizophrenia in Japanese population[20]. In present study, rs915894 and rs422951 were not associated with schizophrenia (Table 1). Our result is pattern similar to previous results that rs915894 or rs422951 was not related to the development of schizophrenia.

The rs915894 SNP is located on exon 3. The AA, AC, and CC genotype frequencies were reported to be 0.517, 0.350, and 0.133 in European, 0.222, 0.511, and 0.267 in Chinese, 0.289, 0.333, and 0.378 in Japanese, and 0.533, 0.317, and 0.150 in Sub-Saharan African,

Table 1. Multiple logistic regression analysis of Notch homolog 4 (Drosophila) (NOTCH4) polymorphisms in schizophrenia patients and normal control subjects.

locus	SNP	Genotype (Case/Control)			Codominant			Dominant			Recessive		
		CC (%)	CR (%)	RR (%)	OR (95% CI)	*P*	Corrected *P*	OR (95% CI)	*P*	Corrected *P*	OR (95% CI)	*P*	Corrected *P*
Lys117Gln	rs915894	84/89 (30%/30%)	138/142 (49%/47%)	61/70 (22%/23%)	0.97 (0.66-1.42)	0.88	1.00	1.01 (0.70-1.43)	0.98	1.00	1.1 (0.75-1.63)	0.62	1.00
Pro204Leu	rs2071282	240/272 (85%/90%)	43/28 (15%/9%)	0/1 (0%/0%)	0.57 (0.35-0.95)	**0.049**	0.19	0.6 (0.36-0.95)	**0.041**	0.16	NA-	0.25	1.00
Thr320Ala	rs422951	156/162 (55%/54%)	110/121 (39%/40%)	17/18 (6%/6%)	1.06 (0.75-1.49)	0.95	1.00	1.05 (0.76-1.46)	0.75	1.00	1 (1.50-1.97)	0.99	1.00
Gly942Arg	rs17604492	218/239 (77%/79%)	61/59 (22%/20%)	4/3 (1%/1%)	0.88 (0.59-1.32)	0.75	1.00	0.87 (0.59-1.29)	0.49	1.96	0.1 (0.16-3.17)-	0.64	1.00

Genotype distributions are shown as number (%). CC, CR, and RR represent the homozygote for common allele (CC), heterozygote (CR), and homozygote for rare allele (RR), respectively. Bold character represents statistically significant value. SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; *P*, p value; Corrected *P*, p value corrected by Bonferroni correction; NA, not applicable.

Table 2. Haplotype frequencies of Notch homolog 4 (Drosophila) (NOTCH4) polymorphisms in schizophrenia patients and normal control subjects.

Block	Haplotype		Control (Freq, %)	SCZ (Freq, %)	Model	OR (95% CI)	*P*	Corrected *P*
rs2071282/ rs915894	HAP1 (GA)	HAP1/HAP1	89 (30)	84 (29.6)	Codominant	1.04 (0.83-1.30)	0.76	1.00
		HAP1/-	142 (47)	138 (48.7)	Dominant	1.10 (0.75-1.63)	0.62	1.00
		-/-	70 (23)	61 (21)	Recessive	1.01 (0.70-1.43)	0.97	1.00
	HAP2 (GC)	HAP2/HAP2	59 (19.6)	42 (14)	Codominant	0.87 (0.69-1.09)	0.23	0.92
		HAP2/-	134 (44.5)	133 (46)	Dominant	0.91 (0.65-1.27)	0.56	1.00
		-/-	108 (35.9)	108 (38)	Recessive	0.71 (0.46-1.10)	0.12	0.48
	HAP3 (AC)	HAP3/HAP3	1 (0.1)	0 (0)	Codominant	1.60 (0.98-2.61)	0.06	0.24
		HAP3/-	2 (9.6)	43 (15)	Dominant	1.68 (1.02-2.78)	**0.04**	0.16
		-/-	272 (90.3)	240 (84)	Recessive	NA (0.00-NA)	0.99	1.00

Block consists of rs2071282 and rs915894. Bold character represents statistically significant value. Freq, Frequency; SCZ, schizophrenia; OR, odds ratio; CI, confidence interval; *P*, p value; Corrected *P*, p value corrected by Bonferroni correction; NA, not applicable.

respectively. The genotype frequencies in Korean population were found to be 0.300, 0.470, and 0.230, which are similar to Japanese population. The rs2071282 SNP is located on exon 4. The CC, CT, and TT genotype frequencies were reported to be 1.000 in European, 0.867 and 0.133 in Chinese, 0.837, 0.140, and 0.023 in Japanese, and 1.000 in Sub-Saharan African, respectively. The genotype frequencies in Korean population were found to be 0.900, 0.090, and 0.001, which are similar to Chinese population. The rs422951 SNP is located on exon 6. The AA, AG, and GG genotype frequencies were reported to be 0.417, 0.433, and 0.150 in European, 0.600, 0.356, and 0.044 in Chinese, 0.568, 0.318, and 0.114 in Japanese, and 0.533, 0.400, and 0.067 in Sub-Saharan African. The genotype frequencies in Korean population were found to be 0.540, 0.400, and 0.060, which are similar to Japanese and Sub-Saharan African population. The rs17604492 SNP is located on exon 18. The rs17604492 SNP was not investigated in previous study to our knowledge. The CC, CT, and TT genotype frequencies were reported to be 1.000 in European, 0.711, 0.200, and 0.089 in Chinese, 0.822, 0.156, and 0.022 in Japanese, and 0.800, 0.117, and 0.083 in Sub-Saharan African. The genotype frequencies in Korean population were found to be 0.790, 0.200, and 0.010, which are similar to Japanese population. Therefore, ethnic difference is present in these SNPs.

We concluded that some genetic variants of the NOTCH4 gene may be weekly associated with schizophrenia. Future research obtained replication results will be needed in other population.

Materials & Methods

Subjects

The schizophrenia patient group consisted of 283

Korean schizophrenia patients (188 males, mean age 42.9 years; 95 females, 42.9) diagnosed according to DSM-IV. The 301 control group (145 males, 39.8; 156 females, 36.6) were also recruited. Control subjects with psychiatric disorders and severe other diseases were excluded. This study was carried out according to the Declaration of Helsinki guidelines. Written informed consent was obtained from each subject. This study was approved by the Institutional Review Board of Kyung Hee University Medical Center, Seoul, Korea.

SNP Selection and Genotyping

DNA was isolated from a peripheral blood using the Core One™ Blood Genomic DNA Isolation Kit (CoreBioSystem™, Seoul, Korea). In the NOTCH4 gene area, 4 missense SNPs [rs915894 (exon 3, Lys117Gln), rs2071282 (exon 4, Pro204Leu), rs422951 (exon 6, Thr320Ala), and rs17604492 (exon 18, Gly942Arg)] were selected using human SNP databases (www.ensemble.org; www.ncbi.nlm.nih.gov/SNP). SNP genotyping was done by direct sequencing with following primers: rs915894 (sense, 5′-TCTGCCAAAATGGAGGCAGCTGCCAA-3′; antisense, 5′-CTGGTGAATGTTGGTTGTGGGTAAGT-3′; 406 bp), rs2071282 (sense, 5′-CCTCAGATGGGCTAGAATTCGTACAA-3′; antisense, 5′-GTGGAGTCTTTCTCTGGCATCAGCTG-3′; 382 bp), rs422951 (sense, 5′-CCAGAAACCTGGACAGGTGAGTTGTT-3′; antisense, 5′-CCACTCACACACACGCAGTGAAAGCT-3′; 245 bp), and rs17604492 (sense, 5′-CCCCCCACTTATCTTTGTATATCCC-3′; antisense, 5′-TGGGACAAAGGGTGAAGGTGAAAGCA-3′; 434 bp). The samples were sequenced by an ABI Prism 377 automatic sequencer (PE Applied Biosystems, Foster City, CA, USA). Sequence data were analyzed using the SeqManII software (DNASTAR Inc., Madison, WI, USA).

Statistical Analysis

Chi-square test was used to evaluate the Hardy-Weinberg equilibrium (HWE) of each genotype in the control group. A linkage disequilibrium (LD) block was confirmed by Haploview version 3.32. Lewontis's |D'| and r^2 between all pairs of bi-allelic loci were calculated. The haplotypes and their frequencies were estimated by the EM algorithm. Multiple logistic regression model controlling age and gender as covariables was used to calculate odds ratio (OR), 95% confidence interval (CI), and corresponding p value. For genetic analysis, SNPstats, Haploview version 3.32, SNPAnalyzer (ISTECH Inc., Goyang, Korea), and Helixtree (Golden Helix Inc., MT, USA) programs were used. The power of sample size was calculated using a genetic power calculator (http://pngu.mgh.harvard.edu/~purcell/gpc), and then the effective sample size was also adjusted (calculated sample size $\times$ 100/95). In this case-control study, we had 0.951 (rs915894, numbers of case for 80% power=182), 0.956 (rs2071282, numbers of case for 80% power =173), 0.756 (rs422951, numbers of case for 80% power=331), and 0.888 (rs17604492, numbers of case for 80% power=232). For multiple test, Bonferroni correction was performed to detect a significance level (α/κ=0.0125) for each SNP (n=4). In statistical tests, significant level was set at 0.05.

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