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Experimental Neurobiology

Original Article

Association between a Missense Polymorphism (rs3924999, Arg253Gln) of Neuregulin 1 and Schizophrenia in Korean Population

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Neuregulin 1 (NRG1) is associated with the pathogenesis of schizophrenia through controlling activation and signaling of neurotransmitter receptors. Influence to schizophrenia development by the NRG1 gene may differ in individuals, and genetic polymorphism is one of the factors affecting their differences. Association between three single nucleotide polymorphisms (SNPs) (rs7014762, -1174 A/T; rs11998176, -788 A/T; rs3924999, Arg253Gln) of NRG1 and the development of schizophrenia was analyzed in 221 schizophrneia and 359 control subjects. Polymerase chain reaction and direct sequencing were performed to obtain genotype data of NRG1 SNPs of the subjects. In analysis of genetic data, multiple logistic regression models (codominant1, codominant2, dominant, recessive, and log-additive model) were applied. SNPStats and SPSS 18.0 were used to calculate odds ratio (OR), 95% confidence interval (CI), and p-value of each model. The genotype distributions of rs3924999 were associated with schizophrenia development (OR=0.67, 95% CI=0.47-0.95, p=0.022 in the dominant model and OR=0.69, 95% CI=0.51-0.93, p=0.013 in the log-addtive model) and allelic distributions also showed significant association (OR=0.70, 95% CI=0.52-0.93, p=0.014). The results suggest that rs3924999 of the NRG1 gene may be associated with schizophrenia susceptibility.

Key words: association, neuregulin 1, schizophrenia, single nucleotide polymorphism

INTRODUCTION

Neuregulin 1 (NRG1) is a ligand for the NEU/ERBB2 protooncogene and closely related to cell-cell signal interactions required for the growth and development of multiple organ systems. NRG1 exerts its effect on the epithelium, cardiovascular system, and central nervous system (CNS) [1]. In early embryogenesis, NRG1 is expressed on neural tissue, respiratory epithelium, and endocardium, and in later stage predominantly expressed in neural tissue [2]. NRG1 has also been studied in the field of modulating function of synaptic plasticity [3]. Neuroplasticity is a keyword for schizophrenia pathogenesis. Genetic factors which promote neuronal development and modulate synaptic plasticity may influence the development and symptoms of schizophrenia. NRG1 plays a role in antipsychotic treatment of schizophrenia [4] and may affect dopamine receptors (D2 and D3) [5]. NRG1 is involved in the abnormal gamma-aminobutyric acid (GABA) neurotransmission in schizophrenia, together with ERBB4, which is synaptic receptor of NRG1 and regulates synaptic maturation [6]. Furthermore, *N*-methyl-D-aspartate (NMDA) receptor functional change is related to schizophrenia [7]. NMDA receptor hypofunction contributes to excessive NRG1-ERBB4 signaling in schizophrenia [8]. Increased NRG1-ERBB4 expression was found

Table 1. Primers for polymerase chain reaction

SNP	Sense (5′-3′)	Anti-sense (5′-3′)	Size (bp)
rs7014762	TGCCAACTTGCAGAATCTTGGG	AATGGGCGATAGATCCACACTG	633
rs11998176	CAGTGTGGATCTATCGCCCATT	AACGCTCTCTCTCCTTGCAGCG	621
rs3924999	GATCCATTTTCGCTCATCCATTT	CCCAAAGAGCTGGGATTACAGTT	693

SNP, single nucleotide polymorphism; bp, base pair.

in the prefrontal cortex of postmortem schizophrenic patients [9].

Genetic association between the NRG1 gene and schizophrenia has been reported in many studies. NRG1 polymorphisms were reported to be associated with susceptibility to schizophrenia in Iceland population [10], and replication studies of Scottish [11] and Chinese [12] populations. In another Scottish population study, NRG1 was associated with bipolar disorder as well as schizophrenia [13].

In this study, we investigated the relationship between NRG1 polymorphisms and the development of schizophrenia in Korean population.

MATERIALS AND METHODS

Schizophrenia and control subjects

A total of 221 schizophrenia patients and 359 control subjects (44.2±6.3 years) were recruited. The schizophrenia group consisted of 122 males and 99 females, and the control group was comprised of 180 males and 179 females. Schizophrenia patients were selected among participants who visited at the Departments of Neuropsychiatry in the East-West Neomedical Center and Kyung Hee Medical Center, Seoul, Korea. Patients were diagnosed with schizophrenia by two psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV). Subjects with other psychiatric disorders, neurological diseases, and any severe diseases were excluded. Controls subjects were recruited among participants who assessed as mentally healthy through a general health examination program. This study was conducted in accordance with the guidelines of the Helsinki Declaration and approved by the Ethics Review Committee of Medical Research Institute, Kyung Hee University Medical Center, Seoul, Korea. Informed consent was obtained from all subjects.

SNP selection and genotyping

We searched the promoter and coding regions of the NRG1 gene in the SNP database of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP, BUILD 135), and selected two promoter SNPs (rs7014762, -1174 A/T and rs11998176, -788 A/T) and one missense SNP (rs3924999,

Arg253Gln) among the NRG1 SNPs. Peripheral blood sample of each subject was collected in heparin or EDTA tubes. DNA Isolation Kit for Cells and Tissues (Roche, Indianapolis, IN, USA) was used for extracting genomic DNA. Polymerase chain reactions (PCRs) were performed as the following condition: 35 cycles at 94°C for 30 sec, 58°C for 30 sec, 72°C for 30 sec, and 1 cycle at 72°C for 5 min for the final reaction. The primer sequences for PCRs were as following: rs7014762 (sense, TGCCAACTTGCAGAATCTTGGG; anti-sense, AATGGGCGATAGATCCACACTG), rs11998176 (sense, CAGTGTGGATCTATCGCCCATT; anti-sense, AA-CGCTCTCTCTCCTTGCAGCG), and rs3924999 (sen-se, GATCCATTTTCGCTCATCCATTT; anti-sense, CC-CAAAGAGCTGGGATTACAGTT) (Table 1). The PCR products were processed through direct sequencing (MACROGEN, Seoul, Korea), and genotypes of each SNP were analyzed with SeqManII software (DNASTAR, Madison, WI, USA).

Statistical analysis

We applied multiple logistic regression models in analysis of genotype data: codominant1 (major allele homozygotes vs. heterozygotes), codominant2 (major allele homozygotes vs. minor allele homozygotes), dominant (major allele homozygotes vs. heterozygotes+minor allele homozygotes), recessive (major allele homozygotes+heterozygotes vs. minor allele homozygotes), and log-additive (major allele homozygotes vs. heterozygotes vs. minor allele homozygotes) models [14]. Odd ratios (OR), 95% confidence intervals (CI), and p-values were calculated with SNPStats (http://bioinfo.iconcologia.net/index.php?module=Snpstats) and SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Haploview version 4.2 (Daly Lab, Cambridge, MA, USA) was used in determining linkage disequilibrium (LD) block and haplotypes. The p-value<0.05 was considered significant.

RESULTS

Two hundred twenty one schizophrenia patients and 359 control subjects were analyzed. The age of the schizophrenia group was 42.0±10.6 (mean age±standard deviation) years and that of the control group was 44.2±6.3 years (data not shown).

Table 2. Genotype and allele frequencies of NRG1 SNPs in controls and schizophrenia

SNP	Type	Control n (%)	Schizophrenia n (%)	Model	OR (95% CI)	p	Fisher's exact p
rs7014762	T/T	155 (43.9)	97 (44.1)	Codominant1	1.05 (0.73-1.51)	0.79	
-1174 A/T	A/T	157 (44.5)	100 (45.5)	Codominant2	0.92 (0.51-1.63)	0.76	
	A/A	41 (11.6)	23 (10.4)	Dominant	1.02 (0.72-1.44)	0.90	
				Recessive	0.89 (0.52-1.54)	0.69	
				Log-additive	0.99 (0.76-1.27)	0.92	
	T	467 (66.1)	294 (66.8)		1		
	A	239 (33.9)	146 (33.2)		0.97 (0.75-1.25)	0.82	
rs11998176	A/A	309 (86.1)	183 (82.8)	Codominant1	1.30 (0.81-2.08)	0.27	
-788 A/T	A/T	48 (13.4)	37 (16.7)	Codominant2	0.85 (0.08-9.54)	0.89	1.00
	T/T	2 (0.5)	1 (0.5)	Dominant	1.28 (0.81-2.04)	0.29	
				Recessive	0.82 (0.07-9.15)	0.87	
				Log-additive	1.24 (0.80-1.93)	0.33	
	A	666 (92.8)	403 (91.2)		1		
	T	52 (7.2)	39 (8.8)		1.24 (0.80-1.91)	0.33	
rs3924999	A/A	198 (55.2)	143 (64.7)	Codominant1	0.70 (0.49-1.01)	0.05	
Arg253Gln	A/G	139 (38.7)	71 (32.1)	Codominant2	0.45 (0.19-1.09)	0.08	
	G/G	22 (6.1)	7 (3.2)	Dominant	0.67 (0.47-0.95)	**0.022**	
				Recessive	0.51 (0.21-1.23)	0.12	
				Log-additive	0.69 (0.51-0.93)	**0.013**	
	A	535 (74.5)	357 (80.8)		1		
	G	183 (25.5)	85 (19.2)		0.70 (0.52-0.93)	**0.014**	

NRG1, neuregulin 1; SNP, singe nucleotide polymorphism; OR, odds ratio; CI, confidence interval. The p-values were calculated from logistic regression analysis adjusted by age and sex. The bold number is significant p-value.

Table 2 shows the genotype and allele frequencies of the three examined SNPs (rs7014762, -1174 A/T; rs11998176, -788 A/T; rs3924999, Arg253Gln) of NRG1 in the control group and the schizophrenia group. The genotype distributions of the three tested SNPs were consistent with the Hardy-Weinberg equilibrium (HWE) in the control group (rs7014762, HWE p=0.91; rs11998176, HWE p=0.71; rs3924999, HWE p=0.78, data not shown).

The genotype (T/T:A/T:A/A genotypes) distributions of rs7014762 in the control and schizophrenia groups were 43.9%:44.5%:11.6% and 44.1%:45.5%:10.4%, respectively. The allele (T:A alleles) distributions of rs7014762 in the control and schizophrenia groups were 66.1%:33.9% and 66.8%:33.2%. The genotype (A/A:A/T:T/T) distributions of rs11998176 in the control and schizophrenia groups were 86.1%:13.4%:0.5% and 82.8%:16.7%:0.5%, respectively. The allele (A:T) distributions of rs11998176 in the control and schizophrenia groups were 92.8%:7.2% and 91.2%:8.8%, respectively. The genotype and allele distributions of two SNPs (rs7014762 and rs11998176) were not significant between controls and schizophrenia patients (p>0.05, Table 2). However, the genotype (A/A:A/G:G/G) distributions of rs3924999 in the control and schizophrenia groups were 55.2%:38.7%:6.1% and 64.7%:32.1%:3.2%, respectively. The

genotype distributions of rs3924999 showed differences. The differences were significant association (OR=0.67, 95% CI=0.47-0.95, p=0.022 in the dominant model and OR=0.69, 95% CI=0.51-0.93, p=0.013 in the log-additive model). The allele (A:G) distributions of rs3924999 in the control and schizophrenia groups were 74.5%:25.5% and 80.8%:19.2%. The difference of allele distributions showed significant association (OR=0.70, 95% CI=0.52-0.93, p=0.014) (Table 2). The results suggest that rs3924999 of NRG1 may contribute to risk for schizophrenia susceptibility.

LD block was determined using Haploview 4.2, and one LD block between rs7014762 and rs11998176 was constructed (Fig. 1). In order to investigate association between the haplotypes of LD block SNPs and schizophrenia, haplotype analysis was performed. There were three haplotypes (TA, 0.585; AA, 0.336; TT, 0.078 in frequency). The haplotypes showed no significant difference between the control group and the schizophrenia group (Table 3).

DISCUSSION

NRG1 is well-known schizophrenia-related gene, and there have been association studies of NRG1 SNPs with schizophrenia susceptibility from a decade ago [10-13]. A genome-wide

association study (GWAS) used 33 families consisted by 110 patients in Iceland was performed in the year 2002 and the researchers identified NRG1 as a schizophrenia candidate gene. They concluded that the 'at-risk' haplotypes at the 5' end of the NRG1 gene is responsible for the analysis result [10]. Replication study was conducted in Scottish population, and they found that the 'at-risk' haplotype and rs35753505 SNP were still significant [11]. However, in another replication study conducted in Han Chinese populations, they did not show significance, instead, they found a new haplotype 'Hap china 3' consisted by three groups of microsatellite markers [12]. In another Scottish population study, rs6988339 and rs3757930 SNPs, which were in near the 3' region of the NRG1 gene and not previously studied in Iceland population, were associated with bipolar disorder as well as schizophrenia [13]. Such differences suggest that various SNPs of NRG1 may contribute or not to the schizophrenia development in certain populations.

In our study results, two promoter SNPs (rs7014762, -1174 A/T and rs11998176, -788 A/T) selected from the NRG1 gene were not statistically associated with risk of schizophrenia, and one missense SNP (rs3924999, Arg253Gln) was associated with the development of schizophrenia.
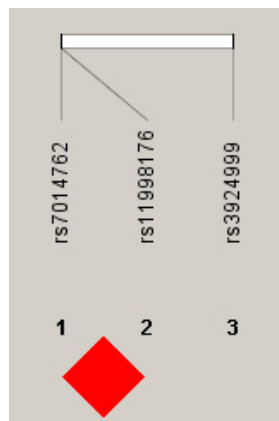
Fig. 1. Linkage disequilibrium (LD) analysis among rs7014762, rs11998176 and rs3924999. One LD block was constructed between rs7014762 and rs11998176 (D'=1.0, r-squared=0.044).

The rs7014762 SNP was once reported to be associated with bipolar disorder, however, not with schizophrenia [15]. And our study result on schizophrenia was consistent with the previously mentioned study. There was no prior study of rs11998176 (promoter, -788 A/T). Therefore this is the first study to investigate the relationship between rs11998176 and schizophrenia. However, the study result showed no association between rs11998176 and schizophrenia development in Korean population. The rs3924999 SNP has been frequently reported in schizophrenia-related researches. In a NRG1 genetic study in Chinese population it was reported that rs3924999 and rs2954041 were associated with schizophrenia susceptibility [16]. Another Chinese population study showed that rs3924999 was associated with schizophrenia risk, especially in the subgroup of patients with positive symptom. In the other study of Chinese population, rs3924999 was associated with the prepulse inhibition (PPI), which is a measurable schizophrenia phenotypic feature [17]. On the contrary, a Germany group reported that PPI was not related with rs3924999 [18]. Along with PPI, auditory P300 is externally measurable feature in schizophrenia [19]. Association of rs3924999 with auditory P300 in schizophrenia was reported [20]. In our study result, the G allele of rs3924999, which is minor allele, showed less odds ratio in the schizophrenia group than that of the control group in the dominant model (OR=0.67, 95% CI=0.47-0.95, p=0.022) and the log-additive model (OR=0.69, 95% CI=0.51-0.93, p=0.013). Allelic distribution of rs3924999 was significantly different between schizophrenia and controls (OR=0.70, 95% CI=0.52-0.93, p=0.014). These results were slight association, however, it might be suggested from the result that the variant allele G may have a dominant effect and more protective effect from schizophrenia than the majority allele A of rs3924999, or the A allele of rs3924999 might be an allele with increased risk of schizophrenia development. The G allele of rs3924999 changes original 253th amino acid arginine to variant amino acid glutamine, and its change might have modified the cell signaling of NRG1.

In conclusion, this study results showed that a missense SNP rs3924999 (Arg253Gln) of NRG1 was weakly associated with

Table 3. Haplotype analysis of NRG1 SNPs

Haplotype	Frequency	Schizophrenia		Control		Chi-square	p
		+	-	+	-		
TA	0.585	422.6	295.4	256.3	185.7	0.088	0.7674
AA	0.336	243.4	474.6	146.7	295.3	0.060	0.8064
TT	0.078	52.0	666.0	39.0	403.0	0.946	0.3307

NRG1, neuregulin 1; SNP, singe nucleotide polymorphism. Haplotypes were consisted of rs7014762 and rs11998176.

the development of schizophrenia in Korean population. Two other SNPs of NRG1 (rs7014762 and rs11998176) did not show significant associations. The results suggest that rs3924999 of the NRG1 gene may contribute to schizophrenia development in Korean population.

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