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Original Article

Presence of a genetic association between NRG-3 SNP rs17101193 and schizophrenia

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Abstract

Aim: NRG-3 gene is a member of neuregulin signaling pathway and alleles of several single nucleotide polymorphisms (SNP) located in this gene are suspected to be associated with schizophrenia. During this work, we have done a study to investigate the potential association of the SNP *rs17101193* of NRG-3 gene.

Material and Methods: We used the DNA samples from 301 unrelated schizophrenia patients and 326 healthy control samples collected from Malatya-Turkey to determine the SNP genotypes. After genotyping, we compared the genotypes and alleles in terms of distributions between cases and controls. We also applied the recessive models for both alleles to get a more clarified result. We used Chi-Squared test and Fisher's exact test for hypothesis testing.

Results: Even though the difference between the case and control groups were not significant in context of distributions of two alleles of *rs17101193*, the distributions of genotypes were significantly different (p = 0.046). On the other hand, one of the genotypes (AA) was seen only in the patients.

Conclusion: The results of our analyses and statistical test indicated that *rs17101193* SNP of NRG-3 gene has a potential to be associated with schizophrenia and homozygosity of "A" allele seems to be a risk factor in our population. More evidence from separate case-control studies from different populations may be required to strengthen this idea.

Keywords: Association; genetics; NRG3; polymorphism; schizophrenia; SNP

INTRODUCTION

Schizophrenia (MIM181500) is a chronic psychiatric disease with clinical characteristics including cognitive deficits as well as positive and negative symptoms. The prevalence of schizophrenia is 0.5-1% in the general population (1). Schizophrenia is one of the complex and multifactorial diseases which is affected by several susceptibility genes, epigenetics, environmental and stochastic factors (2). The estimated heritability of disorder is close to 80% (3). There is a lot of genes showed association with schizophrenia (4).

Despite several studies which include the ones in case-control format as well as genome wide association studies (GWAS), and meta-analysis indicated several candidate genes for schizophrenia, the molecular pathology of disease is still unclear. Many association studies performed so far have revealed controversial results. Hence, the results of studies showing positive or negative associations remain to be replicated in different populations with larger number of subjects (5).

Since the association of the gene coding for neuregulin-1 (NRG1) with schizophrenia was shown (6 and 7), the other members of neuregulin pathway have been suspected and investigated in different populations to explain the molecular basis of disease phenotype (8). One of these candidate genes is NRG3 (responsible for synthesis of neuregulin 3 protein). NRG3 belongs to the neuregulin gene family. This gene family has several members and includes EGF (Epidermal Growth Factor) like signaling molecules which take part in cellular communication (9). Expressions of the genes belong to this family are seen in multiple tissues and NRG1,2 and 3 are known to act in functioning and development of the central nervous system (10).

In this work, our group has done a study in case-control format to shed a light on the association of schizophrenia with the SNP *rs17101193* of NRG3 gene. The SNP is a C to A change located in the genomic position "chr10:82985170" (GRCh38.p12) which results in the amino acid change N552K (Asp 552 Lys: the change of Asparagine at the 552nd amino acid position to Lysine). This SNP attracted

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our attention since its minor allele was seen in multiple affected members of several unrelated families in a next-generation sequencing study (11). According to our scientific literature data base search, our work is the first case-control study to be done on the association of the NRG3 SNP *rs17101193* and schizophrenia.

MATERIAL and METHODS

The local ethics committee has ethically approved all applications and procedures in this study according to Declaration of Helsinki (Protocol #2014/132). Each volunteer provided written informed consents forms. Clinical diagnosis and evaluation of patients and controls were performed by the second author who is a senior psychiatrist in Psychiatry Department at Inonu University, School of Medicine.

The case group was consisting of 301 unrelated schizophrenia patients from Malatya (Turkey) (ages between 18 and 64 years - average = 36.9, SD: 11.6). For diagnosis criteria, The Structured Clinical Interview for the DSM-IV (SCID-I) has been used (12).

All patients were followed for more than 3 years (average follow-up length in years was fifteen and standard deviation was ten). In average, the patients were diagnosed at 24.4 years of age (standard deviation: 8.6). The case group included the patients who have been diagnosed with a subtype of schizophrenia. All patients were in Turkish ethnic origin. Patients excluded from this study were the ones who have been diagnosed to have schizophrenia form disorder, mood disorder showing psychotic features, psychotic disorder which was substance-induced, schizoaffective disorder, paranoid personality disorder, psychotic disorder due to a general medical condition, schizoid disorder and schizotypal disorder. In the group of controls, we had 326 healthy people who were in Turkish ethnic origin, but not related to each other.

DNA extraction and determination of SNP genotypes

The peripheral blood samples collected in EDTA coated tubes used for extraction of total DNA. For protecting the confidentiality of the volunteers, we applied a coding system to DNA and blood samples. DNA was isolated using Invitrogen Purelink DNA mini kit (California, USA).

We determined the genotypes of SNP using a genotyping assay designed by Applied Biosystems for the SNP "*rs17101193*" (TaqMan ® Catalog number: 4351379) with appropriate PCR Master Mix (Catalog number: 4304437) on a Real-time PCR machine (Applied Biosystems, StepOne Plus. California, USA). We applied the instructions of manufacturer for reactions. The total reaction volume was 10 µL, genomic DNA used 2 µL (concentration: 20 ng/µL), TaqMan ® Universal PCR Master Mix: 5 µL, TaqMan ® genotyping assay: 0.5 µL, and water: 2.5 µL. PCR was started with 10 minutes at 95°C and 40 cycles carried out under the conditions: 15 seconds at 95°C and 1 minute at 60°C. SNP detection was completed with an endpoint plate read for each reaction after PCR amplification has finished. For analyses of SNP association, real-time PCR Genotyping results of controls were compared with that of patients.

Statistical analysis

Distributions of alleles and genotypes are represented by counts and frequencies. Chi-Square method was used for testing Hardy–Weinberg equilibrium and version 4.2 of Haploview software used to confirm the results (13). For determining possible disease associations, control and case groups were compared for allele and genotype distributions. The data collected from genotyping experiments as well as allele counts calculated from these data were subjected to chi-squared (Pearson) test for determining statistical significance of differences seen between the patient and control groups. We used Fisher's exact test method for testing the recessive model for "A" allele. Statistical power was calculated using the G*Power software (14).

RESULTS

We have determined the genotypes of 301 schizophrenia patients and 326 healthy control samples collected from Malatya which is a city located in Turkey for testing a possible association between schizophrenia and the SNP *rs17101193* of NRG3 gene. We have done the comparison of our control and case groups for distributions of genotypes and alleles of the SNP. To our knowledge there are no studies in the literature done in case-control format carried out in samples from any part of Turkey for investigating the genetic association with this SNP and schizophrenia.

Table 1. Distributions and frequencies of genotypes found in the patient and control groups

	Genotype Counts (Frequencies)			p-value* HWE**	p-value* Genotype
	C/C	A/C	A/A		
Patients	253 (0.84)	43 (0.143)	5 (0.017)	0.056	0.046
Controls	286 (0.88)	40 (0.12)	0	0.24	

*: Chi-Squared test
**: Hardy Weinberg Equilibrium

Table 2. Distributions and frequencies of alleles found in the patient and control groups

	Allele Counts (Frequencies)		p-Value (Allele)*
	C	A	
Patients	549 (0.91)	53 (0.09)	0.07
Controls	612 (0.94)	40 (0.06)	

*: Chi-squared test

The SNP we have screened in this study was in Hardy-Weinberg Equilibrium in both control and case groups (p>0.05). Distributions of genotypes expressed as counts and frequencies obtained from control and patient groups are given in Table 1. Table 2 shows the distributions of alleles in two groups. The results of Pearson's Chi-Squared tests comparing the allele and genotype distributions between patient and control groups were given in both tables in the p-value columns. Even though there was no significant difference between the patient and control groups in distributions of alleles (p = 0.07), the difference seen in the distributions of genotypes were significant (p = 0.046). When we tested the AA genotype against the other genotypes for recessive model, we have seen a significant increase in the patients since there is no homozygotes for the A allele in any control sample (Table 3). For detecting a locus with an effect size of 0.3, the statistical power of the sample we studied was >80% (significance level = 0.05).

Table 3. Comparison of patient and control groups for distribution of homozygotes for A and C recessive models

A-Recessive Model	A/A	C/A + C/C	P-Value
Case	5	296	0.025*
Control	0	326	
C-Recessive Model	**C/C**	**C/A + A/A**	
Case	253	48	0.18**
Control	286	40	

*: Fisher's exact test
**: Chi-squared test

DISCUSSION

Abnormalities in brain development are thought to be related with schizophrenia. Protein products of neuregulin gene family are important actors for formation of specific brain circuitries and several genes which are the members of the family are linked to the disease (15). After positional cloning (6) and a screening study done in a large Scottish sample (7) the gene coding for neuregulin-1 which is called NRG1 was implicated as one of the genes susceptible for schizophrenia. In a study done with families with multiple members who were diagnosed for schizophrenia, exons of the genes which are the members of neuregulin gene family were sequenced and specific alleles of several SNPs in different genes were carried by multiple affected members of unrelated families (11). One of those SNPs was *rs17101193* SNP in the NRG-3 gene.

Our aim in the present work was to investigate the potential association of *rs17101193* SNP and schizophrenia in a case-control study. We have genotyped the SNP in groups of controls and cases and compared two groups for the distribution of alleles of the SNP and their genotypes. Hardy-Weinberg equilibrium was approved in both control and case groups (p>0.05). When we compared the distributions of A and C alleles of the polymorphism, any significant difference did not appear between two groups.

However, the comparison of two groups for the genotypes revealed an interesting difference. The homozygosity of A allele was seen only in the patients group. The AA genotype was not present in the control group thus gave rise to a difference between two groups. The statistical significance of this difference was identified by Pearson's goodness of fit Chi-Square test which indicated that the difference was significant and was not caused by the margins (p=0.046). We have also applied the recessive models for both alleles. The recessive model for "A" allele clarified the picture better since the difference of two groups was much more obvious when we compared the counts of AA genotype with the total of two other genotypes (AC and CC). The statistical significance of this difference was validated by the Fisher's exact test (p=0.025).

CONCLUSION

The results of experiments, analyses and statistical tests we have carried out suggest that the *rs17101193* SNP located in the NRG-3 gene is associated with schizophrenia in our patients and the "AA" was identified as a risk genotype. Since the frequency of minor allele is relatively low, confirmation of this result with larger case and control groups from different populations may strengthen the idea of association between *rs17101193* SNP and schizophrenia.

Competing interests: The authors declare that they have no competing interest.

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Ethical approval: This study was approved by the Institutional Ethics Committee and conducted in compliance with the ethical principles according to the Declaration of Helsinki.

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