



Association analysis of two polymorphisms in neuregulin-1 gene in a Turkish schizophrenia sample

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Abstract

Aim: NRG1 is one of the genes that was reported to have susceptibility alleles effecting the phenotype of schizophrenia. There are many SNPs (single nucleotide polymorphisms) located in the NRG1 gene with suspected alleles which were associated to schizophrenia. In this study, we investigated the potential associations between schizophrenia and NRG1 polymorphisms rs4560751 and rs3802160.

Materials and Methods: We collected DNA samples of 96 patients diagnosed for schizophrenia and 100 healthy controls living in Malatya-Turkey. We genotyped two NRG1 SNPs mentioned above and compared case and control groups for distributions of alleles and genotypes. We also tested the potential associations in dominant models. We used Chi-Squared test for hypothesis testing.

Results: No significant differences were seen between the groups of patients and controls for the distributions of alleles and genotypes of two NRG1 SNPs we have screened.

Conclusion: The results of our experiments and statistical tests we have done indicated that in the population we have screened there was no sufficient evidence to support the association of NRG1 SNPs rs4560751 and rs3802160 with schizophrenia.



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Introduction

Schizophrenia is a multifactorial chronic psychiatric disease (MIM181500) with a prevalence of 0.5-1% in the general population. Cognitive deficits and positive and negative symptoms are major clinical characteristics of the disease [1]. Since it is a complex disorder, genetic, epigenetic, environmental, or stochastic factors affect schizophrenia [2]. The estimated heritability of schizophrenia is about 80% [3]. Like the other multifactorial diseases, instead of only one or a few responsible genes, a lot of susceptibility genes and their risk alleles have been found to be associated with schizophrenia [4]. Nevertheless, the pathology of disease at the molecular level is unclear, despite several candidate genes have been revealed after case-control studies, genome wide association studies (GWAS), and meta-analyses carried out up to now [5]. Because of the controversy between the results of independent genetic association studies, confirmation of positive or negative associa-

tions helps to clarify the effects of genetic factors on the disease [6, 7].

The first gene reported to be linked to schizophrenia is the gene coding for neuregulin-1 (NRG1) [8, 9]. Following this, several NRG1 SNPs have been screened to test their associations with schizophrenia. In one of those studies 49 markers including SNPs in NRG1 gene among a list of other SNPs in 5 other genes were screened in case-control format. In addition to epistatic associations, interaction between schizophrenia and two NRG1 SNPs (rs4560751 and rs3802160) were reported [10]. We have screened these two single nucleotide polymorphisms in a case-control group consisted of patients diagnosed for schizophrenia and controls from Malatya-Turkey to reveal if there is any single-SNP association with schizophrenia in our population. The first SNP we have screened called rs4560751 is a G to T alteration in the genomic position “chr8: 31672512” and the second SNP rs3802160 is an A to G change located in the genomic position “chr8:32547111” (GRC h38.p13).

Our scientific literature database search showed that, as a case-control study, the present work is the first one to be carried out for investigating the potential associations of

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NRG1 SNPs rs4560751 and rs3802160 with schizophrenia in the patients and control samples from a Turkish population.

Materials and Methods

All procedures and applications in this study have been ethically approved according to Declaration of Helsinki by the local ethics committee (Malatya Clinical Research Ethics Committee, Protocol : 2021/08). The volunteers included in both case and control groups provided written informed consents forms.

The patients and controls were clinical diagnosed and evaluated by the second author who is a senior psychiatrist at Inonu University, School of Medicine, Department of Psychiatry.

In the group of patients, we had 96 unrelated schizophrenia cases from Malatya (Turkey) (average of ages: 36.9 years, 18 min, 64 max - SD: 11.6). The diagnoses were carried out according to The Structured Clinical Interview for the DSM-IV (SCID-I) [11]. The patients included in this study were followed for more than 3 years (average: 15 years, SD: 10). The age in which patients were diagnosed is 24.4 in average (SD: 8.6). All the patients declared that they had Turkish ethnic origin.

In this study, we excluded the patients with schizophrenia form disorder, schizoid disorder, mood disorder showing psychotic features, paranoid personality disorder, psychotic disorder which was substance-induced, schizoaffective disorder, schizotypal disorder, and psychotic disorder due to a general medical condition. The control group was consisted of 100 healthy people with Turkish ethnic origin according to their self-identification. None of the samples in control group were related to each other.

Isolation of DNA and SNP genotyping

The total DNA samples were isolated using peripheral blood collected in the tubes coated with EDTA. The coding systems were applied to all DNA and blood samples for protecting the confidentiality of the volunteers. Invitrogen Purelink DNA mini kit (California, USA) was used for DNA isolation.

We genotyped each sample using specific genotyping assays designed by Applied Biosystems for the SNPs rs4560751 (TaqMan® Catalog number: C__28957721_10) and rs3802160 (TaqMan® Catalog number: C__27503359_10) with the TaqMan® PCR Master Mix (Catalog number: 4304437) on Step One Plus real-time PCR system of Applied Biosystems (California, USA). The reactions were done under the conditions suggested by manufacturer (in total of 10 µL, 2 µL of genomic DNA (concentration: 20 ng/ µL), 5 µL of TaqMan® Universal PCR Master Mix, 0.5 µL of TaqMan® genotyping assay and 2.5 µL of water). We applied a hot start (10 minutes at 95 °C) before PCR cycles and completed 40 cycles of: 15 seconds at 95 °C and 1 minute at 60 °C. By the end of the PCR amplification, SNP detection was performed with endpoint plate reads. For the analyses of SNP association, genotyping data collected from real-time PCR were compared between the groups of patients and controls.

Statistical analysis

The genotypic and allelic distributions are represented as counts and frequencies. The allele counts and frequencies of the SNPs in the case and control groups were calculated using real-time PCR genotyping data. The accordance of both groups with Hardy–Weinberg equilibrium was tested with Pearson’s Chi-Square method. When the groups of patients and controls differ in counts of SNP genotypes and alleles, the statistical significances of differences were also determined by Chi-Squared test. We have used G*Power software to calculate the statistical power of this study [12].

Results

In this study, we have screened the genotypes of two SNPs (rs4560751 and rs3802160) located on the NRG1 gene in a group of schizophrenia patients and a group of controls to find potential single-SNP associations. In this sample, the statistical power was greater than 80% for detecting a locus with an effect size of 0.3 (significance level = 0.05).

The results are reported as distributions of genotypes and alleles expressed as counts (n) and frequencies in the Table 1. The Pearson’s Chi-squared test results as p-values showing the statistical significance of differences are given in the lines marked as (P) in the same table. These tests indicated that none of the differences seen between the case and control groups in the distributions of genotypes and alleles of SNPs rs4560751 and rs3802160 were statistically significant. The case and control groups were both in Hardy-Weinberg equilibrium (Chi-Square p-values are given in Table-1, column labelled “HWE-p”). To test the accordance with Hardy-Weinberg Equilibrium, observed counts of each genotype were compared with its expected value which was calculated according to Hardy-Weinberg Law (Table 2). We have also tested the potential disease associations in dominant models by comparing two groups for counts of carriers and non-carriers of each SNP allele (Table 3). After chi-squared tests done, this model did not reveal any significant difference between the case and control groups either.

Discussion

In this article, we present the results of a study done in case-control format to examine the potential associations between two SNPs in the NRG1 gene (rs4560751 and rs3802160) and schizophrenia. To our knowledge, this is the first study to investigate whether if there is an association of these SNPs with schizophrenia in a Turkish population.

NRG1 is the first gene found to be linked and associated with schizophrenia [8, 9]. Following these two studies, more polymorphisms in the NRG1 gene have been screened in various populations [13, 14]. The meta-analysis studies also supported the disease association of NRG1 gene [5]. Among the NRG1 SNPs, rs4560751 and rs3802160 were reported to show an interaction with schizophrenia in a case-control sample from Germany [10]. Nevertheless, in the main SNP databases we searched, no other studies appeared to report screening of these SNPs in any other populations. Also, a genetic association found between a

Table 1. The distributions (and frequencies) of genotypes and alleles of rs3802160 and rs4560751 SNPs in the case and control groups.

	Genotype				Allele	
	GG	GT	TT	HWE-p	G	T
rs4560751 MAF*: 0.20 (T)						
Case	75 (0.78)	20 (0.2)	1 (0.01)	0.79	170 (0.9)	22 (0.1)
Control	73 (0.73)	25 (0.25)	2 (0.02)	0.93	171 (0.85)	29 (0.15)
P-Value	0.66				0.37	
rs3802160 MAF*: 0.33 (G)	AA	AG	GG	HWE-p	A	G
Case	40 (0.42)	40 (0.42)	16 (0.17)	0.28	120 (0.625)	72 (0.375)
Control	43 (0.43)	41 (0.41)	16 (0.16)	0.25	127 (0.635)	73 (0.365)
P-Value	0.98				0.84	

*MAF: Global Minor allele Frequency reported in 1000 Genomes Project (www.ensembl.org).

Table 2. Hardy-Weinberg expected distributions of SNP genotypes in the case and control groups.

rs3802160	AA	AG	GG
Case	37.5	45	13.5
Control	40.3	46.4	13.3
rs4560751	GG	GT	TT
Case	75.26	19.48	1.26
Control	73.1	24.8	2.1

Table 3. Comparison of case and control groups for dominant models of rs4560751 and rs3802160 alternate alleles. Numbers of carriers of each allele are given as “Present” and non-carriers as “Absent”. The frequencies are given in parentheses.

rs3802160	A-Present	A-Absent	G-Present	G-Absent
Case	80 (0.83)	16 (0.17)	56 (0.58)	40 (0.42)
Control	84 (0.84)	16 (0.16)	57 (0.57)	43 (0.43)
p	0.9		0.85	
rs4560751	G-Present	G-Absent	T-Present	T-Absent
Case	95 (0.99)	1 (0.01)	21 (0.22)	75 (0.78)
Control	98 (0.98)	2 (0.02)	27 (0.27)	73 (0.73)
p	-		0.4	

SNP and a disease in one population may not appear in another one. Hence, the data obtained from different populations with multiple studies are required to reveal the involvement of a SNP to development of a disease [6, 7]. For the cases of rs4560751 and rs3802160, to fill this gap, we have screened a group of schizophrenia patients (n=96) and a matched group of control samples (n=100) from a Turkish population. The volunteers were from Malatya, Turkey (a city in the Eastern Anatolian Geographical Region of the country). The results of this study may not reflect the entire population of Turkey because the samples were collected from a limited geographical region.

The case and control groups were found similar for the frequencies of alternative alleles of rs4560751 and rs3802160

SNPs in our association analyses at single-SNP levels. Between two groups, there were no significant differences for distributions of alleles, or there was no evidence that supports the presence of an allelic association in this cohort. When frequencies of genotypes at each SNP locus were compared between the case and control groups, no significant differences were seen. In other words, no sufficient evidence to support any disease associations at genotypic level were found.

In addition, we also tested the potential disease associations of the alleles of both SNPs in dominant models. When we compared numbers of the carriers of each allele regardless of the individuals’ zygosity, we were not able to see any significant differences between the case and control groups either.

The possible reasons for associations not to appear in our study might be the inadequate sample size, the differences of populations in risk alleles, the heterogeneity seen in the clinical diagnosis of schizophrenia and its clinical presentation and regional limitations. This is because we have used patient and control groups which were considerably smaller and collected only from Malatya region of Turkey. Therefore, a cohort in a larger size to be collected from an extended geographical region may still reveal presence of genetic associations with the rs4560751 and rs3802160. Apart from the small sample size and geographical limitations, another potential reason for not finding a genetic association between these two SNPs and disease phenotype in our population is the multifactorial and polygenic nature of schizophrenia. This can be supported by the presence of many other genes associated with schizophrenia such as ERBB4 (the receptor for Nrg1 Protein), NRG3, COMT, DRD2, DRD3, DRD4, DAOA, BDNF and TLR2 with hundreds of risk alleles of various SNPs (for example, rs707284, rs839523 and rs7598440 SNPs in the ERBB4 gene, rs17101193 in the NRG3 gene or rs111200466, rs5743708, rs121917864, and rs5743704 SNPs in the TLR2 gene) [4, 15, 16, 17, 18]. Furthermore, when we focused only on the NRG1 gene, a number of SNPs associated with schizophrenia can be seen in the literature [20, 21]. This suggests, the patients who do not carry the risk alleles of rs4560751 and rs3802160 SNPs, may have the risk alleles of other SNPs in the same gene, or the risk hap-

lotypes constructed by those alleles of several SNPs. On the other hand, the epistatic interactions between various SNP-allele combinations of several genes can be effective for the development of schizophrenia phenotype [22].

The presence of disease association of a gene in one population may not always appears in all other populations and this absence of association does not always provide the sufficient evidence for the complete absence of involvement of that gene to the development of disease phenotype. In the case of *NRG1*, one of the recent functional studies showed that *NRG1* haploinsufficient mouse models exhibit behavioral phenotypes similar to schizophrenia deficits [23]. Considering this, we can still think that the variants of *NRG1* gene could affect schizophrenia phenotype, but this involvement requires more SNPs in the gene to be screened and analyzed at the levels of haplotypes and epistatic interactions.

Conclusion

In conclusion, our study did not reveal any significant evidence for the presence of a single SNP association between development of schizophrenia and rs4560751 and rs3802160 SNPs of the *NRG1* gene in the samples that collected from Malatya - Turkey. Screening more SNPs in the *NRG1* and the other related genes followed by the haplotype and epistatic association analyzes may help to understand better the genetic background of schizophrenia.

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Conflict of interests

Absence of any conflict of interest has been declare by all authors.

Ethics approval

It was approved by the Malatya Clinical Research Ethics Committee with the Protocol Code of 2021/08. All applications were conducted in compliance with the principles of ethics according to the Helsinki Declaration.

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