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Investigation of Neuregulin-1 Gen rs6994992 Polymorphism in Gifted Students in Turkish Population

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Abstract

***Aim**:* The aim of this study is to investigate whether neuregulin-1 gene (NRG1) rs6994992 polymorphism has any role in gifted students in Turkish population.

***Methods**:* During the 2017-2018 academic year, 97 gifted students (experiment group) and 99 students with normal development (control group) attending 5th grade of different public schools participated in the research. Genomic DNA was extracted from the oral epithelial cells. Genotyping of the molecular variant was performed by Polymerase Chain Reaction- Agarose gel electrophoresis-DNA sequencing techniques.

***Results**:* Genotype difference in gifted students was estimated as odds ratio and 95% confidence interval using binary logistic regression models. While the frequencies of CC (wild type), CT (heterozygous), TT (polymorphic type) genotypes were 33.0, 53.6 and 13.4%for the experimental group, respectively, they were observed as 33.0, 60.0 and 7.0% in the control group. In the Turkish population, no association could be detected for both genotype and allele distribution for NRG1 rs6994992 C/T polymorphism between gifted and control group (OR: 0.538, CI: 0.190-1.525, p = 0.244).

Conclusion: According to the results of our study, there was no relationship between NRG1 rs6994992 polymorphism and gifted students in the Turkish population.

Keywords: NRG1, rs6994992, SNP, gifted, intelligence, Turkish population

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1. INTRODUCTION

Neuregulins are ligands that bind to ErbB receptors, enable activation, but also mediate cell-to-cell interactions. They are paracrine, autocrine, and juxtrachrin signal peptides linked to the Epidermal Growth Factor (EGF) family. They are encoded with four different genes: NRG1, NRG2, NRG3 and NRG4 [1, 2, 3]. Neuregulin-1 (NRG1) is a gene with more than 20 exons and large introns located in the 8p13 region on the chromosome. It belongs to the Epidermal Growth Factor Receptor (EGFR) family, and encodes an endogenous glycoprotein of 44 kD. These proteins are mostly secreted from the nervous system, cardiovascular system, intestines, and kidneys, and operate signal pathways that will cause stimulation or inhibition of functions such as proliferation, apoptosis, migration, differentiation, and adhesion in the cell. NRG1 is effective in many physiological and pathophysiological mechanisms, and in the nervous system. By providing endothelial barrier function, it plays a role in protecting blood brain barrier and brain microvascular structures and increasing permeability [4, 5]. Neuregulin protein, also known as the glial growth factor encoded by the NRG1 gene, is an endogenous protein that plays a critical role in the intercellular communication and signaling system. It is also expressed on motor axons in the nerve and is required for Schwann cell development. NRG1 is also a ligand for the NEU/ERBB2 protooncogene. It has been shown that NEU/ ERBB2 is closely related to EGFR. NRG1 produces at least fifteen developmentally regulated proteins [3]. Functionally, various products of this gene have been implicated in various biological processes such as embryogenesis, angiogenesis, breast cancer, nervous system development, myogenesis and gonadogenesis [6]. Neuregulins interact with the transmembrane tyrosine kinase receptors of the ErbB family and send signals to target cells. Receptor-ligand interactions stimulate heterodimerization of receptor monomers, thereby stimulating proliferation, migration and differentiation in cells. NRG1 can acquire specificity by affecting the biological functions of the signal path as well as its specific interest in the receptor types [7]. Transcription factors that bind to these regions of ERBB4 and NRG1 genes predispose to schizophrenia or have a protective effect on the disease [8]. As a result of oxygen-glucose deficiency cytotoxicity and expression of ErbB4, the NRG1 gene has been shown to protect the brain against cerebral ischemia disease [9]. Significant results have been found in the rs35753505 polymorphism genotypes of the NRG1 gene in patients with hepatocellular carcinoma [10]. Another study has discovered that NRG1 gene helps in functional recovery after spinal cord injury [11].

The aim of this study is to investigate whether NRG1 gene rs6994992 polymorphism has any role on intelligence in gifted students attending the 5th grade. This study is the first study that explores the relationship between intelligence and genetics among the Turkish population.

2. MATERIALS AND METHODS

2.1. Subjects

The current study has been carried out in the city of Kocaeli, Turkey, between the years 2017-2019 with the gifted (n= 97) students and students with normal development (n= 99), whose age ranged from 10 to13 and who attended the 5th grade. The experimental group was made up of students who had previously taken Wisc-r and group intelligence test and were diagnosed as gifted. The control group consisted of students attending different public school. No intelligence test was applied to the control group. This can be listed as a limitation of our study.

Prior to data collection research, KOU GOKAEK Non-Interventional Ethics Committee permission numbered 2017/375 was granted and MoNE and Governorship approvals dated 10.05.2018 and numbered 99332089/605.01/9238429 were obtained. All participants provided voluntary consent forms before participating in the study.

2.2. DNA Isolation

Students who participated in the study were asked to clean their teeth and mouths with disposable toothbrushes before saliva samples were

collected. After waiting for 30 minutes, 200 µl saliva samples were taken from each student. The samples were kept in cool containers until they were taken to the laboratory for DNA isolation.

DNA isolation was performed using the EURx GeneMATRIX Tissue & Bacterial DNA Purification Kit (Gdansk Poland, Cat no. E3551) following the procedures recommended by the company.

2.3. Polymerase Chain Reaction (PCR)

Primers were designed using Primer3web version 4.1.0 program [12]. PCR was performed using primers of the NRG1 gene from the isolated DNA. The primers and PCR conditions used in the study can be seen in Table 1 and Table 2. 5x FIREPol Master Mix (Solis BioDyne) was used to prepare the PCR reaction mixture. PCR reaction mix 5x Master Mix: 6µl, 10 µM primer (sense): 0.5 µl, 10 µM primer (antisense): 0.5 µl Mold DNA: 2 µl were used. Bidistillated water was added to 30 µl for the PCR mixture of each gene region. PCR products were run for 30 minutes at 100 volts in agarose gel electrophoresis and visualized with a UV transluminator.

Table 1
Forward and reverse primers of the NRG1 gene

Gene Name	SNP number	Primer sequence
NRG1	rs6994992	Forward- 5'-CCTCCCAAAAAGT CGAGTCA-3' Reverse-5'-CGCTTCAGGAGAA GATCACC-3'

Table 2
PCR conditions for NRG1 gene rs6994992 polymorphism

	Temperature (℃)	Time (second)	Cycle
Predenaturation	94	240	1
Denaturation	95	30	35
Annealing	55	30	
Elongation	72	30	
Final Elongation	72	350	1

2.4. DNA Sequence Analysis

PCR products were purified by the BM Lab in accordance with the kit procedures used with the ExoSAP-ITTM PCR Product Cleanup Reagent (Thermo Fisher Scientific, USA) purification enzyme. Sequence analysis was performed using forward and reverse primers of the purified PCR products NRG1 gene. For the Sanger sequencing, the ABI 3730XL Sanger sequencing device (Applied Biosystems, Foster City, CA) and the BigDye Terminator v3.1 Cycle Sequencing Kit were used in the Macrogen Netherlands laboratory. DNA sequence analysis was performed on DNA samples that were amplified in agarose gel electrophoresis. Genotypes were identified.

Genotypes of NRG1 gene rs6994992 polymorphism were revealed by imaging with Chromas 2.6.6 program. The DNA sequence that includes the NRG1 gene rs6994992 polymorphism (C/T) is shown in Figure 1. The sections underlined show forward and reverse primers.

Figure 1 NRG1 gene sequence including rs6994992 polymorphism

2.5. Statistical Analysis

SPSS 22 statistical software was used for statistical analysis. Homozygous and heterozygous genotype frequencies and allelic mutation frequencies between experiment and control groups were compared using Chi-square analysis. Odds ratio (OR) and binary logistic regression analysis were performed to calculate 95% confidence intervals for each polymorphism. $p<0.05$ was considered for statistical significance. Michael H. Court's (2005-2008) online calculation engine was used because of any deviations from the Hardy-Weinberg equation

[13]. On the result of power analysis which was performed for detecting an association between gifted children and the studied polymorphisms, sample size was found to be sufficient for experiment and control groups consisting of 97 and 99 individuals (α: 0,05 and 1-β: 0,98).

3. RESULTS

While 35% of gifted students (i.e., the experimental group) were female students and 65% were male students, these values were 55% for girls and 45% for boys in the control group. Since the numbers in girls and boys were not equal between groups, p = 0.003 was found statistically significant. The difference between two groups were not statistically significant either in terms of height, weight and waist circumference (Table 3).

Hardy-Weinberg equation was calculated as $\chi 2$ = 1.281, p = 0.257 in the gifted group and $\chi 2$= 8,596, p = 0.0003 in the group with normal development. The reason for the deviation from HWE may be that the group with normal development in this study was formed from five different schools.

Table 3
Demographic characteristics of gifted students and students with normal development

	Gifted group N=97 (%)	**Normal Development group N=99 (%)**	**p value**	**OR (95% CI)**
Gender				
Girl	34 (35)	55 (55)		
Male	63 (65)	44 (45)	**0.003***	2.3 (1.337-4.179)
Size	143.9±7.15	143.3±7.67	0.809	0.801-0.901
Weight	38.39±9.00	37±7.86	0.840	0.789-0.891
Waist circumference	65.67±11.14	64.31±7.83	0.535	0.466-0.604

Genotype and allele frequencies of NRG1 gene C/T rs6994992 single nucleotide polymorphism in gifted students and students with normal development are shown in Table 4.

Table 4
Genotype and allel distributions of NRG1 gene rs6994992 polymorphism in students with Special Ability and Normal development

NRG1 rs6994992	**Gifted group N=97 (%)**	**Normal Development Group N=99 (%)**	**p value**	**OR (95% CI)**
Genotype				
CC	32 (33.0)	32 (33.0)		
CT	52 (53.6)	60 (60.0)	0.244	0.538 (0.190-1.525)
TT	13 (13.4)	7 (7.0)	0.132	0.467 (0.173-1.257)
Allel				
C	116 (59.8)	124 (62.6)		
T	78 (40.2)	74 (37.4)	0.565	0.888 (0.591-1.333)

*When compared to the control group, it is significantly different at p <0.05 level.

When Table 4 is analyzed, it can be seen that for the NRG1 gene rs6994992 single nucleotide genotype frequencies for gifted students, CC (wild type) is 33.0%, CT (heterozygous) is 53.6%, and TT (polymorphic type) is 13.4%, while these values were found 33.0%, 60.0% and 7.0% for students with normal development, respectively. Allel frequencies were C 59.8% and T 40.2% in gifted students, and C 62.6% and T 37.4% in students with normal development. No statistically significant difference was found in genotype and allele distributions between gifted students and students with normal development (p> 0.05).

Genotypes were determined by investigating electropherograms of NRG1 gene rs6994992 single nucleotide polymorphism separately for both experimental and control groups. Figure 2 shows the CC, CT and TT genotypes, respectively.

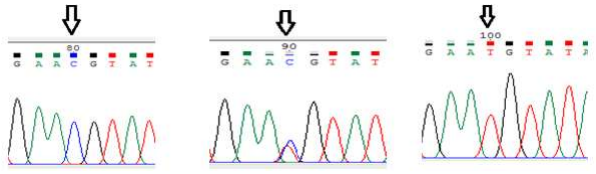

Figure 2 Arrows show CC, CT and TT genotypes of NRG1 gene rs6994992 SNP, respectively from left to right

4. DISCUSSION

NRG1 gene, which has been proposed as a potential gene for psychosis, plays a role in neuronal development, synaptic plasticity, glutameric neurotransmission and glial function. In particular, the TT genotype of rs6994992 of functional promoter polymorphism; NRG1 has been associated with increased gene expression, an increased risk of psychosis and other psychological and neurological phenotypes. NRG1 gene, which has an extremely important effect on brain development, can also cause individual differences [6]. NRG1 gene rs6994992 has been found to be related with creativity in intellectually successful individuals with high promoter polymorphism. According to the study by Kéri (2009), the NRG1 gene, which is regarded as a potential gene for glial functions that help nerve cells, has been examined to determine the role it plays in neural development. To this end, creativity tests were carried out, in which the IQ scores of the participants were measured together with the schizotypic features. Among these, healthy participants with high intellectual and academic performance were identified and the study investigated whether there was a relationship between creativity and NRG1 promoter polymorphism. The results of this study has revealed that NRG1 gene has an effect on functions that impact both creativity and some psychopathology [14].

The prefrontal cortex of the brain is important in cognitive inhibition and creativity, and the rs6994992 promoter polymorphism of the NRG1 gene affects the function of this region of the brain [15, 16]. In 2012, a study conducted Yokley et al., which investigated the effects of NRG1 gene, has found that it may have an impact on psychopathology and intelligence, thus arguing for a relationship between variants in NRG1 and cognitive domains [17]. In the literature on the relationship between genetics and gifted people, Durdiakova et al. (2013) and Celec et al. (2013) have two studies that focus on that, apart from NRG1, androgen receptor repeat polymorphism, ESR1, ESR2, SRD5A2 and SHBG variants [18, 19]. Both Kéri (2009) and Yokley et al. (2012) found significant results for NRG1 gene rs6994992 polymorphism in their studies in terms of creativity and / or psychopathology [14, 17]. In our study, we investigated the same gene polymorphism and, unlike them, included gifted children in our research. Unfortunately, as mentioned earlier, there were some limitations of our study such as the disclosure of IQ scores by the institutions and not being able to giving IQ tests to students that show normal development.

As a result, no statistically significant differences were found between the gifted students and the students with normal development in terms of NRG1 rs6994992 promoter polymorphism. In the Turkish population, there was no other study, to our best knowledge, that focused on the genetic polymorphism that had the sample of gifted students This study is one of the pioneering genetic studies conducted with gifted individuals in our country. In a larger sample, we recommend researchers interested in intelligence and genetics to study genes that are thought to be related to intelligence such as NRG1, APO E, ESR1, ESR2, SRD5A2 and SHBG.

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The Declaration Conflict of Interests/Common Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this paper.

Authors' Contribution

All authors have contributed in experimental study and writing of the manuscript equally.

Research and Publication Ethics

All applicable international, national, and/ or institutional guidelines for non-invasive clinical studies were followed.

Ethics Committee Approval

Prior to data collection research, KOU GOKAEK Non-Interventional Ethics Committee permission numbered 2017/375 was granted and MoNE and Governorship approvals dated 10.05.2018 and numbered 99332089/605.01/9238429 were obtained.

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