



ORIGINAL ARTICLE

Neuregulin1 gene variants as a biomarker for cognitive impairments in patients with schizophrenia



E.S. Ananloo^{a,b}, S. Yoosefee^{c,d,*}, M. Karimipour^e

^a Department of Psychosomatic, Imam Khomeini Hospital Complex (IKHC), School of Medicine, Tehran University of Medical Sciences (TUMS), Tehran, Iran

^b Department of Neuroscience, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences (TUMS), Tehran, Iran

^c Neuroscience Research Center, Qom University of Medical Sciences, Qom, Iran

^d Spiritual Health Research Center, Qom University of Medical Sciences, Qom, Iran

^e Department of Molecular Medicine, Pasteur Institute of Iran, Tehran, Iran

Received 21 September 2019; accepted 18 December 2019

Available online 16 January 2020

KEYWORDS

Cognitive impairments;
Executive function;
neuregulin1;
Schizophrenia;
Wisconsin card sorting test

Abstract

Background and objectives: This study aimed to determine whether the selected neuregulin1 (NRG1) gene variants (rs2439272 and rs6988339) are associated with schizophrenia, and Cognitive Impairments Associated with Schizophrenia (CIAS).

Methods: Participants with schizophrenia (N = 276) and healthy controls (N = 193) were enrolled. Cognitive assessment using the Wisconsin card sorting test (WCST) and Wechsler adult intelligence scale and genotyping for selected variants of *NRG1* were performed.

Results: For both the rs2439272 and rs6988339, compared to healthy controls, we found significant higher frequencies of the G allele and the GG genotype in all and male participants with schizophrenia, and with all Wechsler adult intelligence score (WAIS) and Wisconsin card sorting test (WCST) scores (except for non-perseverative score) in all, male and female participants.

Conclusions: There was an association between rs2439272, rs6988339 of *NRG1* variants and schizophrenia and CIAS (intelligence and executive functions).

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* Corresponding author.

E-mail address: Syoosefee@muq.ac.ir (S. Yoosefee).

Introduction

Schizophrenia (Online Mendelian Inheritance in Men; OMIM: 181500) is a disabling psychiatric disorder characterized by positive and negative symptoms, and also cognitive impairments. A cluster of early features including cognitive impairments appear to anticipate schizophrenia symptoms and may constitute a core risk profile of this disorder.¹ Cognitive impairments characterize the patients with schizophrenia throughout the course of disorder.^{2,3} Evidence shows that the cognitive impairments are present at disorder onset and are not caused by medication, progression of disorder, or other nonspecific factors.³ From the point of view that schizophrenia is a disorder of information processing, it makes sense to add neuropsychological impairments to other indicators in order to calculate a 'risk profile'.⁴ Multiple domains of cognitive functions are impaired in schizophrenia.⁵ For example, the cognitive impairments in multiple domains were observed in a cohort study.⁶ These impairments include dysfunctions of abstraction, attention, and memory^{7–10} as the different domains of executive function.^{7,8}

Although the etiology of schizophrenia is not clearly known, but it has a high heritability estimates approximately 80–85%.¹¹ Chromosome 8p22-p12 is shown to be a susceptibility locus for schizophrenia. Neuregulin1 gene (*NRG1*) [chr8p12] plays a critical role in the growth and development of multiple organ systems, and is involved both in neurodevelopment, synaptic plasticity and neurotransmitter mechanisms in the brain. So, *NRG1* may play a role in the pathogenesis of schizophrenia {Stefansson, 2003 #2339}.^{12–20} Stefansson et al. reported the overrepresentation of at-risk haplotype ('HapICE') constructed from five single-nucleotide polymorphisms (SNPs) and two microsatellite markers in *NRG1* gene for schizophrenia in an Icelandic population.²¹ Other research groups found different haplotypes in different ethnic groups.^{22–24} A number of studies have investigated the effect of *NRG1* on cognitive impairments related to schizophrenia, but the results were inconsistent.^{25–30} There is evidence showing that the two selected SNPs (rs2439272 and rs6988339) located in intron 3 on *NRG1* gene SNPs have significant effects on schizophrenia.^{31,32} The purpose of this study was to gain insight into the role of these two SNPs and schizophrenia and CIAS, including intelligence quotient (IQ) and executive functions in an Iranian population.

Materials and methods

Participants

Four hundred sixty nine Iranian participants, including 276 unrelated participants with schizophrenia and 193 healthy matched controls were evaluated. Schizophrenia patients recruited from the Roozbeh Psychiatry Hospital, Tehran University of Medical Sciences, Tehran, Iran. In the case group, the inclusion criteria were the schizophrenia diagnostic criteria based on the fourth edition of diagnostic and statistical manual of mental disorders-text revised (DSM-IV-TR) using the schedule for affective disorders and schizophrenia, and

no other mental disorder, the use of the lifetime version of the Schizophrenia, and Affective Disorders Schedule. Healthy controls were recruited from the same geographical area. In this group, the inclusion criteria were not suffering from any mental disorders based on DSM-IV-TR criteria and not having first-degree relatives with any mental disorder, doing clinical interviews, pedigree, past medical and psychiatric history evaluations by two expert psychiatrists, the researchers assessed the presence of all types of mental disorders. The exclusion criteria for the both groups of case and control were WAIS IQ score below 70, history of any other medical conditions, because of possibility to mimic the symptoms of schizophrenia, substance addiction or use in the past one year to abuse/dependence, and severe head trauma. Demographic features are shown in supplementary Table 1.

Clinical assessments

The clinical diagnosis of schizophrenia was made independently by two expert psychiatrists according to the DSM-IV-TR criteria. We assessed the psychopathology, including positive and negative symptoms, general psychopathology and total score, using the positive and negative syndrome scale (PANSS) in patients.

Cognitive assessments

Cognitive impairments, the IQ and executive functions were assessed in both groups of cases and controls using the Wechsler adult intelligence scale (WAIS-III) and the WCST, respectively.

DNA preparation, SNPs selection and genotyping

All blood samples were taken by vacuum tube pre-filled with the anticoagulant EDTA. High molecular weight genomic DNA was prepared from venous blood using the salting out procedure.³³ Two SNPs (rs2439272 and rs6988339) were selected by using the related literature. Genotyping was performed blind to status by using polymerase chain reaction (PCR), restriction fragment length polymorphism-PCR (RFLP-PCR), and DNA sequencing. Primers were designed using the Primer3; <http://biotools.umassmed.edu/bioapps/primer3-www.cgi>. Primers sequences, PCR product sizes, corresponding restriction fragment enzyme and digested sizes for each SNP are shown in supplementary Table 2. Annealing temperatures were 56 °C to 57 °C. 100% of the genotypes were callable, and minor allele frequency was greater than 2 %. The digested fragments were fractionated on 2 % agarose gel. In addition, in order to eliminate the genotyping errors, all raw genotyping data were independently read by two experts and the suspect genotypes were retyped. Furthermore, for each SNP, a random group of samples were also re-genotyped by direct sequencing to confirm the genotyping results of restriction fragment enzyme method.

Statistical analysis

The Hardy–Weinberg equilibrium for genotypic distributions in two populations was tested using the χ^2 goodness-of-fit test. Odds ratio and 95 % confidence interval (CI) was calculated to evaluate the effect of different genotypes. The allelic association and linkage disequilibrium (LD) were estimated using the COCAPHASED (UNPHASED) program.³⁴ The genotype frequencies between cases and controls were compared using the CLUMP22 software³⁵ by running 1000 Monte Carlo permutation. Genotypic association was tested by the χ^2 , or by Fisher's exact test to assess the significance of the results. The independent-samples *t*-test procedure was used to compare the means of the quantitative test scores for two groups of case and control. A general linear regression model (Univariate) was used to assess the relation between a categorical grouping (carriers or not of the risk alleles) vs. quantitative outcomes, including the IQ and WCST scores. Means and standard deviations of WAIS and WCST scores were calculated based on the genotypes. Cohen's *d* was used as an effect size to indicate the standardized difference between each two means.

$$d = \frac{M_1 - M_2}{\sqrt{(SD_1^2 + SD_2^2)/2}}$$

The significance level for all statistical tests was $P < 0.05$. The power analysis for rs2439272 and rs6988339 were %60 and %80 respectively.

Results

Clinical assessments

All of the patients had the diagnosis of chronic schizophrenia with more than 8 years, and had a drug regimen of 4–6 mg of Risperidone (Chlorpromazine equivalents treatment). Each subscales of PANSS scores [Positive Symptoms ($F = 57.05$), Negative Symptoms ($F = 46.69$), General Psychopathology Symptoms ($F = 13.75$), and Total score ($F = 30.99$)] in patients were significantly higher compared the healthy controls ($p < 0.001^*$).

Regarding the cognitive impairments between patients with schizophrenia and healthy controls

All scores of WAIS (including verbal IQ, performance IQ and total IQ) were decreased significantly in all, male and female patients with schizophrenia vs. healthy controls ($P < 0.001$). In all participants, in patients with schizophrenia vs. healthy controls, the verbal IQ scores (Mean $\pm$ SD) (74.35 ± 11.07 and 105.19 ± 7.25 respectively) were decreased significantly ($P < 0.001$). The performance IQ scores (Mean $\pm$ SD) (74.19 ± 8.72 vs. 108.48 ± 5.66) were also decreased significantly ($P < 0.001$). Furthermore, the total IQ scores (Mean $\pm$ SD) (74.35 ± 11.07 vs. 105.19 ± 7.25) were decreased significantly as well ($P < 0.001$). In male participants, in patients with schizophrenia vs. healthy controls, the verbal IQ scores (Mean $\pm$ SD) (74.16 ± 11.59 and 104.40 ± 7.26 respectively) were decreased signifi-

cantly ($P < 0.001$). The performance IQ scores (Mean $\pm$ SD) (74.31 ± 10.14 vs. 107.92 ± 5.78) were also decreased significantly ($P < 0.001$). In addition, the total IQ scores (Mean $\pm$ SD) (74.15 ± 11.07 vs. 112.97 ± 6.39) were decreased significantly as well ($P < 0.001$). In female participants, in patients with schizophrenia vs. healthy controls, the verbal IQ scores (Mean $\pm$ SD) (74.64 ± 10.29 and 106.55 ± 7.06 respectively) were decreased significantly ($P < 0.001$). The performance IQ scores (Mean $\pm$ SD) (74.01 ± 6.00 vs. 109.45 ± 5.36) were also decreased significantly ($P < 0.001$). And the total IQ scores (Mean $\pm$ SD) (73.84 ± 9.63 vs. 114.21 ± 6.10) were decreased significantly as well ($P < 0.001$).

Moreover, all scores of WCST (including perseveration, non-perseveration and total errors) were increased significantly in all, male and female patients with schizophrenia vs. healthy controls. The exceptions were for non-perseveration errors that were decreased significantly in all and female patients with schizophrenia vs. healthy controls. In all participants, in patients with schizophrenia vs. healthy controls, the perseveration errors (Mean $\pm$ SD) (23.12 ± 6.75 and 5.50 ± 0.56 respectively) were increased significantly ($P < 0.001$). The non-perseveration errors (Mean $\pm$ SD) (4.61 ± 2.60 vs. 5.50 ± 0.15) were decreased significantly ($P < 0.001$). And the total errors (Mean $\pm$ SD) (27.83 ± 5.43 vs. 11.00 ± 0.71) were increased significantly ($P < 0.001$). In male participants, in patients with schizophrenia vs. healthy controls, the perseveration errors (Mean $\pm$ SD) (22.93 ± 6.81 and 5.55 ± 0.50 respectively) were increased significantly ($P < 0.001$). The non-perseveration errors (Mean $\pm$ SD) (6.64 ± 2.83 vs. 5.51 ± 0.14) were also increased significantly ($P < 0.001$). And the total errors (Mean $\pm$ SD) (27.88 ± 4.80 vs. 11.06 ± 0.63) were increased significantly too ($P < 0.001$). In female participants, in patients with schizophrenia vs. healthy controls, the perseveration errors (Mean $\pm$ SD) (23.40 ± 6.67 and 10.90 ± 0.83 respectively) were increased significantly ($P < 0.001$). The non-perseveration errors (Mean $\pm$ SD) (4.56 ± 2.22 vs. 5.48 ± 0.18) were decreased significantly ($P = 0.001$). And the total errors (Mean $\pm$ SD) (27.75 ± 6.28 vs. 10.90 ± 0.83) were increased significantly ($P < 0.001$).

Association of genetic variants with schizophrenia

We compared the allele and genotype frequencies in patients with schizophrenia vs. healthy controls. For the SNP rs2439272, we found a significant difference in G allele frequencies between patients with schizophrenia (0.53) and healthy controls (0.44) in all subjects ($P = 0.009$), and between patients with schizophrenia (0.53) and healthy controls (0.43) in male samples ($P = 0.019$). For the same SNP, we found significant difference in GG genotype frequencies between patients with schizophrenia (0.26) and healthy controls (0.16) in all subjects ($P = 0.025$), and between patients with schizophrenia (0.28) vs. healthy controls (0.16) in male samples ($P = 0.024$).

For the SNP rs6988339, we found significant differences in G allele frequencies between patients with schizophrenia (0.44) and healthy controls (0.33) in all subjects ($P = 0.0007$), and between patients with schizophrenia (0.45) and healthy controls (0.29) in male samples ($P = 0.005$). For the same SNP, we found significant differences in GG genotype

Table 1 Allele and genotype frequencies between the schizophrenia patients (case) and healthy controls (Control) for NRG1 gene selected SNPs.

SNP (NCBI number)	Groups	Allele frequency				Genotype frequency				
		G	A	COCAPHASE		GG	GA	AA	CLUMP	
				χ^2	P				χ^2	P
rs2439272										
All	Case (n=276)	0.53	0.47	6.729	0.009*	0.26	0.54	0.20	6.97	0.025*
	Control (n=193)	0.44	0.56			0.16	0.57	0.27		
Male	Case (n=166)	0.53	0.47	5.494	0.019*	0.28	0.54	0.18	7.54	0.024*
	Control (n=122)	0.43	0.57			0.16	0.51	0.33		
Female	Case (n=110)	0.52	0.48	1.458	0.22	0.23	0.55	0.22	3.33	0.16
	Control (n=71)	0.46	0.54							
rs6988339										
All	Case (n=276)	0.44	0.56	11.484	0.0007*	0.15	0.58	0.27	12.40	0.002*
	Control (n=193)	0.33	0.67							
Male	Case (n=166)	0.45	0.55	15.319	0.005*	0.18	0.52	0.30	9.78	0.006*
	Control (n=122)	0.29	0.71							
Female	Case (n=110)	0.44	0.56	0.227	0.63	0.10	0.70	0.20	4.33	0.11
	Control (n=71)	0.39	0.61							

* Significant.

frequencies between patients with schizophrenia (0.15) and healthy controls (0.09) in all subjects ($P=0.002$). These genotype frequencies were significantly different between patients with schizophrenia (0.18) vs. healthy controls (0.07) in male samples ($P=0.006$). In female samples, for both of the SNPs, no significant differences in allele and genotype frequencies were detected (Table 1). Because there was no linkage disequilibrium between the two selected variants in our analysis, we did not do haplotype analysis.

Association of genetic variants with WAIS scores

For the both SNPs, our findings indicate that there is a significant impact on verbal IQ ($P<0.001$), performance IQ ($P<0.001$) and total IQ ($P<0.001$) in all, male and female subjects. These findings suggest a direct association between the rs2439272 and rs6988339 GG genotype with the verbal, performance and total IQ impairments (Table 2).

Cohen's d showed that for both SNPs, A allele is protective and G allele is associated with poorer WAIS scores. The maximum standard differences were observed between AA and GG genotypes.

Association of genetic variants with WCST scores

For the SNP rs2439272, our findings indicate that there is a significant impact of GG genotype on perseverative ($P<0.001$), and total errors ($P<0.001$) in all, male and female subjects. But for the non-perseverative errors, we also found significant impact in all ($P=0.019$), male ($P=0.009$) and female ($P=0.001$) participants. These findings suggest a direct association between the rs2439272 GG

genotype with the perseverative, non-perseverative, and total errors based on the WCST scores in all, male and female participants (Table 3). Regarding the effect of rs6988339, we found significant impact of GG genotype on perseverative ($P<0.001$), non-perseverative ($P<0.001$) and total errors ($P<0.001$) in all, male and female subjects. But for the non-perseverative errors, we also found significant difference in male ($P=0.005$), and female participants ($P<0.001$). These findings suggest a direct association between the rs6988339 GG genotype with the perseverative, non-perseverative, and total errors based on the WCST scores in male and female, and perseverative, and total errors in all participants (Table 3). Cohen's d showed that for both SNPs, A allele is protective and G allele is associated with poorer WCST scores. The maximum standard differences were observed between AA and GG genotypes.

Discussion

In the current study, we investigated the association between two selected NRG1 gene SNPs (rs2439272 and rs6988339) and schizophrenia, and CIAS, including IQ and executive functions. The WAIS and WCST test scores revealed that cognitive impairments have significant differences in different domains between the case and control groups. Both SNPs were in a region of 52824 base pairs (bp) spanning the NRG1 gene, were polymorphic, and included in analysis. We found that these SNPs have significant effects on the risk of schizophrenia development and CIAS. These effects were detected in all, male and female samples.

In the case of rs2439272, our results showed that the G allele, which is the major allele, may be the risk allele with

Table 2 The effect of the NRG1 SNPs on cognitive test scores (WAIS) in patients with schizophrenia (case) vs. healthy controls (Control): A general linear model analysis.

SNPs: Statistics:	Case (Mean±SD)	Control	rs2439272 F (dfn; dfd) (P value) (Corrected Model)	rs6988339 F (dfn; dfd) (P value) (Corrected Model)
WAIS				
All				
VIQ ^a	74.35 ± 11.07	105.19 ± 7.25	385.55 (3; 465) (<0.001*)	388.30 (3; 465) (<0.001*)
PIQ ^b	74.19 ± 8.72	108.48 ± 5.66	766.97 (3; 465) (<0.001*)	781.84 (3; 465) (<0.001*)
TIQ ^c	74.02 ± 9.43	113.43 ± 6.30	858.10 (3; 465) (<0.001*)	865.08 (3; 465) (<0.001*)
Male				
VIQ ^a	74.16 ± 11.59	104.40 ± 7.26	214.87 (3; 284) (<0.001*)	217.28 (3; 284) (<0.001*)
PIQ ^b	74.31 ± 10.14	107.92 ± 5.78	361.70 (3; 284) (<0.001*)	366.37 (3; 284) (<0.001*)
TIQ ^c	74.15 ± 9.32	112.97 ± 6.39	526.39 (3; 284) (<0.001*)	530.45 (3; 284) (<0.001*)
Female				
VIQ ^a	74.64 ± 10.29	106.55 ± 7.06	178.33 (3; 177) (<0.001*)	174.93 (3; 177) (<0.001*)
PIQ ^b	74.01 ± 6.00	109.45 ± 5.36	541.46 (3; 177) (<0.001*)	551.02 (3; 177) (<0.001*)
TIQ ^c	73.84 ± 9.63	114.21 ± 6.10	335.28 (3; 177) (<0.001*)	330.84 (3; 177) (<0.001*)

^a Verbal IQ.^b Performance IQ.^c Total IQ.

* Significant.

Table 3 The effect of the NRG1 SNPs on cognitive test scores (WCST) in patients with schizophrenia (case) vs. healthy controls (Control): A general linear model analysis.

SNPs: Statistics:	Case (Mean±SD)	Control	rs2439272 F (dfn; dfd) (P Value) (Corrected Model)	rs6988339 F (dfn; dfd) (P Value) (Corrected Model)
WCST				
All				
PE ^a	23.12 ± 6.75	5.50 ± 0.56	434.74 (3; 465) (<0.001*)	439.80 (3; 465) (<0.001*)
NE ^b	4.61 ± 2.60	5.50 ± 0.15	3.35 (3; 465) (0.019*)	7.00 (3; 465) (<0.001*)
TE ^c	27.83 ± 5.43	11.00 ± 0.71	609.16 (3; 465) (<0.001*)	614.22 (3; 465) (<0.001*)
Male				
PE ^a	22.93 ± 6.81	5.55 ± 0.50	264.79 (3; 284) (<0.001*)	265.32 (3; 284) (<0.001*)
NE ^b	4.64 ± 2.83	5.51 ± 0.14	3.93 (3; 284) (0.009*)	4.00 (3; 284) (0.005*)
TE ^c	27.88 ± 4.80	11.06 ± 0.63	494.69 (3; 284) (<0.001*)	491.00 (3; 284) (<0.001*)
Female				
PE ^a	23.40 ± 6.67	5.42 ± 0.65	170.00 (3; 177) (<0.001*)	171.00 (3; 177) (<0.001*)
NE ^b	4.56 ± 2.22	5.48 ± 0.18	5.67 (3; 177) (0.001*)	6.00 (3; 177) (<0.001*)
TE ^c	27.75 ± 6.28	10.90 ± 0.83	166.45 (3; 177) (<0.001*)	172.00 (3; 177) (<0.001*)

^a Perseverative Error.^b Non-perseverative Errors.^c Total errors.

* Significant. ** Trend toward the significant effect.

increased risk of schizophrenia development and CIAS, and allele A, may have a protective effect, especially in males samples. Rs2439272 is one of the six core SNPs (rs6994992, SNP8NRG221132, SNP8NRG241930, rs3924999, rs10503929, and rs2439272) within the NRG1 gene identified as promising schizophrenia risk gene.¹³ Yoosefee and colleagues³⁶ showed a significant effect of rs2439272 GG genotype on negative symptoms in schizophrenia (especially in male participants). Roussos et al.³¹ have reported that reduced prepulse inhibi-

tion (PPI) – a well validated schizophrenia endophenotype – was related to rs2439272 G allele. Haplotype analysis confirmed that the two other SNPs (rs10503929 and rs3924999) were also associated with PPI reductions, when combined with rs2439272. Cho et al. have reported a significant association between rs2439272 and the ‘working memory’ domain in the group of healthy participants.³⁰

Regarding the SNP rs6988339, our results showed that the G allele, which is the minor allele, may be the risk

allele with increased risk of schizophrenia development and CIAS, and allele A, may have a protective effect, especially in males samples. Hatami et al.³⁷ showed a significant effect of rs6988339 GG genotype on negative symptoms in schizophrenia. Our results are consistent with the study of Walker et al.³² These authors have reported that the haplotype comprising two SNPs (rs6988339, and rs3757930), which is in near the 3' region of the NRG1 gene, was associated with schizophrenia in two Scottish samples.

The role of prefrontal region in cognitive impairments in schizophrenia has been identified.³⁸ This region together with the hippocampus are abnormally activated.³⁹ Hippocampus-prefrontal cortex (PFC) pathway is very important in executive functions.^{40,41} The most neurocognitive tests assessing the PFC functions are typically worse in schizophrenia,⁴² and the WCST is the standard task to detect cognitive impairments in this area of the human brain.⁴³ Neuregulin1 gene is involved in different neurodevelopmental functions⁴⁴ and is expressed in synaptic regions in most brain areas, including the hippocampus, PFC and paraventricular region.^{45,46} Neuregulin1 is expressed by new-born neurons in radial glial cells,^{47,48} and regulate its morphology and function. These cells play critical role in the development of the cerebral cortex, such as the supporting of new-born neurons and acts as neural and glial precursors.⁴⁹ On the other hand, several studies have shown defects in brain cytoarchitecture in schizophrenia.^{50,51} These lines of evidence suggest that defects in NRG1-erbB signalling during brain development could lead to alterations in neuronal migration and cortical development. This could disrupt cortical connectivity and, subsequently, lead to behavioural defects such as cognitive impairment. In our study, according to significant differences in cognitive executive function (WCST scores) between the cases and the control groups, the risk alleles of two selected SNPs may be involved in cognitive impairments. Down-regulation of oligodendrocyte-related mRNA species within the dorsolateral PFC of patients with schizophrenia has shown.⁵² Oligodendrocytes express ErbB receptors and respond to oligodendrocytes are mainly responsible for axon myelination in the brain. Neuregulin1 and this gene is involved in the proliferation and survival of oligodendrocyte precursors.⁵³ While myelination in human brain continues until adolescence or early adulthood,^{54,55} a period corresponding to the peak time for onset of schizophrenia. This late myelination is most evident in the frontal and temporal lobes.⁵⁴ It seems that dysregulation of myelination may lead to change in the white matter density, decrease transmission and processing of information, and subsequently, in cognitive impaired functions. Recently, it has been revealed that NRG1/ErbB4 activity in the hippocampus is critical for learning and memory.⁵⁶ On the other hand, it was shown that white matter changes exist in schizophrenia.⁵⁷ It may be concluded that decreased of information transmission and processing measured by WCST, are somehow related to the selected SNPs. The roles of NRG1 on GABAergic neurotransmission,^{58,59} establishment of excitatory synapses in GABAergic interneurons and development of balanced excitatory/inhibitory tone in the brain,^{60,61} and also its role in regulating acetylcholine receptors in the central nervous system⁶² have been shown. Interestingly, studies have demonstrated dysfunctions in

these neurotransmitter systems in schizophrenic cases.^{63–66} On the hand in the field of Pharmacogenetics, it has been shown that acetylcholinesterase inhibitors and N-methyl-D-aspartate (NMDA) receptors antagonists, may be effective in the treatment of CIAS.^{67,68} Collectively, the studies on glutamatergic, GABAergic and cholinergic neurotransmission, in addition to neuroprotective effect of NRG1 for cortical neurons⁶⁹ revealed that the imbalance among these neurotransmission systems could influence information processing and thus contribute to cognitive impairments. It the present study, the NRG1 selected SNPs have significant effects on the risk of development of schizophrenia in male rather than female samples. However, relatively few animal studies of NRG1 showed the several sex-specific differences including spatial cognition.⁷⁰ These findings may suggest the role of NRG1 is sex specific. In animal studies, the role of ovarian hormones to affect corticosterone response to stress has been showed. Estrogen has also been shown to modulate both excitatory and inhibitory states in neurons.⁷¹ It may be that circulating gonadal hormones modulate NRG1-induced changes in GABAergic neurotransmission during development and adulthood to produce some of these sex-specific findings including cognitive impairments.

There are several limitations to the current study. The main limitation was the small sample size, especially the females, both participants with schizophrenia and controls, which impact the statistical power of our study. The multiple testing is another limitation. The other limitation was that all participants with schizophrenia were recruited from hospitals. So there are inherent selecting bias and information bias that are unavoidable problems. We matched the patients and healthy groups based on the age, sex, height, and handedness. However, we could not match these two groups for other variables such as ethnicity.

Conclusion

Although a few studies about the role of NRG1 gene in Iranian schizophrenic population has taken place so far⁷² according to our knowledge, this is for the first time that the association of two NRG1 SNPs in intron 3 (rs2439272 [A/G] and rs6988339 [A/G]) are reported with the risk of schizophrenia and its cognitive impairments. These findings are consistent with the theory that the NRG1 gene variants may mediate risk of schizophrenia at least in part through its effect on cognitive impairments.

Ethical considerations

This study was carried out in accordance with the approved guidelines of Ethical Committees of Tehran University of Medical Sciences, Tehran, Iran. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from all participants.

Funding

The research used no funding or financial support in any stage.

Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgments

We thank staffs of the Department of Adult Psychiatry, the Department of Genomic Psychiatry and Behavioural Genomics (DGPBG) at the Roozbeh Psychiatric Hospital in Tehran University of Medical Sciences (TUMS), the Section of Molecular Medicine at the Pasteur Institute in Tehran, for their cooperation. We also express our sincere gratitude for the late Mrs. Narges Karamghadiri for her effective collaboration and wish God blessings for her.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.ejpsy.2019.12.004>.

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