Research Paper: Association of Candidate Single Nucleotide Polymorphisms Related to Candidate Genes in Patients With Schizophrenia

Seyedeh Sara Karimian[1*], Mohammad Taghi Akbari[2,3], Seyed Saeed Sadr[4], Gholamreza Javadi[1]

1. Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
2. Department of Medical Genetics, Faculty of Medical Sciences, Tarbiat Modarres University, Tehran, Iran.
3. Tehran Medical Genetics Laboratory, Tehran, Iran.
4. Department of Psychiatry, Imam Hussein Hospital, Shahid Beheshti University, Tehran, Iran.

Use your device to scan and read the article online

Citation: Karimian, S. S., Akbari, M. T., Sadr, S. S., & Javadi, Gh. (2020). Association of Candidate Single Nucleotide Polymorphisms Related to Candidate Genes in Patients With Schizophrenia. *Basic and Clinical Neuroscience, 11*(5), 595-608. http://dx.doi.org/10.32598/bcn.9.10.470

http://dx.doi.org/10.32598/bcn.9.10.470

Article info:
Received: 28 May 2018
First Revision: 10 Jun 2018
Accepted: 06 Apr 2019
Available Online: 01 Sep 2020

Keywords:
Biomarkers, SNPs, NrCAM, PRODH, ANKK1, ANK3, Schizophrenia

ABSTRACT

Introduction: Schizophrenia is a chronic heterogenic neurodevelopment disorder. Many genes interfere in the development of SCZ. All four genes, NrCAM, PRODH, ANK3, and ANKK1, which were evaluated in this study, were previously reported to be associated with Schizophrenia. The NrCAM contributes to creating cognitive deficiencies through the CAM's signaling pathway. PRODH plays a vital role in creating SCZ negative symptoms through the signaling pathway of glutamatergic and NMDA receptors. ANK3 affects ion channel and molecular adhesion in Ranvier and initial segments of axons, leading to mental retardation, sleep disorder, and SCZ. ANKK1 encodes a protein kinase and was reported to be associated with alcohol addiction, Attention Deficit Hyperactivity Disorder (ADHD), and SCZ.

Methods: The subjects were selected from Schizophrenic patients referring to the Psychiatric Ward of Imam-Hussein Hospital and Schizophrenic Patients Support Institution (AHEBBA). 95 (30 Schizoaffective patients, 57 Paranoid patients, and 8 disorganized) patients were recruited as the subjects in the present case-control association study. 120 healthy subjects were recruited from the Tehran Medical Genetics Laboratory staff and a group of students from the Islamic Azad University of Science and Research in Tehran. The genotypes were determined with molecular genotyping techniques of PCR-RFLP, ARMS-PCR, and Cycle sequencing. Results were analyzed by the Chi-Square test using SPSS V. 24 and R, SNP STATE Package to investigate significant differences between cases and controls.

Results: The incidence of schizophrenia was 68% and 32% among men and women, respectively. The evaluation of the allelic association between schizophrenia and all the candidate SNPs showed a significant association between NrCAM's SNP rs10235968 and SCZ (P=0.001). Haplotype T, T, C in rs10235968, rs6967368, rs3763463, respectively, within the NrCAM gene, showed significant association with schizophrenia disorder (P=0.0001).

Conclusion: No association was found between other candidate SNPs and SCZ among the subjects.

*** Corresponding Author:**
Seyedeh Sara Karimian, PhD.
Address: *Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran.*
Tel: *+98 (912) 3309710*
E-mail: *mtakbari@modares.ac.ir*

Highlights

- Schizophrenia (SCZ) is a chronic mental disorder.
- Many genes and environmental factors are involved in SCZ development.
- The CAM's signaling pathway is a candidate pathway involved in SCZ.

Plain Language Summary

Schizophrenia (SCZ) is a chronic mental disorder. Many genes and environmental factors are involved in SCZ development. The CAM's signaling pathway is a candidate pathway involved in SCZ to be linked to both psychosis and neurocognitive dysfunctions. Using GWAS and signaling pathways data, researchers reported several signaling pathways contributing to the development of SCZ. One of the most potent risk factors for SCZ is 22q11 deletion syndrome. The administration of NMDA antagonists results in cognitive deficiency and SCZ syndromes among healthy people, leading to dopaminergic neurons transmission. ANKK1 encodes a serine-threonine kinase protein and belongs to a receptor-interacting serine/threonine-protein kinase family, which contributes to cell division and cell differentiation. ANKK1 is located on the chromosome 11q32.2 and includes 10 exons. It was reported to be associated with alcohol addiction, eating disorder, SCZ, and Attention Deficit Hyperactivity Disorder (ADHD). This study aimed to investigate the association between the candidate SNPs within the NrCAM gene, and in the PRODH gene.

1. Introduction

Schizophrenia (SCZ) is a chronic mental disorder. Many genes and environmental factors are involved in SCZ development. NrCAM is a candidate gene for schizophrenia and has been reported in many studies with conflicting results (Ayalew, Le-Niculescu, et al. 2012). NrCAM encodes the neuron cells' adhesion protein molecule and is located on 7q31.1. The Northern Blotting technique showed a 2 kb transcript of NrCAM in all brain tissues. The 7.0 kb transcript of NrCAM is highly expressed in the brain medulla, adrenal, and adrenal cortex (Lane, et al., 1996, Wang, Williams, Du, Terrett, & Kenwrick, 1998). NrCAM owns 36 exons and is involved in WNT and CAM's signaling pathways (Chen & Zhou 2010, Zhang et al., 2015). NrCAM is one of the twelve genes active in CAM's pathway and has a significant relationship with schizophrenia. It acts as an interneurons connection and a signal transmission in CAM's pathway (Zhang et al., 2015).

The CAM's signaling pathway is a candidate pathway involved in SCZ, repeatedly reported to be linked with both psychosis and neurocognitive dysfunctions (Zhang et al., 2015). CAM's pathway plays a vital role in the brain's cognitive functions, including the formation of memory, attention, learning, reasoning, and thinking disrupted in SCZ (Hargreaves et al., 2014). The gene NrCAM is active in interneurons, neuron-glial adhesion, and growth cone motility (Kamiguchi & Lemmon, 1997). NrCAM has also been demonstrated to participate in many cellular processes of central and peripheral nervous systems, inclusive neurite growth, exon routing, myelination, and cellular migration (Kamiguchi & Lemmon 1997).

Using GWAS and signaling pathways data, researchers reported several signaling pathways contributing to the development of SCZ, including ionic channel pathway (Askland, Read, O'Connell, & Moore, 2012), myelination pathway (Yu et al., 2014), apoptosis factor pathway, adhesion molecule pathway, growth factor signaling pathway, and glutamate metabolism pathway (Jia, Wang, Meltzer, & Zhao, 2010). Cell adhesion molecules are glycoproteins expressed on the cell surface and significantly contribute to biological processes, including immune responses, inflammation responses, embryonic development (Elangbam, Qualls Jr, & Dahlgren, 1997). NrCAM is mostly reported about autism (Sakurai, et al., 2006).

NrCAM's SNPs rs3763463, rs10235968, rs6967368 are located in the promoter area, and rs1269634 is located in intron area. They were evaluated among an Iranian sample of patients with schizophrenia. An association study on NrCAM's SNPs and SCZ was conducted in the Korean population, and no association was found between NrCAM's SNPs and SCZ (Kim et al., 2009).

In another study conducted at the University of California, NrCAM's SNP rs646558 showed association with SCZ (Atz, Rollins, & Vawter, 2007). Yoo et al, evaluated the association between NrCAM's SNPs and personality disorder, addiction to amphetamines. Their results showed a significant association between NrCAM's SNP rs129634 and symptomatic amphetamine addiction (Yoo et al., 2012). One of the most potent risk factors for SCZ is 22q11 deletion syndrome (Levinson, et al., 2011). One-third of patients with 22q11 deletion syndrome (which is also called DiGeorge or Velocardiofacial syndrome) are suffering from Schizophrenia/Schizoaffective (Pulver et al., 1994). while the prevalence ratio of 22q11 deletion syndrome among the populations is 1/4000, this rate is about 1% among schizophrenic cases (Christofolini et al., 2011). 22q11 deletion syndrome was proposed as a genetic kind of SCZ; therefore, all the sequences located in the 22q11 deletion area are addressed to be linked with schizophrenia. PRODH is one of the genes located on the chromosome 22q11.2 and includes 15 exons. PRODH encodes the Proline dehydrogenase enzyme and Catalyzing conversion of Proline into Glutamate. Hyperprolinemia is regarded as involved in the development of SCZ (Jacquet et al., 2002, Kempf et al., 2008). On the one hand, Proline is a modulator of glutamine neurotransmitters (Liu et al., 2002). In particular, the hypofunction of the NMDA receptor, a glutamatergic receptor, is also involved in the development of SCZ (Galderisi, Merlotti, & Mucci, 2015).

The administration of NMDA antagonists results in cognitive deficiency and SCZ syndromes among healthy people, leading to dopaminergic neurons transmission (Galderisi, Merlotti, & Mucci, 2015). The association of PRODH's SNP rs238731 located in the exon 12 and SCZ is supported in numerous studies (Shashi, Berry, & Keshavan, 2009; Ota et al., 2014).

This study aimed to investigate the association between the candidate SNPs rs10235968, rs1269634, rs6967368, rs3763463 within the NrCAM gene, and rs2238731 in the PRODH gene in an Iranian sample of patients with schizophrenia.

ANKK1 encodes a serine-threonine kinase protein and belongs to a receptor-interacting serine/threonine-protein kinase family, which contributes to cell division and cell differentiation (Hamosh et al., 2002; Jasiewicz et al., 2014). ANKK1 is located on the chromosome 11q32.2 and includes 10 exons. ANKK1 was reported to be associated with alcohol addiction, eating disorder, SCZ, and Attention Deficit Hyperactivity Disorder (ADHD) (Jasiewicz et al., 2014; Arab & Elhawary 2015). c.562C>T within the ANKK1 gene located in exon 3 argued to have a significant association with susceptibility to SCZ (Shirzad, Beyraghi, Ataei, & Akbari, 2017). ANK3 is located on chromosome 10q21.2, includes 52 exons, and was reported to be associated with mental retardation and SCZ (Iqbal et al., 2013). The protein encoded by ANK3 assumes to be connected to integral proteins and locates in the Ranvier node and axons (Iqbal et al., 2013). c.7649 G>T within ANK3 locates in the exon 13 was shown to influence SCZ susceptibility (Shirzad, Beiraghi, et al. 2017). Another goal of this study is to investigate the frequency of recurrence of two novel variants c.562 C> T and c.7649 G> T within genes ANKK1, ANK3 respectively, in the two groups Iranian case and control.

2. Materials and Methods

2.1. Patients and controls

This study was a case-control association study where none of the patients were relatives. A total of 95 patients (men=65, women=30) with average age 32±12.18 were recruited from the Psychiatric ward of Imam Hussein Hospital and Schizophrenic Patient Support Institution (AHEBBA) in Tehran from August 2015 to May 2016. (30 Schizoaffective, 57 Paranoid, and 8 disorganized). The patients' demographic specifications, including gender, age, place of birth, and age at onset, were recorded. Patients were identified by drawing pedigree and interviews to recognize any physical illness or a family history of psychiatric disorders. Patients were clinically diagnosed with SCZ by an expert psychiatrist, according to the approach of Diagnostic and Statistical Manual of Mental Disorders (DSM–5) symbolism. Positive and Negative Syndrome Scale (PANSS) was used to recognize the severity of Positive and Negative Syndromes among schizophrenic cases.

A total of 120 healthy subjects (men=78, women=42) with an average age of 35±14.14 were recruited from Tehran Medical Genetics Laboratory staff and a group of students from Islamic Azad University of Science and Research in Tehran. These subjects were demographically matched by age and gender. They were identified by drawing pedigree and interviews to recognize any previous psychological or a family history of psychiatric disorders and physical diseases. Case and control groups were matched in terms of age and gender. None of the participants had a specific physical illness. The specifications of case and control subjects are introduced in Table 1.

Table 1. Demographics and characteristics of cases and controls

Variable	N	Frequency (%)	N	Frequency (%)
	SCZ Patients		Controls	
Gender	95		120	
Female	30	31.4 %	42	34.5%
Male	65	68.6 %	78	65.5%
Age	95	32 ±12.18	120	35(±14.14)
Age at onset	80	21.43 ±7.32		
Family History (%)	80	31.1%		
PANSS score	80	80 ±10.0039		

NEUROSCIENCE

SCZ: schizophrenia, N: sample size, PANSS: positive and negative syndrome scale. As shown, 65 males and 30 females have participated in the case group with average age 32 ± 12.18, PANSS score with average score 80 ±10.0039, and age at onset with average age 21.43 ±7.32 And 31.1% of patients had a Family History of schizophrenia disorder. Also, among the controls group, 78 males and 42 females have participated with an average age (35 ±14. 14).

2.2. Ethical considerations

The consent Letter was taken from all patients and their caregivers. This research was conducted in agreement with the Declaration of Helsinki. This investigation was authorized by the medical ethics committee of the Science and Research Branch of Islamic Azad University of Tehran.

2.3. DNA extraction

The blood samples (5 ml) were obtained from all cases and controls in EDTA tubes and were prepared for DNA extraction. In this study, MagCore HF16 Automatic Nucleic Acid Extractor system (RBC Bioscience Corp, Taiwan) with MagCore blood Genomic DNA Extraction Kit (RBC Bioscience Corp, Taiwan) was used to extracting of Genomic DNA samples. Then they were stocked at a temperature of -20°C. Nanodrop and 1% agarose gel electrophoresis were used to specify the quality and the quantity of the purified DNA.

2.4. Primer design

The software Gene Runner (www.SNPs.com) and primer-blast-NCBI-NIH databases were used to designing primers for PCR-RFLP, ARMS-PCR, and Cycle-Sequencing methods.

SNPs rs3763463, rs2238731, and novel variant c.562 C>T were genotyped through the PCR-RFLP method (Table 2). The PCR-RFLP primers were designed according to the recognition site and the fragments' length after enzymatic digestion. Remaining SNPs rs1269634, rs6967368, rs10235968, and novel variant c.7649 G>T were genotyped through the ARMS-PCR method (Table 3). Two forward primers and one reverse common primer were designed for any polymorphisms which were examined by ARMS-PCR (i.e. wild type [Wt] and mutant [mut]). A mismatch would appear in the 3' terminal of mutant primer. However, more changes inside the last five nucleotides at the 3′ terminal of the mutant primer will lead to enhance the specificity (Little 1995; Table 3). A fragment of the β-globin gene was chosen as an internal control. Necessary primers for Cycle sequencing were designed for all the polymorphisms and were listed in Table 4.

2.5. PCR-AFLP

While Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) was used for genotyping target SNPs, it needs to be digested using certain restriction enzymes. Their recognition site and the length of fragments obtained from the enzymatic digestion are described in Table 5.

Samples were evaluated for wild and mutant alleles in each PCR run. Two tubes containing positive and negative control were replicated along with other tubes. The appropriate restriction enzymes in agreement with the manufacturer's instructions were used to digesting the PCR products. A 12% polyacrylamide gel electrophoresis and silver staining protocols were used to visualizing digested products. The PCR conditions for PCR-AFLP:

- Reaction volume: 20 µl
- PCR mix: 19.05 µl

Table 2. List of primers used for PCR-RFLP method.

Gene	SNP	Primer sequences (5'-3')	Product length (bp)	Restriction Enzyme
NRCAM	rs3763463 (C/G)	Forward: 5'.. GCAGCAAGCAGTGTGTTTACTC.. 3' Reverse: 5'. CTTCGAAATTCATCAGTTGGG..... 3'	320 bp	sstII
PRODH	rs2238731(G>T)	Forward: 5'.... GGACAGAGGTTGGAGGCCC..... 3' Reverse: 5'... GTTGATGGGGTCCTCATAGCC..... 3'	315 bp	Hin1II (NIaIII)
ANKK1	c.562 C>T	Forward: 5'... ACCCTGGAACAGGCAGATAGC... 3' Reverse: 5'.. GTTCACACAGTCCCAGGCAAG..... 3'	444 bp	Dde I

NEUROSCIENCE

Table 3. List of primers used for ARMS-PCR method

Gene	SNP	Primer sequences (5'-3')	Product length (bp)	Tm °C
NRCAM	rs1269634 (A/G)	Forward (Wt): 5'... GTTTAAGTAATTTTCATGCGCGA..... 3' Forward (Mut): 5'... GTTTAAGTAATTTTCATGCGTGG..... 3' Reverse(C): 5'. TCATAAGGATGGTAGACAGATTATGC.. 3'	260 bp	58
NRCAM	rs6967368 (A/T)	Forward(Wt): 5'.....TCTTTTTCATTTGGCAAACCCTT... 3' Forward Mut: 5'.....TCTTTTTCATTTGGCAAACCCTA... 3' Reverse(C): 5'.....CATGGGAAGGACAGGCAAGAC.... 3'	331 bp	60
NRCAM	rs10235968 (C/T)	Forward(Wt): 5'.....CTTAGCCTGACCCTTGGTTCC... 3' Forward(Mut): 5'.....CTTAGCCTGACCCTTGGTTCT.... 3' Reverse(C): 5'.....AAGGCTCCGCTGAGCTCAC.... 3'	126 bp	62
ANK3	c.7649 G>T	Forward(Wt): 5'.....CATCCACATGGCATGTTTTAGAC.... 3' Forward (Mut): 5'.....CATCCACATGGCATGTTTTAGAA... 3' Reverse (C): 5'.....GAGTCATTGCCTTCTTATCTGGAG... 3'	471 bp	58
β- globin gene (Internal control)		Forward Control primer: 5'.....CAATGTATCATGCCTCTTTGCACC... 3' Reverse Control primer: 5'...GAGTCAAGGCTGAGAGATGCAGGA... 3'	800bp	

NEUROSCIENCE

Forward (Wt): Forward wild type primer, Forward Mut: Forward mutant primer, Reverse (C): Reverse common primer

• Primer concentration: 0.15 µl (each forward and reverse)

• Taq polymerase: 0.15 µl

• Template DNA: 0.5 µl (~16.5ng)

The thermal cycling:

• Primary denaturation: 5 minute at 95°C

• No. of cycles: 30

• Denaturation: 30 seconds at 94°C

• Annealing: 30 seconds at 61°C

• Extension: 30 seconds at 72°C

• Terminal extension: 10 minute at 72°C

The enzymatic digestion conditions for digestion of PCR products directly after amplification:

• Add:

PCR reaction mix: 5 µl

Nuclease-free water: 4.7 µl

10xBuffer G: 1 µl

Restriction enzyme: 0.3 µl

• Mix softly and spin down for a few second

• Incubate at 37°C for over night

Thermal inactivation: Restriction enzyme is inactivated by incubation at 65°C for 20 min.

Table 4. List of primers used for cycle sequencing method.

Gene	SNPs	Primer sequences (5'-3')	Product length (bp)	Tm
NrCAM	rs1269634 (A/G)	Forward: 5'..... GGATGGTAGACAGATTATGCTTCA... 3' Reverse: 5'..... GCAGTTCAGAGTGATGATAAATGC.... 3'	515 bp	58
NrCAM	rs6967368 (A/T)	Forward: 5'.. AATCTGCTCCTAACTTATCTCTCCATT... 3' Reverse: 5'. AAATTGTCCTCAAAGAAGTGAAATTTT.. 3'	236 bp	59
NrCAM	rs3763463 (C/G)	Forward: 5'..... GCAGCAAGCAGTGTGTTTACTC..... 3' Reverse: 5'..... CTTCGAAATTCATCAGTTGGG..... 3'	320 bp	60
	rs10235968 (C/T)	Forward:5'..... TGGTGAGGAGCTCAGAAAATGTT..... 3' Reverse:5'.. ATTTGCTTATTTACAAATGGGGGAGTA.. 3'	353 bp	60
PRODH	rs2238731(G/T)	Forward: 5'..... GGACAGAGGTTGGAGGCCC..... 3' Reverse: 5'..... GTTGATGGGGTCCTCATAGCC..... 3'	315 bp	61
ANKK1	c.562 C>T	Forward: 5'... ....ACCCTGGAACAGGCAGATAGC..... 3' Reverse: 5'..... GTTCACACAGTCCCAGGCAAG..... 3'	444 bp	61
ANK3	c.7649 G>T	Forward: 5'..... GGGTCTGATAAGCGGTCCAG..... 3' Reverse: 5'... .....ACCATTTTTAGGGCGTGCC..... 3'	366 bp	59

NEUROSCIENCE

• Electrophoresis: 10x10 cm 12% polyacrylamide, 1 hour at 150 volts

• Staining: 0.1% silver nitrate.

2.6. ARMS-PCR

ARMS-PCR was performed in triplicates for all specimens. The results showed Wt [1] allele or Mut [2] allele, depending on the amplicon bands detected on a polyacrylamide gel. Samples carrying the Wt/Wt alleles or Mut/ Mut alleles showed a single band on a polyacrylamide gel, while two bands with the same molecular size were expected for samples carrying both Mut and Wt alleles. A real negative is suggested by applying to replicate the internal control region and lack of amplification through the ARMS primers. The 12% polyacrylamide gel electrophoresis and silver staining protocols were used to visualizing amplified products [1], Wild type allele, and [2] Mutant type allele.

The PCR conditions for ARMS-PCR are:

• Reaction volume: 20 µl

• PCR mix: 18.21 µl

• Primer concentration: 0.25 µl (each forward (Mut/ Wt) and reverse); 0.07 µl (each forward and reverse for β-globin)

• Taq polymerase: 0.15 µl

• Template DNA: 1 µl (~13ng)

Thermal cycling:

Table 5. Restriction enzymes and their recognition site

SNP	Restriction Enzyme	Recognition Site	The length of the fragments		
			Wild Type	Heterozygote	Polymorphic Type
rs2238731	Hin1 II (Nia III)	5'... ↑C A T G ↓ ...3' 3'... ↑G T A C↓ .....5'	317nt	112nt, 205nt, 317nt	205nt, 112nt
rs3763463	SacII	5'...CC GC↓G .....3' 3'...GG↑CG CC.....5'	168nt, 152nt	320nt, 168nt, 152nt	320n
rs897218854	DdeI	5'.....C↓TNAG.....3' 3'.....GANT↑C.....5'	182nt, 16nt, 246nt	198nt, 246 nt, 16nt, 182nt	198nt, 246nt

SNP: Single nucleotide polymorphism

NEUROSCIENCE

Table 6. Genotype distribution for the candidate SNPs in the study

Gene		SNPs	P
NrCAM	rs10235968	SCZ	0.82
		Controls	0.016
		All subject	0.093
NrCAM	rs1269634	SCZ	0.83
		Controls	1
		All subject	0.78
NrCAM	rs6967368	SCZ	0.22
		Controls	0.073
		All subject	0.034
NrCAM	rs3763463	SCZ	1
		Controls	0.6
		All subject	0.71
PRODH	rs2238731	SCZ	1
		Controls	1
		All subject	1

NEUROSCIENCE

- Primary denaturation 5 minute at 95°C
- No. of cycles: 30
- Denaturation: 30 seconds at 94°C
- Annealing: 30 seconds at 65°C
- Extension: 30 seconds at 72°C
- Terminal extension: 10 minute at 72°C
- Electrophoresis: 10x10 cm 12% polyacrylamide, 1 hour at 150 volts
- Staining: 0.1% silver nitrate

2.7. Sequencing

To confirm the results obtained by ARMS-PCR and PCR-AFLP, Applied Biosystems incorporation (ABI) and 3130 Genetic Analyzer were used to sequencing one-third of samples.

The PCR conditions for Sequencing:

- Reaction volume: 20 µl
- PCR mix: 19.05 µl
- Primer concentration: 0.15 µl (each forward and reverse),
- Taq polymerase: 0.15 µl
- Template DNA: 0.5 µl (~20ng)

The thermal cycling:

- Primary denaturation 5 minute at 95°C
- No. of cycles: 30
- Denaturation: 30 seconds at 94°C
- Annealing: 30 seconds at 65°C
- Extension: 30 seconds at 72°C
- Terminal extension: 10 minute at 72°C

The PCR products were transferred to the advanced genomic department of Tehran Medical Genetic Laboratory for cycle sequencing. The cycle sequencing was implemented based on Applied Biosystem.

TheApplied Biosystem Protocol:

- 96°C for 1 min
- 25 cycles of:
- 96°C for 10 seconds.
- 50°C for 5 seconds.
- 60°C for 4 minute
- 4°C - hold

EDTA-Ethanol protocol was used for purifying products. After adding 12 µl Formamide, purified products were denatured at 95°C for 5 minutes and then cooled off to 4°C. Finally, products were run using the 3130 Genetic Analyzer.

2.8. Statistical analysis

The Chi-square test was used to determine departure from Hardy–Weinberg Equilibrium (HWE) for all SNPs (Wittke-Thompson, Pluzhnikov, & Cox, 2005; Rodriguez, Gaunt, & Day, 2009). To evaluate normality distributions, the Kolmogorov-Smirnov test using SPSS v. 24 was conducted. For analyzing the results, the Chi-square test using the R, SNP STATE Package to investigate the association of the candidate SNPs with SCZ. The Allele homozygosity and heterozygosity were specified. The OR (Odds Ratio) and 95% CI (Confidence Interval) were determined for all genotypes. Calculated Probability P≤0.05 was considered significant.

3. Results

3.1. Hardy–Weinberg disequilibrium

The genotype distribution for all candidate SNPs, among case and control, did not deviate from those predicted by Hardy–Weinberg (P>0.05), except for SNP rs10235968 in NrCAM, in control group (P=0.016) and, SNP rs6967368 in NrCAM in all subject (case+control) (P=0.034). The P-value for genotype distribution was calculated for the candidate SNPs and listed in Table 6. The rest of the candidate SNPs did not pervert from Hardy-Weinberg equilibrium in both case and control groups.

3.2. Allele and genotype frequency of the candidate SNPs

Incidence of Allele C and Allele T for NrCAM's SNP rs10235968 among patients was 0.66 and 0.34, respectively, while the incidence of allele C and T was equal among the healthy group (0.5/0.5). Genotyping frequency of two genotypes CC and TT for SNP rs10235968 among patients was 0.43 and 0.11, respectively, while the frequency for the two genotypes among the healthy group was both 0.19. The genotype and allele frequencies of all candidate SNPs were presented in Table 7.

Table 9 shows genotype association of SNP rs10235967 in NrCAM with schizophrenia. In codominant inheritance: the genotype CT, 3.01 times, will increase the risk of schizophrenia, compared to the genotype CC. The genotype TT 4.1 times will increase the risk of schizophrenia compared to the genotype CC (P=0.0006). In dominant inheritance: the genotypes CT+TT, 3.21 times will increase the risk of schizophrenia, compared to the genotype CC (P=0.0001). In overdominant inheritance: the genotype CT, 1.87 times, will increase the risk of schizophrenia, compared to the genotypes CC+TT (P=0.025).

3.3. Haplotype analysis

According to the haplotype analysis, shown in Table 10, three alleles, T, T, and C, in the variants rs10235968, rs6967368, rs3763463, respectively, within the NrCAM gene showed a strong association with schizophrenia disorder (P=0.0001).

3.4. Linkage disequilibrium (LD)

The SNPs rs10235968, rs6967368, rs3763463 located in the NrCAM gene promoter (Upstream variant 2KB) were analyzed for LD. NrCAM displayed LD in two blocks rs69677368 and rs3763463 (P=0.0371).

3.5. Frequency of recurrence of two novel variants c.562 C> T and c.7649 G> T

After genotyping two novel variants, c.562 C> T and c.7649 G> T within genes ANKK1, ANK3, among patients and healthy people. We have found no recurrence frequency of two novel variants, c.562 C> T and c.7649 G>T.

4. Discussion

According to the results of the present study and other studies investigating NrCAM's SNPs and schizophrenia association, all SNPs' frequency in this study has shown no significant association between the candidate SNPs and SCZ except for SNP rs10235968 in NrCAM, which was significantly associated with SCZ. It seems that the allele T in rs10235968 associated with schizophrenia, suggested be-

Table 7. Genotype and allele frequencies of the all candidate SNPs

Gene		Polymorphisms	N	Genotype frequency (%)			Allele frequency	
				CC	CT	TT	C	T
NrCAM	SCZ	rs10235968	95	41(43)	44(46)	10 (11)	0.66	0.34
	Controls		119	22 (19)	71 (62)	22 (19)	0.5	0.5

Gene		Polymorphisms	N	Genotype frequency (%)			Allele frequency	
				AA	AG	GG	A	G
NrCAM	SCZ	rs1269634	95	17(18)	49(52)	29(13)	0.44	0.56
	Controls		119	16(13)	56(47)	47(39)	0.37	0.63

Gene		Polymorphisms	N	Genotype frequency (%)			Allele frequency	
				TT	TA	AA	A	T
NrCAM	SCZ	rs6967368	95	70(74)	21(22)	4(3)	0.15	0.85
	Controls		117	92(79)	21(18)	4(3.4)	0.12	0.88

Gene		Polymorphisms	N	Genotype frequency (%)			Allele frequency	
				CC	GC	GG	C	G
NrCAM	SCZ	rs3763463	95	76(80)	18(19)	1(1)	0.89	0.11
	Controls		118	94(80)	24(20)	0(0)	0.9	0.1

Gene		Polymorphisms	N	Genotype frequency (%)			Allele frequency	
				CC	CT	TT	C	T
PRODH	SCZ	rs2238731	95	93(98)	2(2)	0(0)	0.99	0.01
	Controls		119	117(98)	2(1.7)	0(0)	0.99	0.01

Gene		Polymorphisms	N	Genotype frequency (%)			Allele frequency	
				CC	CT	TT	C	T
ANKK1	SCZ	rs897218854	95	94(98.9)	1(1.1)	0(0)	0.99	0.005
	Controls		114	114(96.6)	0(0)	0(0)	1	0

Gene		Polymorphisms	N	Genotype frequency (%)			Allele frequency	
				GG	GT	TT	G	T
ANK3	SCZ	c.7649 G>T	95	94(98.9)	1(1.1)	0(0)	0.99	0.005
	Controls		114	114(96.6)	0(0)	0(0)	1	0

N: Sample size, SCZ: Schizophrenia

NEUROSCIENCE

Table 8. Association between the candidate SNPs and schizophrenia

Gene	Chr	SNP/Mutation	A1/A2	Common allele	P Value	OR (95%CI)	$_x$ 2
NrCAM	7	rs10235968	C/T	C	0.001	1.969 (1.323-2.929)	11.284
NrCAM	7	rs126934	A/G	A	0.144	1.407 (0.88-2.22)	2.134
NrCAM	7	rs6967368	T/A	T	0.593	0.831 (0.42-1.63)	0.285
NrCAM	7	rs3763463	G/C	C	0.781	0.817 (0.05-13.28)	0.078
PRODH	22	rs2238731	T/C	C	0.881	0.81 (0.05-13.02)	0.022

NEUROSCIENCE

OR: Odds Ratio, CI: Confidence Interval, Chr: Chromosome, SNP: Single Nucleotide Polymorphism, A1/A2: allele1/allele2

Table 9. Genotypic association of SNP rs10235967 in NrCAM with schizophrenia

rs10235968 genotype association with SCZ					
Model	**Genotype**	**Case**	**Control**	**OR (95% CI)**	**P-value**
Codominant	C/C	41 (43.2%)	22 (19.1%)	1.00	6e-04
	C/T	44 (46.3%)	71 (61.7%)	3.01 (1.59-5.70)	
	T/T	10 (10.5%)	22 (19.1%)	4.10 (1.65-10.18)	
Dominant	C/C	41 (43.2%)	22 (19.1%)	1.00	1e-04
	C/T-T/T	54 (56.8%)	93 (80.9%)	3.21 (1.73-5.95)	
Recessive	C/C-C/T	85 (89.5%)	93 (80.9%)	1.00	0.08
	T/T	10 (10.5%)	22 (19.1%)	2.01 (0.90-4.49)	
Over dominant	C/C-T/T	51 (53.7%)	44 (38.3%)	1.00	0.025
	C/T	44 (46.3%)	71 (61.7%)	1.87 (1.08-3.25)	

NEUROSCIENCE

SNP: Single Nucleotide Polymorphism, OR: Odds Ratio, CI: Confidence Interval, P-value: A small p-value (typically ≤ 0.05) shows strong evidence against the null hypothesis, so the null hypothesis is rejected.

Table 10. Haplotype Analysis of the candidate SNPs

Haplotype Association With SCZ						
	rs10235968	**rs6967368**	**rs3763463**	**Frequency**	**OR (95% CI)**	**P-value**
1	C	T	C	0.4544	1.00	---
2	T	T	C	0.3104	3.70 (2.08 - 6.57)	<0.0001
3	T	A	C	0.1169	1.31 (0.67 - 2.54)	0.43
4	C	T	G	0.0984	1.68 (0.79 - 3.58)	0.18
5	C	A	C	0.0151	4.66 (0.46 - 47.20)	0.19

Global haplotype association p-value: 0.00015

NEUROSCIENCE

ing risk in the Iranian population (P=0.001). The decreased frequency of genotype TT for SNP rs10235968 in NrCAM was seen among the patient's group (Table 7). Reduced homozygous CC and TT and increased heterozygote CT were observed among the healthy group in NrCAM's SNP rs10235968 (Table 7).

The Haplotype T, T, C in rs10235968, rs6967368, rs3763463, respectively, within the NrCAM gene, showed significant association with schizophrenia disorder (P=0.0001). rs10235968 deviated from those predicted by Hardy–Weinberg equilibrium in the control group (P=0.016). Probably, it is because of the small sample size. Many studies have been conducted aim to genotype rs10235968. However, the results are a little different in different populations. According to the NCBI dataset, the common allele for SNP rs10235968 is C with allelic frequency 0.52. in 1000 genome projects, the allele frequency of C and T was 0.521 and 0.479, respectively. Nevertheless, in Africa, the frequency of allele C of rs10235968 is 0.446, and allele T is 0.554. The allele C may probably not be regarded as an ancestral allele, and more probably, rs10235968 is a balancing polymorphism.

The prominent role of SNP rs10235968 in the development of schizophrenia is still unknown. Although the candidate SNPs have been previously studied about SCZ, no study has been conducted on the association of these SNPs with SCZ among Iranian patients.

Two types of research evaluated the association of NrCAM's SNPs with SCZ: Atz, Rollins, & Vawter (2007) in California University reported the association of SNP rs646558 in NrCAM with the susceptibility to SCZ, and Kim et al. (2009) evaluated the association of 13 SNPs in NrCAM and SCZ among Korean population and no association was found between the examined polymorphisms and SCZ. The latter study's Korean population results are not in line with those drawn from this study among Iranian patients. SNP rs10235968 is located in the promoter of NrCAM. The role of nucleotide changes in the upstream region of NrCAM is not entirely known. This region is not translated; however, since it is located in the gene's promoter region, the epigenetic changes may affect gene translation (Barbeau, Liang, Robitalille, Quirion, & Srivastava, 1995).

The NrCAM gene expression strongly decreases in the the brain of patients with SCZ, and the abnormal ratio of the synaptic NrCAM proteins found in the hippocampus of schizophrenic patients (Honer et al., 1997; Vawter, Howard, Hyde, Kleinman, & Freed, 1999; Vawter, 2000). The association of CAM's signaling pathway and SCZ among Chinese and European populations were previously reported (O'Dushlaine et al., 2011, Zhang, et al., 2015). NrCAM is involved in CAM's signaling pathway, which has an essential role in the brain's cognitive function, an attribute that is disrupted in SCZ. It is hypothesized that the SNPs of NrCAM can affect CAM's pathways; by how they can affect the brain's cognitive function. NrCAM SNPs are likely to affect the function of the protein or gene expression, through which they can affect the process of transmitting signals between neuron cells (Schmid & Maness 2008).

The cell-cell adhesion molecule, which is encoded by the NrCAM gene, is vital for the formation of neurons and their axons, synaptic flexibility, myelination, and highly coordinated function of the brain, such as the brain's cognitive functions of memory and learning (Benson, Schnapp, Shapiro,& Huntley, 2000). Previous research showed that the disruption of neuronal connection's adhesion during neuronal cell growth in the nervous system may result in neuronal circuit dysfunction and can be the etiological foundation of many neurological disorders (Yang, Hou, Jiang, & Zhang, 2014). Hargreaves et al (2014) reported that mutations or abnormal expression of NrCAM are likely to cause alteration in synapse formation. Disruption in the NrCAM function can be associated with psychiatric disorders, including SCZ, Autism, Alzheimer, Mathematic learning disability, and drug addiction (Sakurai, 2012). Furthermore, the protein NrCAM is a stimulator for the division of astrocyte neuronal cells. Glucocorticoid Receptor (GR) pathway contributes to NrCAM´s ability to stimulate cell division as NrCAM proteins adhere to neuron cell, intracellular

The NrCAM mRNAs decrease, whereas calreticulin mRNAs and glutamine mRNAs increase. These two genes are active in the Glucocorticoid Receptor (GR) pathway, which is one of the ten signaling pathways identified as a biomarker in the Veripsych kit within the blood serum of drug-Naïve schizophrenic patients (Tomasik, Schwarz, Guest, & Bahn, 2012; Sabherwal, English Föcking, Cagney, & Cotter, 2016). It may be postulated that the activity of NrCAM and the two proteins in question affect each other, and disruption in either of them will disrupt the intracellular chain of events and cause a complication. As a result, the decreased expression of NrCAM through the GR pathway can also disrupt the nervous system's development.

PRODH is one of the critical known genes concerning SCZ. The role of PRODH in the development of SCZ was frequently reported in different populations (Bassett, Marshall, Lionel, Chow, & Scherer, 2008). SNP rs2238731 is situated in exon 12 of PRODH and was reported as a functional missense mutation (Bender et al., 2005). SNP rs2238731 is situated in a translated exon. rs2238731 (V427M) affects the function of proline dehydrogenase, the protein which is encoded by PRODH. Bender and Almashanu reported that the SNP V427M leads to a 30%-70% decrease in the Proline Dehydrogenase Enzyme (POX) among the schizophrenic patients (Bender et al., 2005).

The reduction in the proline dehydrogenase enzyme activity contributes to the development of SCZ by affecting the glutamatergic pathway and, in particular, by affecting the NMDA receptors (Coyle, Tsai, & Goff, 2003; Zinkstok, et al. 2008). In a study carried out on the relationship between polymorphisms in PRODH and brain cortical volumes, only SNP V427M (rs2238731) was found to be significantly associated with the cortical thickness. Cortical thickness in patients with genotype GA was reported to be smaller compared to those carrying genotype GG. However, this finding needs to be confirmed in larger sample sizes (Ota et al., 2014). The frequency of allele A in rs2238731 is 0.02 in the American population, and it was reported as the predisposing allele for SCZ (Bender, et al., 2005). Ota et al. (2014) suggested that allele G in rs2238731 could be a protective allele for SCZ. Despite the role of rs2238731 in the development of SCZ, no significant association was found between rs2238731 and SCZ among Iranian patients.

The frequency of alleles G and A in rs2238731 displayed no meaningful difference between the two patients and healthy groups (P=0.881). Novel variants c.562 C>T and c.7649 G>T in ANKK1 and ANK3 respectively, neither schizophrenic nor non-schizophrenic people were carrying this mutated allele. Recently, the c.562 C>T was registered by characteristic rs89721885408642 in NCBI.

Novel variant c.7649 G>T is situated in exon 13 of ANK3. Proteins encoded by the ANK3 are those connected to cellular integral proteins. In a study conducted in the Norwegian population, the role of ANK3 in susceptibility to SCZ was reported. They examined the expression of ANK3 in blood and found that protein ANK3 increased in the blood of those suffering from SCZ. As a result, they suggested that ANK3 mRNA might be one of the diagnostic biomarkers for diagnosing SCZ (Athanasiu et al., 2010).

ANK3 is associated with other mental disorders such as autism and mental retardation (Bi et al., 2012; Iqbal et al., 2013). Increased expression of ANK3 was previously reported in blood patients with SCZ. It can be regarded as a factor for the formation of psychological disorders such as mental retardation, autism, and SCZ (Iqbal et al., 2013).

5. Conclusion

In this study, for the first time, the NrCAM's polymorphisms were interrogated among a sample of Iranian patients in terms of association with SCZ. Our results suggest the association of NrCAM's SNP rs10235968 with schizophrenia disorder in an Iranian sample. NrCAM expression should be evaluated to understand the role of SNP rs10235968 further. The results obtained in this study are different from other similar studies conducted in other populations and indicate genetic diversity in different populations.

The NrCAM's SNPs can play essential roles in the development of schizophrenia through numerous biological pathways. This study's restriction was the small sample size and the low number of polymorphisms that were evaluated in this research. In our investigation, the sample size was too small to come to a decisive conclusion. Hence, further researches are suggested to support the results of this investigation. Besides corroborating the affiliation between NrCAM polymorphisms and SCZ among the Iranian, more polymorphisms should be evaluated.

Ethical Considerations

Compliance with ethical guidelines

All ethical principles are considered in this article. The participants were informed about the purpose of the research and its implementation stages. They were also assured about the confidentiality of their information and were free to leave the study whenever they wished, and if desired, the research results would be available to them.

Funding

This research was extracted from the research project (Grant No. 94001), Funded by the University of Tehran Medical Genetics Laboratory.

Authors' contributions

All authors equally contributed in preparing this article.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

We appreciate the all patients who have participated in this investigation. We also appreciate Imam Hussein Hospital's Psychiatry ward and the Iranian Society for Supporting Schizophrenic Patients (AHEBA). Also, we appreciate the personnel of the Tehran Medical Genetics Laboratory for their support.

References

Arab, A. H., & Elhawary, N. A. (2015). Association between ANKK1 (rs1800497) and LTA (rs909253) genetic variants and risk of Schizophrenia. *BioMed Research International, 2015*:821827. [DOI:10.1155/2015/821827] [PMID] [PMCID]

Askland, K., Read, C., O'Connell, C., & Moore, J. H. (2012). Ion channels and schizophrenia: A gene set-based analytic approach to GWAS data for biological hypothesis testing. *Human Genetics, 131*(3), 373-91. [DOI:10.1007/s00439-011-1082-x] [PMID] [PMCID]

Athanasiu, L., Mattingsdal, M., Kähler, A. K., Brown, A., Gustafsson, O., & Agartz, I., et al. (2010). Gene variants associated with schizophrenia in a Norwegian genome-wide study are replicated in a large European cohort. *Journal of Psychiatric Research, 44*(12), 748-53. [DOI:10.1016/j.jpsychires.2010.02.002] [PMID] [PMCID]

Atz, M. E., Rollins, B., & Vawter, M. P. (2007). NCAM1 association study of bipolar disorder and schizophrenia: Polymorphisms and alternatively spliced isoforms lead to similarities and differences. *Psychiatric Genetics, 17*(2), 55. [DOI:10.1097/YPG.0b013e328012d850] [PMID] [PMCID]

Ayalew, M., Le-Niculescu, H., Levey, D. F., Jain, N., Changala, B., & Patel, S. D., et al. (2012). Convergent functional genomics of schizophrenia: from comprehensive understanding to genetic risk prediction. *Molecular Psychiatry, 17*(9), 887-905. [DOI:10.1038/mp.2012.37] [PMID] [PMCID]

Barbeau, D., Liang, J. J., Robitalille, Y., Quirion, R., & Srivastava, L. K. (1995). Decreased expression of the embryonic form of the neural cell adhesion molecule in schizophrenic brains. *Proceedings of the National Academy of Sciences, 92*(7), 2785-9. [DOI:10.1073/pnas.92.7.2785] [PMID] [PMCID]

Bassett, A. S., Marshall, C. R., Lionel, A. C., Chow, E. W., & Scherer, S. W. (2008). Copy number variations and risk for schizophrenia in 22q11. 2 deletion syndrome. *Human Molecular Genetics, 17*(24), 4045-53. [DOI:10.1093/hmg/ddn307] [PMID] [PMCID]

Bender, H. U., Almashanu, S., Steel, G., Hu, C. A., Lin, W. W., & Willis, A., et al. (2005). Functional consequences of PRODH missense mutations. *The American Journal of Human Genetics, 76*(3), 409-20. [DOI:10.1086/428142] [PMID] [PMCID]

Benson, D. L., Schnapp, L. M., Shapiro, L., & Huntley, G. W. (2000). Making memories stick: Cell-adhesion molecules in synaptic plasticity. *Trends in Cell Biology, 10*(11), 473-482. [DOI:10.1016/S0962-8924(00)01838-9]

Bi, C., Wu, J., Jiang, T., Liu, Q., Cai, W., & Yu, P., et al. (2012). Mutations of ANK3 identified by exome sequencing are associated with autism susceptibility. *Human mutation, 33*(12), 1635-8. [DOI:10.1002/humu.22174] [PMID]

Chen, L., & Zhou, S. (2010). "CRASH" ing with the worm: insights into L1CAM functions and mechanisms. *Developmental dynamics: An official publication of the American Association of Anatomists, 239*(5), 1490-501. [DOI:10.1002/dvdy.22269] [PMID] [PMCID]

Christofolini, D. M., Bellucco, F. T., Ota, V. K., Belangero, S. I., Cernach, M. C., & Gadelha, A., et al. (2011). Assessment of 22q11. 2 copy number variations in a sample of Brazilian schizophrenia patients. *Schizophrenia Research, 132*(1), 99-100. [DOI:10.1016/j.schres.2011.07.007] [PMID]

Coyle, J. T., Tsai, G., & Goff, D. (2003). Converging evidence of NMDA receptor hypofunction in the pathophysiology of schizophrenia. *Annals of the New York Academy of Sciences, 1003*(1), 318-27. [DOI:10.1196/annals.1300.020] [PMID]

Elangbam, C. S., Qualls Jr, C. W., & Dahlgren, R. R. (1997). Cell adhesion molecules—update. *Veterinary Pathology, 34*(1), 61-73. [DOI:10.1177/030098589703400113] [PMID]

Galderisi, S., Merlotti, E., & Mucci, A. (2015). Neurobiological background of negative symptoms. *European Archives of Psychiatry and Clinical Neuroscience, 265*(7), 543-58. [DOI:10.1007/s00406-015-0590-4] [PMID]

Hamosh, A., Scott, A. F., Amberger, J. S., Bocchini, C. A., & McKusick, V. A. (2005). Online Mendelian Inheritance in Man (OMIM), a knowledgebase of human genes and genetic disorders. *Nucleic Acids Research, 33*(suppl_1), D514-D517. [DOI:10.1093/nar/30.1.52] [PMID] [PMCID]

Hargreaves, A., Anney, R., O'Dushlaine, C., Nicodemus, K. K., Gill, M., Corvin, A., & Wellcome Trust Case Control Consortium 2. (2014). The one and the many: Effects of the cell adhesion molecule pathway on neuropsychological function in psychosis. *Psychological Medicine, 44*(10), 2177-87. [DOI:10.1017/S0033291713002663] [PMID] [PMCID]

Honer, W. G., Falkai, P., Young, C., Wang, T., Xie, J., & Bonner, J., et al. (1997). Cingulate cortex synaptic terminal proteins and neural cell adhesion molecule in schizophrenia. *Neuroscience, 78*(1), 99-110. [DOI:10.1016/S0306-4522(96)00489-7]

Iqbal, Z., Vandeweyer, G., van der Voet, M., Waryah, A. M., Zahoor, M. Y., & Besseling, J. A., et al. (2013). Homozygous and heterozygous disruptions of ANK3: At the crossroads of neurodevelopmental and psychiatric disorders. *Human Molecular Genetics, 22*(10), 1960-70. [DOI:10.1093/hmg/ddt043] [PMID]

Jacquet, H., Raux, G., Thibaut, F., Hecketsweiler, B., Houy, E., & Demilly, C., et al. (2002). PRODH mutations and hyperprolinemia in a subset of schizophrenic patients. *Human Molecular Genetics, 11*(19), 2243-9. [DOI:10.1093/hmg/11.19.2243] [PMID]

Jasiewicz, A., Samochowiec, A., Samochowiec, J., Małecka, I., Suchanecka, A., & Grzywacz, A. (2014). Suicidal behavior and haplotypes of the Dopamine Receptor Gene (DRD2) and ANKK1 gene polymorphisms in patients with alcohol dependence–preliminary report. *PLoS One, 9*(11), e111798. [DOI:10.1371/journal.pone.0111798] [PMID] [PMCID]

Jia, P., Wang, L., Meltzer, H. Y., & Zhao, Z. (2010). Common variants conferring risk of schizophrenia: a pathway analysis of GWAS data. *Schizophrenia Research, 122*(1-3), 38-42. [DOI:10.1016/j.schres.2010.07.001] [PMID] [PMCID]

Kamiguchi, H., & Lemmon, V. (1997). Neural cell adhesion molecule L1: Signaling pathways and growth cone motility. *Journal of Neuroscience Research, 49*(1), 1-8. [DOI:10.1002/(SICI)1097-4547(19970701)49:13.0.CO;2-H]

Kempf, L., Nicodemus, K. K., Kolachana, B., Vakkalanka, R., Verchinski, B. A., & Egan, M. F., et al. (2008). Functional polymorphisms in PRODH are associated with risk and protection for schizophrenia and fronto-striatal structure and function. *PLoS Genet, 4*(11), e1000252. [DOI:10.1371/journal.pgen.1000252] [PMID] [PMCID]

Kim, H. J., Kim, H. G., Kim, M. H., Kwack, K. B., Park, J. K., & Kim, T., et al. (2009). Association between neuronal cell adhesion molecule (NRCAM) single nucleotide polymorphisms and schizophrenia in a Korean population. *Psychiatry and Clinical Neurosciences, 63*(1), 123-4. [DOI:10.1111/j.1440-1819.2008.01893.x] [PMID]

Lane, R. P., Chen, X. N., Yamakawa, K., Vielmetter, J., Korenberg, J. R., & Dreyer, W. J. (1996). Characterization of a highly conserved human homolog to the chicken neural cell surface protein Bravo/Nr-CAM that maps to chromosome band 7q31. *Genomics, 35*(3), 456-65. [DOI:10.1006/geno.1996.0385] [PMID]

Levinson, D. F., Duan, J., Oh, S., Wang, K., Sanders, A. R., & Shi, J., et al. (2011). Copy number variants in schizophrenia: confirmation of five previous findings and new evidence for 3q29 microdeletions and VIPR2 duplications. *American Journal of Psychiatry, 168*(3), 302-16. [DOI:10.1176/appi.ajp.2010.10060876] [PMID] [PMCID]

Little, S. (1995). Amplification□refractory mutation system (ARMS) analysis of point mutations. *Current Protocols in Human Genetics, 7*(1), 8-9. [DOI:10.1002/0471142905.hg0908s07] [PMID]

Liu, H., Heath, S. C., Sobin, C., Roos, J. L., Galke, B. L., & Blundell, M. L., et al. (2002). Genetic variation at the 22q11 PRODH2/DGCR6 locus presents an unusual pattern and increases susceptibility to schizophrenia. *Proceedings of the National Academy of Sciences, 99*(6), 3717-22. [DOI:10.1073/pnas.042700699] [PMID] [PMCID]

Nanfack, A. J., Agyingi, L., Noubiap, J. J. N., Ngai, J. N., Colizzi, V., & Nyambi, P. N. (2015). Use of amplification refractory mutation system PCR assay as a simple and effective tool to detect HIV-1 drug resistance mutations. *Journal of Clinical Microbiology, 53*(5), 1662-71. [DOI:10.1128/JCM.00114-15] [PMID] [PMCID]

O'Dushlaine, C., Kenny, E., Heron, E., Donohoe, G., Gill, M., & Morris, D., et al. (2011). Molecular pathways involved in neuronal cell adhesion and membrane scaffolding contribute to schizophrenia and bipolar disorder susceptibility. *Molecular Psychiatry, 16*(3), 286-92. [DOI:10.1038/mp.2010.7] [PMID]

Ota, V. K., Bellucco, F. T., Gadelha, A., Santoro, M. L., Noto, C., & Christofolini, D. M., et al. (2014). PRODH polymorphisms, cortical volumes and thickness in schizophrenia. *PloS One, 9*(2), e87686. [DOI:10.1371/journal.pone.0087686] [PMID] [PMCID]

Pulver, A. E., Nestadt, G., Goldberg, R., Shprintzen, R. J., Lamacz, M., & Wolyniec, P. S., et al. (1994). Psychotic illness in patients diagnosed with velo-cardio-facial syndrome and their relatives. *Journal of Nervous and Mental Disease, 182*(8), 476-8. [DOI:10.1097/00005053-199408000-00010] [PMID]

Rodriguez, S., Gaunt, T. R., & Day, I. N. (2009). Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. *American Journal of Epidemiology, 169*(4), 505-14. [DOI:10.1093/aje/kwn359] [PMID] [PMCID]

Sabherwal, S., English, J. A., Föcking, M., Cagney, G., & Cotter, D. R. (2016). Blood biomarker discovery in drug-free schizophrenia: the contribution of proteomics and multiplex immunoassays. *Expert review of Proteomics, 13*(12), 1141-55. [DOI:10.1080/14789450.2016.1252262] [PMID]

Sakurai, T. (2012). The role of NrCAM in neural development and disorders—beyond a simple glue in the brain. *Molecular and Cellular Neuroscience, 49*(3), 351-63. [DOI:10.1016/j.mcn.2011.12.002] [PMID]

Sakurai, T., Ramoz, N., Reichert, J. G., Corwin, T. E., Kryzak, L., & Smith, C. J., et al. (2006). Association analysis of the NrCAM gene in autism and in subsets of families with severe obsessive–compulsive or self-stimulatory behaviors. *Psychiatric Genetics, 16*(6), 251-7. [DOI:10.1097/01.ypg.0000242196.81891.c9] [PMID]

Schmid, R. S., & Maness, P. F. (2008). L1 and NCAM adhesion molecules as signaling coreceptors in neuronal migration and process outgrowth. *Current Opinion in Neurobiology, 18*(3), 245-50. [DOI:10.1016/j.conb.2008.07.015] [PMID] [PMCID]

Shashi, V., Berry, M. N., & Keshavan, M. S. (2009). Mechanistic approach to understanding psychosis risk in velocardiofacial syndrome. *Current Pediatric Reviews, 5*(2), 89-104. [DOI:10.2174/157339609788185703]

Shirzad, H., Beyraghi, N., Ataei, K. M., & Akbari, M. T. (2017). Family-based whole-exome sequencing for identifying novel variants in consanguineous families with schizophrenia. *Iranian Red Crescent Medical Journal, 19*(2). [DOI:10.5812/ircmj.35788]

Tomasik, J., Schwarz, E., Guest, P. C., & Bahn, S. (2012). Blood test for schizophrenia. *European Archives of Psychiatry and Clinical Neuroscience, 262*(2), 79-83. [DOI:10.1007/s00406-012-0354-3] [PMID]

Vawter, M. P., Howard, A. L., Hyde, T. M., Kleinman, J. E., & Freed, W. J. (1999). Alterations of hippocampal secreted N-CAM in bipolar disorder and synaptophysin in schizophrenia. *Molecular Psychiatry, 4*(5), 467-75. [DOI:10.1038/sj.mp.4000547] [PMID]

Vawter, M. P. (2000). Dysregulation of the neural cell adhesion molecule and neuropsychiatric disorders. *European Journal of Pharmacology, 405*(1-3), 385-95. [DOI:10.1016/S0014-2999(00)00568-9]

Wang, B., Williams, H., Du, J. S., Terrett, J., & Kenwrick, S. (1998). Alternative splicing of humanNrCAMin neural and nonneural tissues. *Molecular and Cellular Neuroscience, 10*(5-6), 287-95. [DOI:10.1006/mcne.1997.0658]

Wittke-Thompson, J. K., Pluzhnikov, A., & Cox, N. J. (2005). Rational inferences about departures from Hardy-Weinberg equilibrium. *The American Journal of Human Genetics, 76*(6), 967-86. [DOI:10.1086/430507] [PMID] [PMCID]

Yang, X., Hou, D., Jiang, W., & Zhang, C. (2014). Intercellular protein–protein interactions at synapses. *Protein & Cell, 5*(6), 420-44. [DOI:10.1007/s13238-014-0054-z] [PMID] [PMCID]

Yoo, B. K., Shim, J. C., Lee, B. D., Kim, C., Chung, Y. I., & Park, J. M., et al. (2012). Association of the neuronal cell adhesion molecule (NrCAM) gene variants with personality traits and addictive symptoms in methamphetamine use disorder. *Psychiatry Investigation, 9*(4), 400. [DOI:10.4306/pi.2012.9.4.400] [PMID] [PMCID]

Yu, H., Bi, W., Liu, C., Zhao, Y., Zhang, D., & Yue, W. (2014). A hypothesis-driven pathway analysis reveals myelin-related pathways that contribute to the risk of schizophrenia and bipolar disorder. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 51*, 140-5.[DOI:10.1016/j.pnpbp.2014.01.006] [PMID]

Zhang, Z., Yu, H., Jiang, S., Liao, J., Lu, T., & Wang, L., et al. (2015). Evidence for association of cell adhesion molecules pathway and NLGN1 polymorphisms with schizophrenia in Chinese Han population. *PloS One, 10*(12), e0144719. [DOI:10.1371/journal.pone.0144719] [PMID] [PMCID]

Zinkstok, J., Schmitz, N., Van Amelsvoort, T., Moeton, M., Baas, F., & Linszen, D. (2008). Genetic variation in COMT and PRODH is associated with brain anatomy in patients with schizophrenia. *Genes, Brain and Behavior, 7*(1), 61-9. https://doi.org/10.1111/j.1601-183X.2007.00326.x

Kay, S. R., Opler, L. A., & Lindenmayer, J. P. (1988). Reliability and validity of the positive and negative syndrome scale for schizophrenics. *Psychiatry Research, 23*(1), 99-110. [DOI:10.1016/0165-1781(88)90038-8]