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Association between Common Single- nucleotide Polymorphism of Reelin Gene, rs736707 (C/T) with Autism Spectrum Disorder in Iranian-Azeri Patients

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Abstract

Introduction

Reelin gene (RELN) codes a large extracellular matrix glycoprotein with serine protease activity and is implicated in the modulation of neuronal signaling, synaptic transmission and plasticity. The reelin plays a fundamental and pivotal role in the development of laminar structures and may be one of the loci contributing to the positive linkage between chromosome 7q and autistic disorder. This study was performed to examine the association of a frequent genetic variation in reelin gene, rs736707 (C/T), with Autism risk among Iranian-Azeri population.

Materials and Methods

A case–control association study included 74 patients with Autism spectrum disorder (ASD) and 86 healthy subjects. Genomic DNA was extracted from peripheral blood samples by salting-out method. Single nucleotide polymorphisms (SNP) genotyping was carried out by Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. The collected data were analyzed through java stant online statistics software, using Chi-square, with a significance level of 0.05.

Results

Significant differences in the allele and genotype frequencies between cases and controls were not observed (P>0.05). The rs736707SNP was not associated with Autism predisposition in Iranian-Azeri children.

Conclusion

Based on our results, the rs736707 SNP could not be used as a useful molecular biomarker to predict genetic susceptibility for autism spectrum disorder in Iranian-Azeri patients.

***Key Words**:* Autism; Molecular marker, Polymorphism, Reelin gene.

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1- Introduction

Autism spectrum disorder (ASD) is a complex childhood neuropsychiatric disorder that is characterized by deficits in verbal and non-verbal communication, reciprocal social interactions, stereotypic behaviors, interests and activities. Autism is a heterogeneous disorder and influences by both environmental (1, 2) and genetic factors (3-5).

ASD has onset in early childhood and usually diagnosed before 3 years. Based on the studies Elsabbagh et al. (2012), the median of worldwide prevalence estimates of ASDs is 62/10,000 with approximately four times higher in males than in females. Various twin studies have illustrated that the risk of autism is higher among siblings of affected individuals than in general population, indicating that autism is one of the most strongly genetic childhood-onset psychiatric disorders with high heritability. Therefore, it is a well-accepted hypothesis (6) that several susceptibility genes are interacting together with a complex mode of inheritance leading to the typical phenotypes of the ASD. There could be involvement of at least two to four genes and perhaps more (6-8).

Reelin gene (RELN) is located on chromosome 7q22 in humans and codes for a large extracellular glycoprotein of approximate molecular mass of 388 kDa (9, 10). Reelin is a large secreted extracellular matrix glycoprotein with serine protease activity and is expressed mainly in the brain as well as in blood, spinal cord and other organs and tissues in the body and is implicated in the modulation of neuronal signaling, synaptic transmission and plasticity (11). The Reelin plays a fundamental and pivotal role in the development of laminar structures, such as the cerebral cortex, hippocampus, cerebellum, and brain-stem nuclei. It is secreted by pioneer neurons like Cajal–Retzius neurons, located in the marginal zone of neocortex and hippocampus. Reelin specifically binds to its several transmembrane receptors present on post-mitotic migratory neurons (12).

Several recent studies suggest Reelin may be one of the loci contributing to the positive linkage between chromosome 7q and autistic disorder. Biochemical analyses of postmortem autistic brain point to Reelin as being involved in the pathology of autism (13).

This study investigated the association of a Single nucleotide polymorphism (SNPs), C/T SNP in intron 59 (db SNP: rs736707), including one splice region SNPs in the Reelin gene with autism.

2- Materials and Methods

2.1-Participants

All subjects with ASD were selected from Tabriz Autism Associations in North West of Iran. All participants were diagnosed with ASD following a detailed psychiatric assessment, developmental history, and a review of the data provided by their teachers and parents. These subjects were then examined and assessed by another psychiatrist and at the end only 74 patients fulfilled the DSM-IV criteria for ASD (20). Oral and written informed consents were obtained from at least one parent of all participants, and the research protocol was approved by the ethics committee of Tabriz University of Medical Sciences.

For control group, volunteers were recruited from local children's hospital in the same age range. They were also examined to rule out any neurological, psychiatric or learning problems. Furthermore, none of these children was on medication, and this information was gathered from one of their parents. Furthermore, all children had a permission to withdraw from the study at any time.

At the end, the patient group consisted of both male and female autistic subject (n=74; mean age=8.57 and range age=3-24 years at the time of sampling, 53 males and 18 females). The control group consisted of 86 children.

2.2-Sample Collection

Peripheral blood samples (2cc) were taken from all the participants and delivered to the laboratory for molecular genetics study.

2.3-PCR and RFLP

Genomic DNA was extracted from peripheral blood using the Proteinase *K* (PROK) and salting out method. The target sequence containing the polymorphic site was amplified by forward 5´-GCAGGGCTGACAGGTTACAC-3´ and reverse5´-TGGTCTCCTCTATCAAAGTTGGC-3´ site-specific primers. Polymerase chain reaction (PCR) was performed in a total volume of 25 ll reaction mixtures containing 2.5 µl 10X reaction buffer, 2 mM MgCl2, 0.2 mMdNTPs, 0.4 µM of each primer, 0.1-0.5 µgDNA and 1.0 U of Taq polymerase (Cinnagen, Iran)**.** PCR was carried out by thermal cycler (Sensoquest, GmbH, Germany) at 35 cycles consisting of steps: denaturation at 94^{o}C for 3 min, annealing for 30 s at 59^{o} C for intron 59 and extension for 30s at 72^{o} C. The reaction was completed by a final extension for 7 min at 72^{o} C. The amplification of rs736707 SNPs results in the product of 564 bp. The first PCR products of are electrophoresed on 2 percent agarose gel containing 5 µg/ml ethidium bromide and visualized under UV-light and then digested with 4 U of BtscI restriction enzyme (bioron, Germany) for rs736707 SNP in a final volume of 10µl at 37 ºC for 16 h and finally fragments are separated on 2 percent agarose gels containing ethidium bromide and visualized under UV-light. On the 564 bp fragment, enzyme had a single recognition site and wild-type allele resulted in two fragments of 243 and 321 bp. Also, variant alleles were present as an undigested 564bp fragment.

2.4-Statistical analysis

Allele and genotype frequencies were analyzed using java stant online statistics package software (http://statpages.org/ctab2x2.html). When the number of samples was equal or less than 5, the Fisher's exact test was applied to compare the variables. P value <0.05 was considered as significant level (Table.1). Also, to assess Hardy– Weinberg equilibrium, an online HWE calculator, http://www.oege.org/software/hardy-weinberg.html, was applied.

Results

To investigate the association of rs736707 (C/T) with increased risk of autism spectrum disorder, we genotyped a total of 74 ASD and 86 healthy subjects in the same age range. In Autistic participants, 71.62% of cases were male and 24.32% were female. Among 86 controls, 75.0% were female and 25.0% were male.

Pearson's chi-square test was performed to compare observed. At the end, the patient group consisted of both male and female autistic subject's genotype and allele frequencies with those that are expected in a population with Hardy–Weinberg equilibrium. The frequencies of genotypes and alleles did not show a deviation from Hardy–Weinberg equilibrium in both patient and control groups (P=0.49). The frequencies of CC, CA and AA genotypes were 55.41, 35.13 and 7.11% in patients and 60.47, 32.56 and 6.9% in controls, respectively.

There was no significant association between rs736707 (C/T) polymorphism and increased risk of autism spectrum disorder (P>0.05).

According to the results of the statistical analysis, no significant difference in the frequency of the two variant alleles between

cases and controls was observed; although, the rs736707 -C allele had higher frequency compared with the T allele in both groups, in fact, the C is the major allele while the T is the alternative allele in the studied population. Consistently, there was not statistically considerable difference in the overall distribution of genotype frequencies between patients and healthy individuals (P>0.05); however, CC homozygous dominants were more frequent than CA heterozygous and AA homozygous recessives, indicating that CC homozygosity Reelin rs736707 (C/T) is over represented and, conversely, AA homozygosity is remarkably under-represented in the general Azeri population (Table 1).

Table 1: Genotype and allele frequencies

rs736707	Case (%)	Control (%)	OR (95% CI)	P-Value
Genotypes				
C/C	41(55.41)	52(60.47)	0.812(0.445-1.482)	0.469
C/T	26(35.13)	28(32.56)	1.122(0.599-2.103)	0.701
T/T	7(8.11)	6(6.98)	1.176(0.37-3.777)	0.762
Alleles				
C	108(72.97)	132(76.74)	0.818(0.411-1.629)	0.539
T	40(27.03)	40(23.26)	1.222(0.614-2.436)	0.539

Estimated relative risks with Odds ratios (OR) and 95 % Confidence intervals (95 % CI) and P values for association between rs25487 and breast cancer risk.

Discussion

During embryonic brain development, Reelin is secreted by the Cajal Retzius cells of the marginal zone and provides signal for proper migration of newly generated post mitotic neurons from the ventricular zone to regulate neuronal migration and brain lamination and promoting dendrite maturation, axonal growth and the establishment of synaptic contacts (11, 14-16) to form distinct brain area. In addition Reelin seems to act after embryonic development insynaptogenesis and synaptic plasticity (17). Reelin expression decreases in several neurodevelopmental disorders such as schizophrenia, bipolar disorder, lissencephaly syndrome, autism (18-22). Studies has detected Reelin expression reduces in several brain regions of subjects with autism (18, 23, 24). Post-mortem studies show that Reelin is reduced in cerebellum (40%), superior frontal (70%)

and parietal (70%) cortices. These brain regions have been related to the three core behaviors that are impaired in autism: social behavior, language and communication, and repetitive and stereotyped behaviors. In particular, the frontal and parietal cortices influence the planning and organization of behavior and are closely related to the recognition of language and memory for words (23).

Consequently, several genetic studies demonstrated that there was an association between Reelin gene and increased risk of autism (25-30). Altogether these genetic and molecular studies suggest that the Reelin deficiency may be a vulnerability factor in

the pathology of autism. Since several reports have documented the presence of vocal and neuromotor abnormalities in patients with autism and suggested that these dysfunctions predate the onset of the syndrome (31), therefore recently we performed a case–control study to assess the possibility of an association between a single nucleotide polymorphism of Reelin gene, rs736707, and autism risk among Iranian-Azeri patients. Our results did not detected any significant association between rs736707 SNP with autism in the studied population (P>0.05) (table 1).

Similarly, Dutta et al. studied SNPs rs736707 in an Indian population and concluded that there is no association of the RELN polymorphism with ASDs (32).

On the other hand, Serajee et al. (2006) found that rs736707 was in significant transmission disequilibrium among Caucasian autistic subjects (33). Also, Sharma et al. found that SNPs rs736707 of Reelin gene has a significantly association with autism disorder in the South African Population (34). Further, in a case–control study, Li and et al. studied eight SNPs of the RELN gene and detected a positive association of intron 59 (rs736707) with autism in the Chinese Han Population (35).

Heterogeneity between study groups in clinical characteristics and gene–environment interactions may also be responsible for the inconsistency of results. It has also been hypothesized that autism is a disorder of polygenic inheritance so the effect of a single SNP could be subtle. Therefore, more studies, including more SNPs of Reelin, should be investigated (36).

Although, in the present study, significant difference in the allele frequency between cases and controls was not detected but the C allele was found to be more prevalent in both Azeri patients and controls compared with the T allele. The present findings are in agreement with result that was previously described by Sharma in the South African Population (34).

Nevertheless, these findings are in contrast with data reported by Dutta et al. in Indian population (32), Serajee et al. among Caucasian families of Autism (33), and Bonora et al. (37) among autistic patients selected from International Molecular Genetic Study of Autism Consortium (IMGSAC) multiplex families of the European autistic population where the C allele was as the minor allele in while the T allele was identified as the major allele.

Conclusion

In conclusion, it should be pointed out that the SNPs rs736707 is not associated with autism spectrum disorder in Iranian-Azeri patients based on our results. The reelin polymorphism, rs736707 could not be utilized as a functional molecular biomarker to predict genetic susceptibility for autism spectrum disorder in Iranian-Azeri patients.

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Conflict of Interest: None.

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