Research paper

Association study of the vesicular monoamine transporter 1 (*VMAT1*) gene with autism in an Iranian population

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A R T I C L E I N F O

A B S T R A C T

Autism Spectrum Disorders (ASD) (MIM 209850) are a group of neurodevelopmental disorders distinguished by destructed social interaction and communication abilities along with peculiar repetitive behavior. Several genetic loci have been linked to this disorder. *Vesicular monoamine transporter 1* (*VMAT1/SLC18A1*) is an attractive candidate gene for psychiatric disorders because of its participation in regulation monoamines. In the present case-control study, we evaluated the link between three non-synonymous single nucleotide polymorphisms (SNPs) (rs2270641 [Pro4Thr], rs2270637 [Thr98Ser] and rs1390938 [Thr136Ile]) and one intronic SNP (rs2279709) across the *VMAT1* gene and ASD in a group of Iranian patients. Allele frequency analyses showed significant over-presentation of rs1390938-G allele in cases compared with controls ($P < 0.001$). The analysis under different genetic models showed that the AA genotype of the rs1390938 was protective against ASD under dominant and recessive models. The rs2270641 SNP was associated with ASD risk only in over-dominant model. Other SNPs showed no significant difference in allele or genotype frequencies between two groups. Haplotype analysis revealed that C A T T and C A T G haplotypes (rs2270637, rs1390938, rs2279709 and rs2270641 respectively) have a protective effect against ASD. Consequently, the functional rs1390938 SNP in *VMAT1* is associated with ASD in Iranian population. Considering the role of VMAT1 in regulation of monoamines, the dysregulated expression of this protein during early stages of brain development might be implicated in ASD.

1. Introduction

Autism Spectrum Disorders (ASD) (MIM 209850) comprise a collection of various neurodevelopmental disorders distinguished by destructed social interaction and communication abilities along with peculiar repetitive behavior (Safari et al., 2017). Heritable factors are supposed to confer significant risk for this kind of juvenile neurologic disorder (reza Safari et al., 2016; Hamedani et al., 2017) in a way that various genetic loci participate in ASD might have distinct roles in different families (Veenstra-VanderWeele et al., 2004). In spite of the robust genetic indications, the known genetic predisposing factors are limited. However, it is evident that the genes participated in the pathogenesis of autism are linked with the global processes of neuronal signal transmission, such as those involved in production or reuptake of neurotransmitters (Adamsen et al., 2014). Among genes involved in monoaminergic endocrine systems, *Vesicular monoamine transporter 1*

(*VMAT1/SLC18A1*) is an attractive candidate gene for psychiatric disorders as independent studies have suggested its contribution in conferring risk of schizophrenia as well as bipolar disorder (Lohoff et al., 2006; Richards et al., 2006; Lohoff et al., 2008). VMAT1 is an integral membrane protein located in secretory vesicles of neuronal and endocrine cells and participates in the transmission of monoamines, such as norepinephrine, epinephrine, dopamine, and serotonin, between the cytosol and synaptic vesicles by means of generating electrochemical gradient across the vesicular membrane (Eiden et al., 2004). Its expression has been verified in the human and rat brain at mRNA and protein levels with highest levels of expression in substantia nigra, followed by amygdala, hippocampus, thalamus, fetal frontal lobe and frontal lobe (Hansson et al., 1998; Lohoff et al., 2006). Considering the role of monoamines dysregulation in the pathogenesis of ASD (Quaak et al., 2013), we hypothesized that polymorphisms in the *VMAT1* gene may influence transporter function and/or expression and

might participate in the etiology of ASD. Consequently, we designed an association study to discover the link between three non-synonymous single nucleotide polymorphisms (SNPs) (rs2270641 [Pro4Thr], rs2270637 [Thr98Ser] and rs1390938 [Thr136Ile]) and one intronic SNP (rs2279709) across the *VMAT1* gene and ASD in a group of Iranian patients.

2. Experimental Procedures

2.1. Subjects

The current study is a case- control study which enrolled 495 Iranian ASD patients and 484 age, gender, and ethnic-matched healthy controls. The appropriate sample size for the current study was calculated using the following notions: 95% confidence ($\alpha = 0.05$), 90% power ($\beta = 10\%$) in 1:1 ratio and least extreme Odds Ratio to be distinguished 2.0. Theoretical fraction of controls with exposure to risk allele described as 0.2 as stated by dbSNP database for the rs2270637 (Sullivan et al., 2009). Patients were diagnosed by at least two clinical psychologists according to the criteria stated in the Diagnostic and Statistical Manual of Mental Disorders 5th edition (Dorahy, 2014), and the Autism Diagnostic Inventory-Revised (ADI-R) (Rutter et al., 2003). Patients with any comorbid genetic syndrome or metabolic disorder were excluded from the current study. Controls were chosen from volunteers and complete medical history evaluation as well as physical examinations ruled out the presence of any neurological disorder in them. Written informed consent forms were obtained from all human subjects. The study was approved by the ethic committee of Shahid Beheshti University of Medical Science.

2.2. Sample Collection and DNA Extraction

Saliva samples have been gathered from all human subjects based on the method described formerly (Noroozi et al., 2016). Then, GeneAll Exgene cell SVmini DNA kit (Cat. No. 106-152) was used for extraction of genomic DNA from buccal epithelial cells in the mouthwash samples. The purity and concentration of extracted DNA were evaluated using WPA Biowave II UV/Visible Spectrophotometer (Serial No. 80-3003-75) by measuring the ratio of the absorbance at 260 and 280 nm (A260/280) and the A260 respectively.

2.3. Genotyping

Genotyping of three of the non-synonymous SNPs (rs2270641 [Pro4Thr], rs2270637 [Thr98Ser] and rs1390938 [Thr136Ile]) and one intronic SNP (rs2279709) across the *VMAT1* gene, was performed using tetra-primer amplification refractory mutation system PCR (T-ARMS-PCR). PCR was carried out in a FlexCycler (Analytik Jena, Germany) system using Taq $2 \times$ red master mix (Ampliqon, Denmark). The PCR program included a primary denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 45 s, specific annealing temperatures for 35 s, and 72 °C for 45 s, with the final extension of 72 °C for 5 min. The primer 1 software (http://cedar.genetics.soton.ac.uk/public_html/primer1.html) was used for primer design. Specific annealing temperatures and primer sequences are listed in Table 1. Ten percent of the samples were sequenced by using ABI 3730xl DNA analyzer (Macrogen, Korea) to confirm the results of 4P–ARMS-PCR method.

2.4. Statistical analysis

Statistical analyses were carried out with the SPSS Statistics software version 20 (SPSS Inc., Chicago, IL). Goodness of fit to the Hardy-Weinberg equilibrium was analyzed with the $\chi 2$ test. Genotype and allele frequencies were compared between patients and healthy subjects by using the $\chi 2$ test for independence. Odds ratio (OR) and 95% confidence intervals (CI) were computed to evaluate the relative risk by using SNPStats online programme (http://bioinfo.iconcologia.net/SNPstats). Haplotype frequencies were calculated using the SNPAnalyzer program (Istech Ltd., Goyang-si, Korea) according to the expectation-maximization algorithm. Rare haplotypes were excluded from analysis as this algorithm does not precisely assess haplotype frequencies < 1% (Fallin and Schork, 2000). Differences were regarded as significant when $P < 0.05$.

3. Results

A total of 495 sporadic ASD cases (male-to-female ratio of 5:1) with a mean age of 10.0 ± 3.5 years and 484 controls with a mean age of 10.0 ± 0.5 years and the adjusted sex ratio as patients participated in the study. The age-at-onset for the disease was 3.0 ± 1.8 years in patient group. All assessed SNPs have been shown to be in Hardy–Weinberg equilibrium in both patient and control groups ($P > 0.05$). Allele frequency analyses showed significant over-presentation of rs1390938-G allele in cases compared with controls ($P < 0.001$). The analysis under different genetic models showed that the AA genotype of the rs1390938 was protective against ASD under dominant and recessive models. The rs2270641 SNP was associated with ASD risk only in over-dominant model. Other SNPs showed no significant difference in allele or genotype frequencies between two groups (Table 2). Linkage disequilibrium (LD) for the mentioned SNPs in the *VMAT1* gene was calculated using Haploview version 4.2 software by describing D′ and r^2 values. No pair-wise linkage disequilibrium has been detected between mentioned SNPs. Haplotype analysis revealed that C A T T and C A T G haplotypes (rs2270637, rs1390938, rs2279709 and rs2270641 respectively) have a protective effect against ASD. The expected haplotype blocks originated from these SNPs and their frequencies in cases and controls are shown in Table 3.

4. Discussion

In the present study, we have shown an association between a functional variant (rs1390938) in *VMAT1* and ASD disorder. VMAT1 has been shown to participate in regulation of monoamines in the central nervous system (Lohoff et al., 2014). Alterations in the levels of amines in the brain during crucial time of development influence the normal functions of distinct areas of brain (Verney et al., 2002). Regulated levels of VMAT1 expression during this critical period might participate in the migration of neurons, development of neurosecretory pathways and the survival of neurons (Lohoff et al., 2006). Dysregulation of these processes has been implicated in neurodeveiopmental disorders such as ASD. Interestingly, expression of VMAT1 has been increased in nerve growth factor (NGF)-differentiated PC12 cells but not undifferentiated cells following valproate treatment with a simultaneous increase in (3)H-dopamine levels (Cordeiro et al., 2004). Consequently, the alterations in VMAT1 expression during critical period of neurodevelopment might be a putative underlying mechanism for the association found between maternal use of valproate during pregnancy and significantly increased risk of ASD in the children (Christensen et al., 2013). Other supporting evidences for such supposition come from studies revealing the link between abnormalities in the dopaminergic system and ASD (Quaak et al., 2013). For instance, ASD patients exhibit high levels of a dopamine metabolite namely homovanillic acid in urine (Kaluzna-Czaplinska et al., 2010). In addition, variations in genes encoding dopamine receptors and the enzyme dopamine beta-hydroxylase have been shown to be associated with ASD risk (Quaak et al., 2013). Finally, medicines recommended for ASD patients such as haloperidol and risperidone, exert antagonistic effects against dopamine (Quaak et al., 2013). However, there are several unsolved questions in this regard. Notably, it has been revealed that the A allele of rs1390938 SNP significantly enhances the presynaptic transport of monoamines in vitro and results in amygdala and medial