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Original Article

Relationship between *P2XR4* Gene Variants and the Risk of Schizophrenia in South-East of Iran: A Preliminary Case-Control Study and in Silico Analysis

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Abstract

Background: Schizophrenia (SZN) is a heterogeneous disorder. Recently, the role of purinergic receptor's signaling in mental disorders has implicated. There is no evidence regarding the association of *P2XR4* single nucleotide polymorphisms (SNPs) and the risk of behavioral disorders. Therefore, this preliminary study, we determined the association of rs1169727A/G and rs25644A/G variants located in *P2XR4* gene with the risk of SZN.

Methods: This case-control study was performed on 150 SZN patient referring to Baharan Hospital, Zahedan (Eastern of Iran) in 2018. Genotyping was done by tetra-amplification refractory mutation system polymerase chain reaction (Tetra ARMS-PCR). Different databases were used to determine the effects of the SNPs on the secondary structure of *P2XR4* pre-mRNA and protein as well as binding of transcriptional regulators.

Results: The G allele of rs1169727 significantly increased the risk of SZN (OR=1.41, 95%CI=1.02-1.93, *P*=0.039), but there was no significant association was found between the other SNP and SZN. Moreover, GG model of rs1169727 (OR=2.46, 95%CI= 1.32-4.62, *P*=0.004) and rs25644 (OR=3.45, 95%CI= 1.12-5.10, *P*=0.013) increased the risk of SZN. The substitution of A and G alleles of rs1169727 significantly altered the secondary structure of pre-mRNA (*P*=0.1). In silico analysis revealed that rs25644A/G could act as an intronic cryptic donor site. Screening for flanking sequence of rs1169727A/G and rs25644A/G predicted a novel enhancer and silencer for both SNPs.

Conclusion: rs1169727A/G and rs25644A/G are linked to SZN susceptibility in a sample of the Iranian population. In-silico analysis indicated that rs25644 have substantial roles in determining the pre-mRNA and protein structure of *P2XR4* gene.

Keywords: Purinergic receptors; Schizophrenia; In-silico

Introduction

Schizophrenia (SZN), as a life-threatening mental disorder, affects almost 1% of the general population (1). This psychological disease is generally known by a variety of symptoms such as hallucinations, delusions and definite changes in emotional reactivity (2). SZN has high heritability with various genes being involved in the development and progression of this condition (3). The genetic vulnerability has been introduced as a substantive contributing factor in the etiology of SZN (4). SZN is mainly caused by various environmental factor-related genes in different ethnicities while several lines of evidence recommend that the transmission mode of SZN is more complicated, regarding genetic heterogeneity and incomplete penetrance to be responsible for its inheritance. As a result of these complexities, segregation- and linkage-analysis would not disclose the genetic etiology of SZN as equivocal results were produced (5, 6).

By constructing the single nucleotide polymorphisms (SNP) arrays, ASTN2, *GSK3B*, MYT1L, *DTNBP1*, *ERBB4*, *GRM3*, *AKT1*, and *COMT* have been reported as candidate genes for SZN since the underlying pathways are involved in brain development and neuronal functioning (2, 7). Purinergic receptors are disputably the most abundant receptors in all living organisms and primarily expressed in central nervous systems (CNS). Recently, a body of evidences have highlighted the importance of purinergic signaling in the pathophysiology of different psychiatric disorders (8) as family-based association studies revealed that SNPs located within a region on chromosome 12, were the most probable genomic region to contain susceptibility genes for these types of neurologic disorders in a sample of French Canadian population (9). *P2XR4* displayed the most common brain tissue distribution among other P2XRs as these receptors induce long-term synaptic potentiation (LTP) (10).

The purinergic receptor *P2XR4* (Gene ID=3087) gene (also known as *P2X4R* and *P2RX4)*, is located on 12q24.31 with 14 exons. According to the concept of the reads per kilobase per million (RPKM), this gene has a ubiquitous expression in the colon (RPKM 15.4) and placenta (RPKM 18.6) and other organs as well as brain tissue (11). Although previous investigations have established the correlation between *P2XR7* mutations and clinical depression susceptibility (12), there is a lack of evidence concerning the association of *P2XR4* variants and behavioral disorders. However, genetic deletion of purinergic receptors attenuated inflammation since psychological stress activates the inflammasome and stimulation of the purinergic type 2x7 receptors (13, 14) which may indicate the effects of dysregulation/mutations of these gene family on the onset of diseases other than CNS-related syndromes. This gene has several variants while two of them rs1169727A/G and rs25644A/G were not yet thoroughly investigated.

In this preliminary work, we aimed to determine the possible association between these two polymorphisms and risk of SZN in a sample of the Iranian population.

Materials and Methods

Subjects and DNA extraction

This case-control study was performed on 150 SZN patient referring to Baharan Hospital, Zahedan (Eastern of Iran) in 2018. Diagnosis was done based on the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (American Psychiatric Association). Moreover, 150 healthy subjects were chosen from the same treatment facility with no history of psychiatric or any other mental disorders. The case and control groups were adjusted in age and gender.

An informed consent form was taken from all enrolling subjects. The protocol of the present work was approved by the Ethics Committee of Zahedan University of Medical Sciences, Zahedan, Iran (IR.ZAUMS.REC.1397.323) as laid down in the 1964 Declaration of Helsinki. For this propose, 5 mL venous whole blood was

collected in each ethylene diamine tetra-acetic acid (EDTA)-containing tube. The salting-out method was used for DNA isolation (15).

Genotyping

For genotyping of two SNPs, extracted genomic DNAs were used. Rs1169727A/G is an intronic variant whereas rs25644A/G is a missense variant causing an S258G substitution in related amino acid sequence. Genotyping of all polymorphisms were done by tetra-amplification refractory mutation system polymerase chain reaction (Tetra ARMS-PCR). For this purpose primers designed with Primer1 server (available at http://primer1.soton.ac.uk). The sequences of four primers designed for rs1169727A/G genotyping were as follows: CCTAGGGTTGTCCAATCTTTTGGCTTTC (forward outer), GGCACCAGGCACTGATCTAAGCTCTTTA (reverse outer), CTGGGCCGCATGCGGCCTGTGTGATA forward inner specific for A allele, 272 bp), and GCTCTACTCTAAGCTTGTCTAACCAAC (reverse inner specific for G allele, 247 bp) producing a 467 bp outer control. Regarding rs25644A/G polymorphism, GTTTCTTACACAGGTGTACATGTGCCAT (forward outer), AAAATACAAAAAAAATTAGCCGAGCGTG (reverse outer), GGTTTCCAGCTTCATCTATGTCCATG (forward inner specific for G allele) and AAAAAGGATGGGTTCATGTTCTTGGT (reverse inner specific for A allele) primers produced 234 and 285bp specific bands related to G and A alleles, respectively. The specificity and quality of primers were checked with GeneRanner software v6.5.48.0. All PCR reactions were performed in 15 µL volume containing 7 µL PCR master mix (Taq 2x premix), 1 µL of each primer (10 pmol/ml, sinaclon co, Tehran, Iran), 3 µL DNase free water, and 3 µL genomic DNA (~80-100 ng/ml). The PCR was done using an Eppendorf thermocycler (Eppendorf AG, Hamburg, Germany) with the following conditions: predenaturation at 94 °C for 5 min, 35 cycles each consisted of denaturation (94 °C, 30 sec), annealing (30 sec) and extension (72 °C, 30 sec) followed by a final extension at 72 °C for 5 minutes. Later, 5 µL of PCR product was loaded in 2% agarose gel stained with safe dye and visualized using a UV transilluminator.

In silico analysis

In silico analysis was conducted to examine the potential functions of two SNPs, rs1169727A/G and rs25644A/G located in the *P2XR4* gene. The nucleotide sequence of the *P2XR4* gene with accession no. NM_198987.2 were deduced from the National Center for Biotechnology Information (NCBI) data bank. RNASNP database was used for prediction of rs1169727A/G and rs25644A/G effects on pre-mRNA secondary structures based on the RNA folding algorithms (16). The outputs of the server are a graphical overview to see the local region which detected with maximum structural change was colored according to the *P*-values. The regions with the $P>0.2$ were colored in black, implicating that not significant structural alteration occurred. The potential functional effect of rs25644A/G polymorphism on protein function was evaluated by the SNAP server. SNAP database predicts the effect of non-synonymous polymorphisms on function using in silico derived protein information (17). Chou–Fasman database was used to predict the effect of rs25644A/G on the secondary structures of the protein. This database, predicts the location of alpha-helices and beta-strands from the relative frequencies of each amino acid in the submitted sequence (18).

To explore the effect of the SNPs on the binding sites of transcriptional regulators in *P2XR4*-mRNA, SpliceAid 2 database (a database of human splicing factors expression data and RNA target motifs) was used (19). Each sequence was represented by an accurate graph, that a positive score was assigned to the target sequences that facilitate exon definition (ESE and ISS motifs), and a negative score to the target sequences that facilitate intron definition (ESS and ISE motifs). Moreover, the conservation of the DNA sequences containing rs1169727A/G and rs25644A/G were illustrated by the WebLogo (20). The intronic sequence of *P2XR4*-mRNA

was analyzed by Human Splicing Finder to predict the effects of these variations on splicing patterns by identifying putative donor and acceptor splice sites, branch sites and cis-acting elements (21).

Statistical analysis

Using the package SPSS software version 16 (Chicago, IL, USA), Mann-Whitney and x^2 tests were performed for analysis of non-normal and qualitative data, respectively. The logistic regression was used to determine the relationship between SNPs and SZN using 95% confidence intervals (CI) and odds ratios (ORs). The haplotype analysis and linkage disequilibrium (LD) were conducted using SNPAnalyzer 2.0. In all assays, *P*-values more than 0.05 were regarded statistically insignificant.

Results

No significant differences were found between SZN and control groups regarding age (*P*=0.837) and gender (*P*=0.719). As shown in Table 1, differences in isolation and depression were significant in SZN subjects compared with the control groups.

Table 1: Clinic demographic characteristics of patients with SZN and healthy controls.

Parameters evaluated	*SZN (n=150) (n±SD)*	*Control (n=150) (n±SD)*	*P-value*
Age (yr)	36.48±10.52	36.40±10.88	0.84
Sex (male/female)	94/56	97/53	0.72
Isolation			0.00
Yes	86 (57.3%)	35 (23.3%)	
No	64 (42.7%)	115 (76.7%)	
Depression			0.00
Yes	111 (74.0%)	31 (20.8%)	
No	39 (26.0%)	118 (79.2%)	

P<0.05 was regarded statistically significant.

Table 2 represents the frequency of genotypes and alleles for rs1169727A/G and rs25644A/G and their role as a risk factor for SZN. Regarding rs1169727, dominant AG (OR=0.41, 95%CI=0.22-0.76, *P*=0.004), and recessive GG (OR=2.46, 95%CI=1.32-4.62, *P*=0.004) models were significantly associated with SZN while the frequency of G allele was significantly higher in SZN subjects compared with the healthy group (OR=1.406, 95%CI=1.02-1.93, *P*=0.039). Moreover, codominant AA (OR=0.55, 95%CI=0.39-0.91, *P*=0.023), and recessive GG (OR=3.45, 95%CI=1.22-9.70, *P*=0.013) models of rs25644 polymorphism are also correlated with SZN susceptibility. Compared with other genotypes, interaction analysis indicated that carriers of GGAA (OR=2.41, 95%CI=1.12-5.10, *P*=0.02) and AGAG (OR=0.39, 95%CI=0.20-0.78, *P*=0.007) genotypes demonstrated a significantly increased risk of SZN (Table 3). Haplotype analysis revealed that GG haplotype of rs1169724 A/G and rs25644 A/G are significantly associated with the increased risk of SZN (OR=2.42 95%CI=1.10-5.41, *P*=0.02) (Table 4). The rate of linkage disequilibrium between two mentioned genetic variants was 0.095 (data have not shown). The association between clinicodemographic features of SZN and genotype frequency for the recessive model in both groups was depicted in Table 5. No significant association was noticed between these characteristics and different genotypes and inheritance models of both SNPs.

Table 2: Genotypes and allele frequencies of *P2XR4* polymorphisms rs1169727 A/G and rs25644 A/G in SZN and control subjects

SNP	*Type*	*SZN (%)*	*Control (%)*	*Model*	*OR (95%CI)*	*P-value*
rs1169727 A/G	AA	44 (29.3)	50 (33.3)	Codominant	0.71 (0.45-1.10)	0.12
	AG	70 (46.7)	83 (55.3)	Dominant	0.41 (0.22-0.76)	0.004
	GG	36 (24.0)	17 (11.3)	Recessive	2.46 (1.32-4.62)	0.004
	A	158 (52.7)	183 (61.0)			
	G	142 (47.3)	117 (39.0)		1.41 (1.02-1.93)	0.039
rs25644 A/G	AA	98 (65.3)	91 (60.7)	Codominant	0.55 (0.39-0.91)	0.023
	AG	36 (24.0)	54 (36.0)	Dominant	0.80 (0.50-1.29)	0.39
	GG	16 (10.7)	5 (3.3)	Recessive	3.45 (1.22-9.70)	0.013
	A	232 (77.3)	236 (78.7)			
	G	68 (22.7)	64 (21.3)		1.07 (0.72-1.58)	0.68

SNP: single nucleotide polymorphism, SZN: schizophrenia, *P2XR4*: purinergic receptor x4, OR: odd ratio, CI: confident interval, $P<0.05$ was regarded statistically significant.

Table 3: Interaction of *P2XR4* rs1169727 A/G, and rs25644 A/G polymorphisms on SZN risk

Rs1169727 A/G	*Rs25644 A/G*	*SZN (%)*	*Control (%)*	*OR (95%CI)*	*P-value*
AA	AA	23(15.3)	32 (21.3)	0.67 (0.36 – 1.10)	0.18
AA	AG	13 (8.7)	17 (11.3)	0.73 (0.38 – 1.59)	0.43
AA	GG	6 (4.0)	1 (0.7)	6.21 (0.74 – 52.1)	0.06
AG	AA	51 (34.0)	48 (32.0)	1.10 (0.68 – 1.76)	0.70
AG	AG	15 (10.0)	32 (21.3)	0.39 (0.20 – 0.78)	0.007
AG	GG	6 (4.0)	3 (2.0)	2.03 (0.50 – 8.30)	0.30
GG	AA	24 (16.0)	11 (7.3)	2.41 (1.12 – 5.10)	0.02
GG	AG	8 (5.3)	5 (3.3)	0.002 (0.001 – 0.006)	0.00
GG	GG	4 (2.7)	1 (0.7)	4.07 (0.44 – 36.96)	0.18

SZN: schizophrenia, OR: odd ratio, CI: confident interval, $P<0.05$ was regarded statistically significant

Table 4: Haplotype analysis of *P2XR4* gene polymorphisms between SZN patients and healthy subjects.

rs1169727 A/G	*rs25644 A/G*	*SZN*	*Control*	*OR (95%CI)*	*P-value*
A	A	0.38	0.48	0.78 (0.55-1.10)	0.12
G	A	0.37	0.30	1.19 (0.85-1.66)	0.28
A	G	0.14	0.12	0.85 (0.54-1.30)	0.45
G	G	0.08	0.08	2.42 (1.10-5.41)	0.02

SZN: schizophrenia, OR: odd ratio, CI: confident interval, $P<0.05$ was regarded statistically significant.

Table 5: Association between *P2XR4* gene polymorphisms and clinical demographic characteristics of SZN and healthy groups

	Genotype	*Isolation (yes/no)*	*Depression (yes/no)*
rs1169727			Yes/no
control	GG	6/11	1/16
	AA+AG	29/104	30/102
	P-value	0.22	0.11
SZN	GG	23/13	30/6
	AA+AG	63/51	81/33
	P-value	0.35	0.13
rs24644			
control	GG	0/5	0/5
	AA+AG	35/110	32/113
	P-value	0.19	0.24
SZN	GG	6/10	12/4
	AA+AG	80/54	99/35
	P-value	0.08	0.91

SNP: single nucleotide polymorphism, SZN: schizophrenia, *P2XR4*: purinergic receptor x4, $P<0.05$ was regarded statistically significant.

In silico analysis showed that rs25644 polymorphism is an A → G substitution of *P2XR4* gene, which leads to a serine-glutamate substitution at codon 242. Predicting *P2XR4* rs1169727A/G and rs25644A/G effects on local *P2XR4* RNA secondary structure disclosed that rs1169727A/G made fundamental changes in the secondary structure of pre-mRNA ($P=0.1$ and $P=0.79$; the $P<0.2$ is significant structural change), but rs25644A/G was not shown significant structural alteration on the secondary structure of *P2XR4* pre-mRNA (*P*: 0.4) (Fig.1). The Chou–Fasman hydrophobic score of *P2XR4* at position 242 was −0.22 for serine compared with −0.4 for glutamate residue (Fig.2). Analysis of the *P2XR4* secondary structure represented that the parameter at residue 242 did not differ between the 242S and 242G phenotypes (Fig.2-B, B'). Moreover,

SNAP servers revealed a significant effect of S242G substitution on the protein structure (Score: 41; Expected accuracy: 71%) (Fig.3). The prediction of the consequence of the rs25644A/G polymorphism via HSF 3 database identified that this polymorphism potentially could activate an intronic cryptic donor site (Fig.4). Screening of the flanking sequences of rs25644A/G and rs1169727A/G for enhancer and silencer motifs by SpliceAid2 tools predicted a novel site of the ESEs such as SR (Serine/Arginine-rich) proteins for rs25644A/G and silencer motifs such as hnRNP for rs1169727A/G (Fig.5). Moreover, the conservation of rs25644A/G and rs1169727A/G SNPs was demonstrated by WebLogo tool and in silico analysis showed well-conserved region across multiple mammalian species (Fig.6).

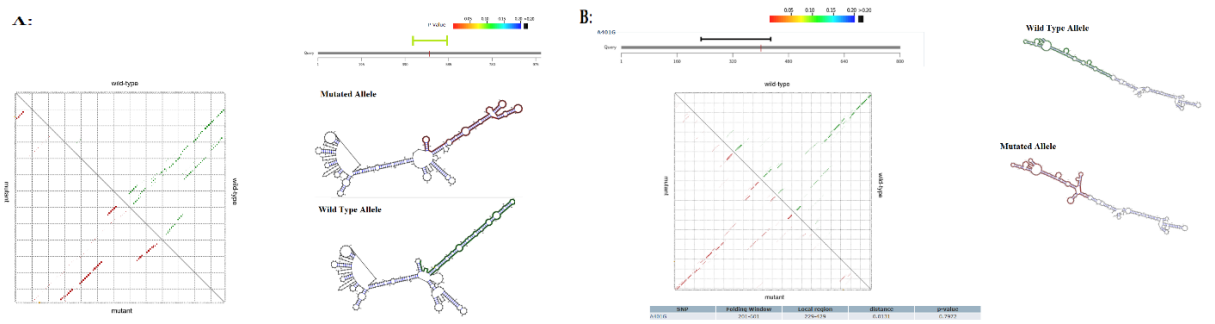


Fig.1: The SNPs effects on local RNA secondary structure were analyzed by RNAsnp. Local region with maximum differences in wild-type and mutant was colored in green and red dye. A: rs25644A/G and B: rs1169727A/G. The p-value< 0.2 is significant structural change

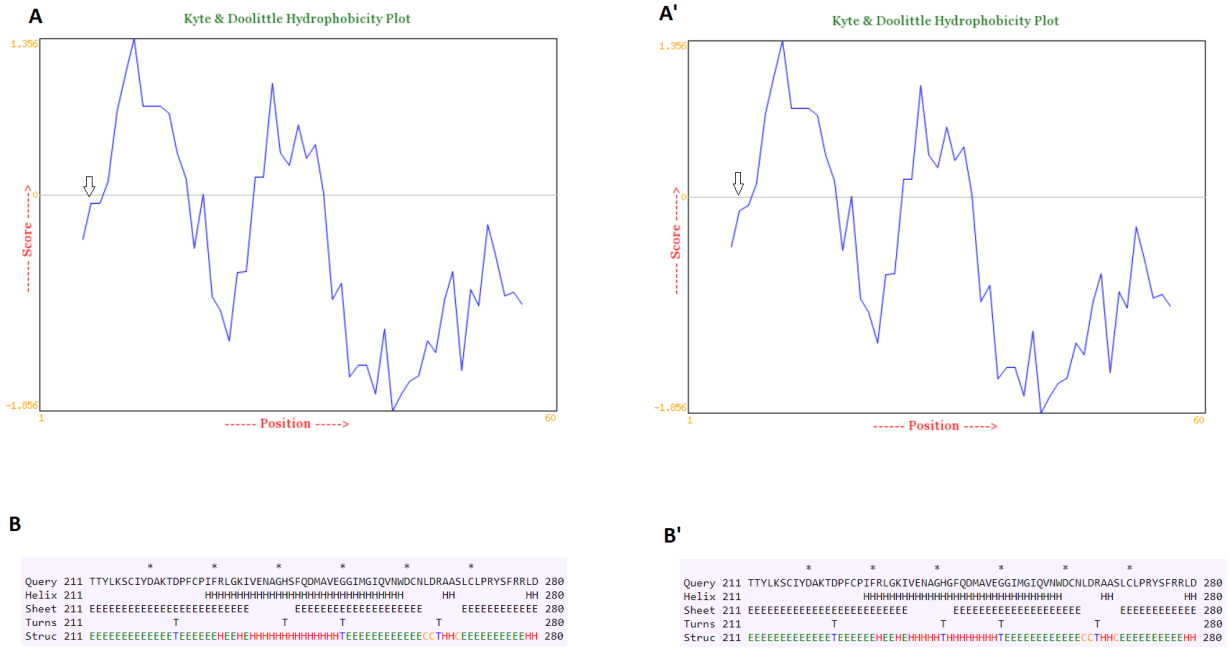
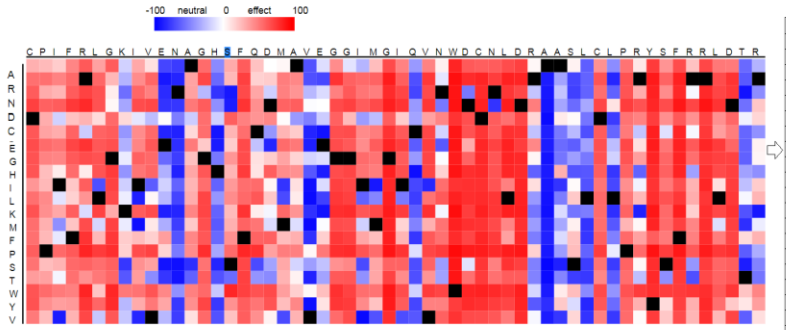


Fig.2: Hydrophobicity plot and secondary structure predictions. (A&A') Hydrophobicity plot for 242S and 242G phenotypes, respectively; (B&B') Chou–Fasman's secondary structure for 242S and 242G phenotypes, respectively; The residue 242 shown by arrowhead



Wildtype Amino Acid	Position	Variant Amino Acid	Predicted Effect	Score	Expected Accuracy
S	242	A	effect	33	66%
S	242	R	effect	28	63%
S	242	N	neutral	-91	97%
S	242	D	neutral	-89	93%
S	242	C	effect	63	80%
S	242	Q	effect	29	63%
S	242	E	effect	20	63%
S	242	G	effect	41	71%
S	242	H	effect	29	63%
S	242	I	effect	40	71%
S	242	L	effect	45	71%
S	242	K	effect	27	63%
S	242	M	effect	31	66%
S	242	F	effect	43	71%
S	242	P	effect	58	75%
S	242	S	neutral	-89	93%
S	242	T	neutral	-58	78%
S	242	W	effect	85	91%
S	242	Y	effect	43	71%
S	242	V	effect	24	63%

Fig.3: The effect of Ser242Glu substitution on protein functions evaluated by SNAP. The result of in silico analysis are shown in table form

HSF Matrices

Sequence Position	cDNA Position	Splice site type	Motif	New splice site	Wild Type	Mutant	If cryptic site use, exon length variation	Variation (%)
392	+392	Acceptor	cgcaggacacagtt	cgcaggacacggTT	76.84	47.89	NA	Site broken -37.68
399	+399	Acceptor	cacagtttccagga	cacggtttccagGA	86.52	86.64	NA	+0.14
400	+400	Donor	acagtttcc	ACGgtttcc	55.7	66.28	NA	New site +18.99

Branch Points calculation is performed using a new algorithm.

Sequence Position	cDNA Position	Branch Point motif	CV for reference sequence (0-100)	CV for mutant sequence (0-100)	Variation
397	+397	gacacAg	73.69	44.06	Site broken

ESE Finder matrices for SRp40, SC35, SF2/ASF and SRp55 proteins

Threshold values:
SF2/ASF: 72.98 SF2/ASF (IgM-BRCA1): 70.51 SRp40: 78.08 SC35: 75.05 SRp55: 73.86
Variation expresses the difference between reference and mutant values. Wild Type value is taken as reference.

Sequence Position	cDNA Position	Linked SR protein	Reference Motif (value 0-100)	Linked SR protein	Mutant Motif (value 0-100)	Variation
396	+396	SC35	ggacacag (84.63)	SC35	ggacacgg (76.64)	-9.44 %
396	+396	SC35	ggacacag (84.63)	SRp40	ggacacg (78.26)	-7.53 %
397	+397			SF2/ASF (IgM-BRCA1)	gacacgg (72.15)	New site
397	+397			SF2/ASF	gacacgg (86.55)	New site
398	+398	SRp40	acacagt (79.46)			Site broken -100
399	+399	SF2/ASF (IgM-BRCA1)	cacagtt (82.46)			Site broken -100
399	+399	SF2/ASF	cacagtt (82.06)			Site broken -100
402	+402			SC35	ggtttcca (83.71)	New site

Fas-ESS hexamers

Sequence Position	cDNA Position	Reference sequence	Set	Mutant sequence	Set	Variation
+402	+402	agttlc	2			Site broken

IEs from Zhang et al.

Sequence Position	cDNA Position	Silencer motif reference sequence	Silencer motif mutant sequence	Variation
401	+401	cagttt		Site broken
402	+402		ggtttc	New Site

Interpreted Data

This table shows only relevant results related to the mutation position and context.
The mutation occurs in the deep intronic positions, the following table show results of splicing and auxiliary sites that could be created by the mutation

Predicted signal	Prediction algorithm	cDNA Position	Interpretation
New Donor Site	1 - HSF Matrices	g a c a c g g t t t c c a g g 398 400 402 404 406 408 410	Activation of an intronic cryptic donor site. Potential alteration of splicing.

Fig.4: The prediction of the rs25644 A/G polymorphisms in *P2XR4* gene via HSF 3 tool

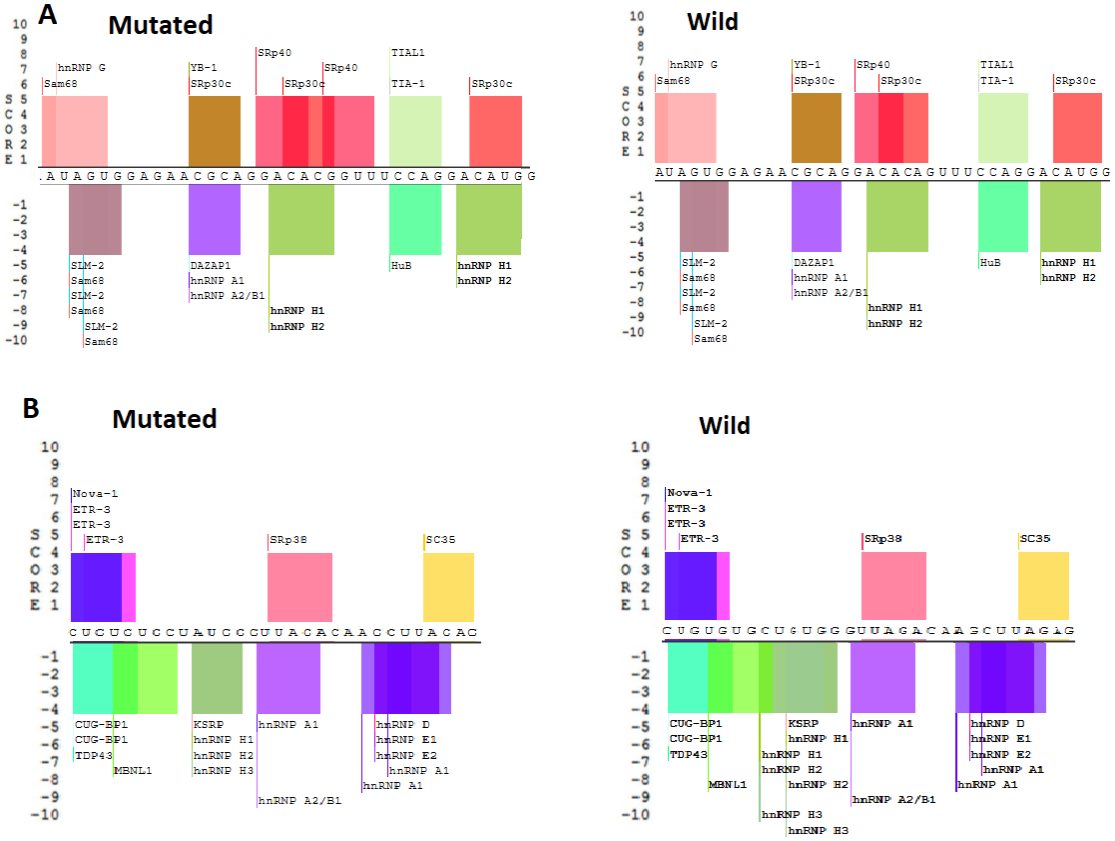


Fig.5: Screening of the flanking sequences in the rs25644 A/G and rs1169727 A/G polymorphisms was revealed enhancer and silencer motifs via SpliceAid 2 tool. (A) rs25644 A/G, (B) rs1169727 A/G polymorphism. The enhancers and silencers motifs are shown by arrowhead

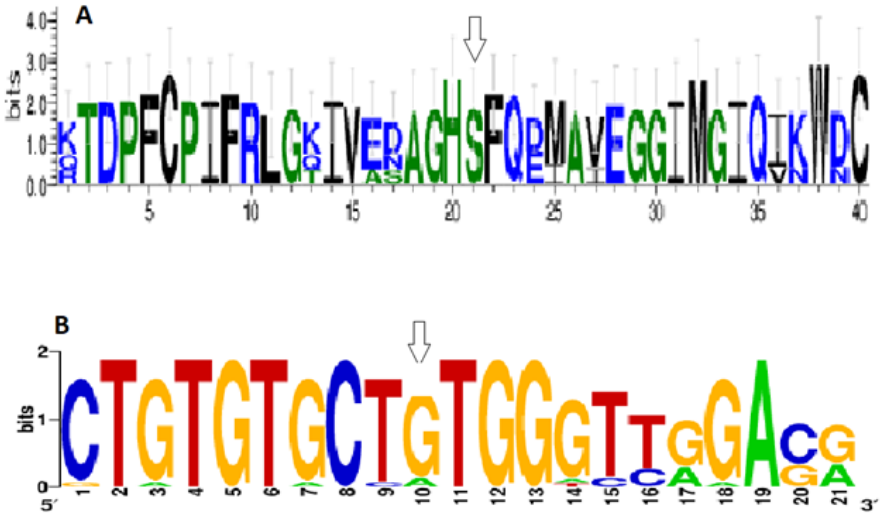


Fig.6: The conservation of the DNA sequences across multiple mammalian species for (A) rs25644A/G, and (B) rs1169727A/G polymorphisms using WebLogo server

Discussion

In this study, we showed that G allele of rs1169727 A/G enhanced SZN susceptibility. Similarly, this allele in the recessive model significantly increased the risk of SZN compared with the healthy group. Although we did not notice any significant correlation between rs25644 A/G alleles and SZN risk, GG genotype of rs25644 A/G enhanced SZN susceptibility. Haplotype analysis showed that the number of subjects who simultaneously carried the two G alleles of both SNPs was increased in SZN patients. GGAA genotype of both variants located in *P2XR4* gene is significantly correlated with the risk of this disease.

SZN has an ill-defined etiology from environmental to genetic factors (22). SZN has a non-mendelian inheritance, and DNA variations have found to play pivotal roles in the development of this psychiatric disorder (23). Both genetic- and copy number variations (CNV) act as risks factor for SZN (23). In addition to common alleles, recent genome-wide association studies (GWAS) have shown some loci that contribute to the risk of SZN (24). Purinergic signaling is considered to be a substantial pathway modulating microglial response in chronic brain diseases as long term changes in synaptic strength can be caused by activation of specific purinergic receptors (25). Purinergic receptors have been located in the pre- and the post-synaptic neuronal membrane that controls the ATP concentration (26). They have seven subunits that can form homo and hetero channels (27). The role of the *P2XR4* gene mutations has been studied comprehensively in some disorders. In the following, we have pointed to some of them. In a study conducted on German Caucasian patients suffering from a recurrent major depressive disorder (MDD), there was not any association between rs25644 with MDD (28), which was in agreement with our findings.

Regarding the significance of ATP concentration in glucose homeostasis, Todd and et al. performed a meta-analysis study and determined the possible association of the mutations in purinergic signaling genes such as *P2XR4* and *P2XR7* with glucose regulation. Data mined from both Meta-Analysis of Glucose- and Insulin-Related Traits Consortium (MAGIC), and the Diabetes Genetics Replication and Meta-analysis (DIAGRAM) consortiums demonstrated that rs25644 (ser258gly) associates with both of glucose homeostasis and type 2 diabetes susceptibility in various models (29). Wesselius et al. evaluated the impact of *P2XR4* and *P2XR7* gene polymorphisms on osteoporosis risk in a sample of Dutch population (30). ATP contributes to the response of bone cells to mechanical loading by its binding to purinergic receptors (31). In another investigation, no difference between the frequency of different genotypes of rs25644 polymorphism in the osteoporosis patients and the control subjects was reported, whereas this SNP in the recessive model dramatically increased the bone mineral density (BMD) value at the lumbar spine in subjects (30). To best of our knowledge, there is no evidence for the association of rs1169727 with any particular disorders.

Our study has several limitations: first, we examined only two SNPs within *P2XR4* gene, whereas there are many SNPs in this genomic region. Second, the total number of 300 SZN and healthy subjects were enrolled in this study. Designing the same study with larger sample size on other ethnicities can be more informative.

Conclusion

We analyzed the association of *P2XR4* gene variation with the risk of SZN in a sample of the Iranian population. We found that G allele only increased the risk of SZN concerning rs1169727A/G polymorphism. The dominant and recessive models of rs1169727 were associated with SZN susceptibility while no significant correlation was discovered concerning rs25644 genotypes and SZN risk in dominant inheritance

model. Moreover, In-silico analysis indicated that rs25644 plays a substantial role in determining *P2XR4* RNA and protein structure.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors disclose no conflict of interest.

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