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Title: Genetic Variations of DAOA (rs947267 and rs3918342) and COMT Genes (rs165599 and rs4680) in Schizophrenia and Bipolar I Disorder

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ABSTRACT

Introduction: Genetic and environmental factors are involved in the incidence of schizophrenia and bipolar disorder. Many reports confirm that several common genes are connected with these two psychotic disorders.
Several neurotransmitters may be involved in the molecular mechanisms of schizophrenia and bipolar disorder. We aimed to estimate the role of two talent genes; DAOA in neurotransmission of glutamate and COMT in neurotransmission of dopamine to guide the treatment of schizophrenia and bipolar disorder.
Methods: Blood samples (N= 100 for schizophrenia, N=100 for bipolar I disorder and N=127 for case control) were collected from individuals unrelated in the south-west of Iran. The SNPs (rs947267 and rs3918342 for DAOA gene/ rs165599 and rs4680 for COMT gene) were genotyped using the PCR-RFLP method. Our finding was studied by Logistic Regression and Mantel-Haenszel chi square assays.

Result: We observed there is an association in rs3918342, rs165599 and rs4680 single nucleotide polymorphisms and schizophrenia and bipolar I disorder. Also, our data demonstrated that the rs947267 was connected to bipolar I disorder but there wasn't any association between this SNP and schizophrenia.

Conclusion: In conclusion, this result supports the hypothesis that variations in DAOA and COMT genes may play a role in schizophrenia and bipolar disorder.

Key Words: DAOA, COMT, Schizophrenia, Bipolar I disorder, Genetics

Running title: Genetic variation of DAOA and COMT genes

1. Introduction

Schizophrenia is a serious mental illness. Nearly 1% of the world's population suffer from this disease (Weihua et al., 2007). Schizophrenia is characterized by delusions, hallucinations, thought disorders and cognitive deficits (Elliott, Michael, & Michael, 2015). Bipolar disorder (BPD) presents with diverse clinical manifestations and is characterized by individuals experiencing episodes of mania or hypomania. Two BPDs (BID, BIID) are often included in studies. BID is mainly characterized by depressive and manic symptoms. Also, patients can experience psychotic characteristics such as delusions and hallucinations. These patients are usually required to be hospitalized. (Jessica, Maria, Terence, & Gustavo, 2015). Data analysis of family, twin and adoption reports uniquely illustrate the connectivity of genetic agents in transition of talentedly to schizophrenia and bipolar disorder (Boks et al., 2007). The heritability of schizophrenia has been reported 80% (Aleman, Kahn, & Selten, 2003). Also, the heritability of bipolar disorder has been estimated 80-90% (Leahy, 2007). Several neurotransmitters, such as glutamate (Nasirinezhad, Mostofi, & Shahbazi, 2016), dopamine, GABA (Rahmanzade et al., 2016) (Dehghani, & Shahbazi, 2016) and serotonin may be involved in the molecular mechanisms of schizophrenia and bipolar disorder (Austin, 2005). Dopamine is an inhibitory neurotransmitter and glutamate is an excitatory neurotransmitter that is involved in a variety of neural processes (Goff & Coyle, 2001). The dopamine and glutamate hypotheses are eminent theories of the pathoaetiology of schizophrenia (Howes, McCutcheon, & Stone, 2015). Based upon neurobiological linkage and association studies, the susceptibility genes for schizophrenia and bipolar disorder can be grouped into two main classes. The first class of genes (DAOA, NRG1, DISC1, Dysbindin and GRM3) exert their effects on the NMDA glutamate receptor. The second class of genes which include COMT, DRD2 and PPP1R1B, are involved in dopamine metabolism and signaling (Harrison & Weinberger, 2005). Two candidate genes; DAOA gene (13q34) and COMT gene (22q11) have recently gained attention. Not only because they have shown connection with psychotic disorders, but also for their key role in glutamatergic and dopaminergic neurotransmissions (Christopher et al., 2006). Chumakov and colleagues recognized DAOA gene (D-amino acid oxidase activator). The DAOA protein is considered an activator of DAAO (D-amino-acid oxidase). DAAO gene (12q24) oxidizes D-serine, a potent activator of the N-methyl-D-aspartate (NMDA). NMDA receptor is a post synaptic glutamate receptor (GluRs) in the human brain (Maderia, Freitas, Vargas-Lopes, Wolosker, & Panizzutti, 2008). Glutamate is an excitatory neurotransmitter that is involved in a variety of neural activities including synaptic flexibility, neuronal development, and neuronal toxicity (Goff & Coyle, 2001). Normal glutamatergic neurotransmission involves enzymes, pre and post-synaptic neurons, glial cells, glutamate receptors and transporters. Disrupting any of the items, may be lead to disruption in the normal glutamatergic neurotransmission (Meador-Woodruff & Healy, 2000). The interaction of DAOA and DAAO genes has been implicated in the NMDA receptor regulation pathway in schizophrenia and bipolar disorder. "Glutamate hypothesis" derived from the NMDA antagonists, such as phencyclidine (PCP) and ketamine, can cause psychotic and cognitive abnormalities of schizophrenia (Christopher et al., 2006). Also "dopamine hypothesis" originated from the identification of D2 receptor blockage. The mechanism of action in D2 receptor blockage is similar to the mechanism of action in antipsychotics (Dashti, Aboutaleb, &

Shahbazi, 2013). Catechol-O-methyl transferase is a unique enzyme for decomposing a number of bioactive molecules such as dopamine. This enzyme is encoded by the *COMT* gene (Lotta, et al., 1995). COMT gene is located on 22q11, a region that was confused in many linkage analysis (Lewis, et al., 2003). Deletions in 22q11 can also lead to the velocardiofacial syndrome, via a heightened risk of psychopathy (Karayiorgou, et al., 1995). However not all studies have supported the DAOA/COMT genes association with schizophrenia or bipolar disorder (Liu, et al. 2006) (Shi, et al. 2008) (Tan, et al., 2014) (Jagannath, et al. 2017), but genome wide association studies of DAOA/ COMT gene with schizophrenia or bipolar disorders published to date (Shifman, et al., 2002) (Glatt, Faraone, & Tsuang, 2003) (Shifman, et al., 2004) (Sacchetti, et al., 2013) (Gott, et al., 2014) (Che-Sheng, et al., 2017) (Jagannath, et al., 2018). To facilitate the treatment of schizophrenia and bipolar disorder we estimate the genetic variation of DAOA gene in glutamate neurotransmission and COMT gene in dopamine neurotransmission and we assessment that's impact on susceptibility for schizophrenia and bipolar disorders.

2. Methods

2.1. Sampling

On the basis of General Health Questionnaire (GHQ) scaled 28 (Lobo, Perez-Echeverria, & Artal, 1986) and Diagnostic and Statistical Manual of Mental Disorders criteria (DSM-IV) blood samples (N=127 for case control, N= 100 for schizophrenia and N=100 for bipolar I disorder) were collected from individuals unrelated in the south-west of Iran. The patients were attended by at least two psychiatrists during their acceptation. All the patients were treated with mood stabilizers drug or anti-psychotic drugs during observation. The control group consisted of 127 non-relative individuals and were screened in two steps. Firstly, they were evaluated through questioning which showed that the individual or his/her first and second-degree relatives do not have a history of at least one of the following: history of consuming psychiatric drugs, history of referral to a psychiatrist or psychologist, psychiatric hospitalization history, history of substance abuse or dependency and history of suicide attempts. Secondly, the screening was completed by General Health Questionnaire. There was no remarkable diversity in sex distribution among cases and controls (55% male in controls, 67% male in schizophrenia patients and 47% male in bipolar disorder patients). Healthful control, bipolar disorder patients and schizophrenia patients subsequently had mean±SD age 37.6±9.6, 34.4±11.2 and 36.9±10.2.

2.2. DNA extraction

The total genomic DNA was extracted of the leukocytes via Diatom DNA Prep extraction kit (Gen Fanavaran, Iran) based on the guidance. Density of the genomic DNA was determined by spectrophotometer.

2.3. SNP genotyping and statistical analyses

We selected single nucleotide polymorphisms (SNPs) from the public SNP database, dbSNP (http://www.ncbi.nlm.nih.gov) and from published finding (Table 1). We chose the markers (rs947267 / rs3918342) for DAOA gene and (rs165599/ rs4680) for COMT gene, because the

DAOA and COMT genes have recently been associated with schizophrenia and bipolar disorder. A large number of studies have recommended that gene polymorphisms are associated with gene expression. The rs4680 is located on exonic region. Exonic SNPs have a direct effect on the characteristics of proteins, while SNPs within untranslated region and introns can affect the expression and splicing of mRNA. The rs3918342 is located upstream of 5′UTR and the rs165599 is located on 3'UTR. The sequences of the UTRs (untranslated regions) of mRNAs play significant roles in posttranscriptional management, but whether an alteration in UTR length can significantly affect the regulation of gene expression is not obvious (Zhenguo & Wen-Hsiung., 2011). The rs947267 is located in the intronic region. Mutations in intronic region, induce abnormal splicing (such as cryptic splice sites or exon skipping) that is obviously different from normal alternative splicing (Cooper, 2010). The DNA samples were used to genotyping by using PCR- RFLP methods. Polymerase chain reaction (PCR) is a technique used in molecular biology to amplify a single copy or a few copies of a segment of DNA for producing thousands to millions copies of a special DNA sequence. As depicted in Table 2, the samples were amplified by two primer pairs. Primers were designed using the Primer3 software or NCBI Primer Blast, (http://www.ncbi.nlm.nih.gov/tools/primer-blast) with the parameters set to create a product. In Restriction fragment length polymorphism analysis (RFLP), the DNA sample is broken into pieces (and digested) by restriction enzymes and the resulting restriction fragments are separated according to their lengths by gel electrophoresis. RFLP analysis can be used as a form of genetic testing to observe whether an individual carries a mutant gene for a disease that runs in his or her family. RFLP analysis was performed to determine genotypes of four polymorphisms: rs947267 by HaeIII restriction enzyme (Figure 1), rs3918342 by BsaAI restriction enzyme (Figure 2), rs165599 by MspI restriction enzyme (Figure 3) and rs4680 by Hin1II restriction enzyme (Figure 4). Also, the data was confirmed by sequencing assay. DNA sequencing is the process of determining the precise order of nucleotides within a DNA molecule. Information were studied by Logistic Regression and Mantel-Haenszel chi square assays. “Hardy Weinberg equilibrium” was estimated using chi-square tests.

3. Results

The aim of our study was to do a more extensive evaluation of the roles of DAOA and COMT genes to the susceptibility to schizophrenia and bipolar I disorder. Genotypic distribution of the SNPs in case and control groups is listed in table 3. Also, the allelic frequency of these SNPs in case and control groups is listed in table 4. All the data in this analysis was found to be in Hardy Weinberg equilibrium. The allelic frequency of SNPs was studied by Logistic Regression and Mantel-Haenszel chi square assays. The results assessed at importance level $P<0.05$. As depicted in table 4, the results of p-values revealed there is a statistically significant association between SNPs rs3918342 ($P=0.001$), rs165599 ($P<0.001$) and rs4680 ($P<0.001$) and schizophrenia. Also, there is a significant association between SNPs rs3918342 ($P<0.001$), rs165599 ($P <0.001$) and rs4680 ($P=0.02$) and bipolar I disorder. Also, our data demonstrated that the rs947267 ($P<0.001$) was importantly communicated with bipolar I disorder but there wasn’t any communication between this SNP ($P=0.09$) and schizophrenia. Our data provide further evidence that the DAOA locus or COMT locus may play a role in the pathophysiology of psychotic disorders. Although no direct link to genetic polymorphism in these genes and NMDA receptor function has been disclosed, the present results support previous reports implicating the DAOA as susceptibility genes for psychotic disorders. Further investigation is warranted to determine the functional variation underlying these results and to relate this to the pathophysiology of psychotic disorders.

4. Discussion

The genetic portion of schizophrenia and bipolar disorder seems to be more complicated and does not have a public heritability. A multi locus model has been suggested to clarify the pattern of heritability in this mysterious disorder. This model offers that a composition of various genetic agents is a prerequisite of these disorders (Risch, 1990). Hence, it is presumed that more than one locus is involved in the creation of schizophrenia and bipolar disorder. Across the glutamatergic pathway researches in Iran, PRODH gene (Rahman zadeh, Mohammadi, karimipour, Heidari keshel, & Omidinia, 2012), DTNBP1 gene (Galehdari, Ajam, Pooryasin, Foroughmand, & Kazeminejad, 2010), GRIN1 gene (Galehdari, 2009), dysbindin gene (Alizadeh, et al., 2012), and NRG1 gene (Shariati, Behmanesh, & Galehdari, 2011) have shown association with schizophrenia. Among dopaminergic pathway researches in Iran, DISC1 gene, hasn't shown association with schizophrenia (Foroughmand, et al., 2010). But MAOA gene has shown association with bipolar disorder (Eslami Amirabadi, et al., 2015). In this study, the key role of two candidate gene; DAOA gene (13q34) and COMT gene (22q11) has gained attention. Our findings were checked with other studies. In one meta-analysis, 13 genetic variants showed genetic overlap between two or more affective disorders (DAOA (rs3918342), COMT (Val158Met), DRD4 48-bp, DAT1 40-bp, SLC6A4 5-HTTLPR, APOE e4, ACE Ins/Del, BDNF (Val66Met), HTR1A C1019G, MTHR C677T, MTHR A1298C, TPH1 218A/C and SLC6A4 VNTR) demonstrating evidence for pleiotrophy in affective disorders (Gott, et al., 2014). Hukic propose an interaction between DAOA and COMT genes, also SNPs in this genes were associated to cognitive dysfunction in bipolar disorder patients. (Hukic, 2016). In an Italian population study described the evidence of association for NMDA-receptor-mediated signalling genes, DAO, PPP3CC, DAOA and DTNBP1 whit schizophrenia (Sacchetti, et al., 2013). Deficiency of the glutamatergic system has been implicated in the pathophysiology of both schizophrenia and bipolar disorder (Tsai G, 2002). The A allele at rs947267 was associated with bipolar I disorder in our study. In Asians following subgroup and Caucasians analysis the genetic polymorphisms of rs947267 in the DAOA gene were not found a statistically significant increased risk of SCZ, BD (Tan, et al., 2014). In one meta-analysis (Shi, Badner, Gershon, & Liu, 2008); composed of 18 association articles, no link was established between rs947267 and bipolar disorder, while there was a remarkable affiliation between rs947267 and bipolar disorder in Iran. Consequently, the association between rs947267 with both of schizophrenia and bipolar disorders is quite different in southwest Iran from Asian population. In this meta-analysis there was an affiliation among rs947267 and schizophrenia. This association has not been observed until now in Iran. Also, the T allele at rs3918342 was associated with schizophrenia and bipolar I disorder in our study. This is the same allele that was found to be associated in the study of Chumakov et al (Chumakov I, et al. 2002). However it should be stated that association between rs3918342 and schizophrenia was not found in the meta-analysis of DAOA studies (Shi, Badner, Gershon, & Liu, 2008). Also, in Caucasians and Asians following subgroup analysis the genetic polymorphisms of rs3918342 in the DAOA gene showed "no statistically significant increased risk of SCZ, BD (Tan, et al., 2014). But in the Taiwanese population, the SNP rs3918342 of the DAOA gene showed significant association with schizophrenia (Che-Sheng, et al., 2017). Also, a

remarkable association has been observed between rs3918342 and bipolar disorder and schizophrenia in the UK (Nicholas, et al., 2009). Related to the COMT gene: the A allele at rs165599 and rs4680 single nucleotide polymorphisms was associated with schizophrenia and bipolar I disorder in our study. One great study of Ashkenazi Jewish patients disclosed an extremely importance of affiliation between COMT gene and schizophrenia (Shifman, et al., 2002). Shifman also showed the positive correlation between rs165599 and the bipolar disorder (Shifman, et al., 2004). Many researchers have studied the rs4680 polymorphism of COMT gene. The association of this variant with schizophrenia is complex and likely be influenced by genetic substructure of human populations (Glatt, Faraone, & Tsuang, 2003). Shifman observed the association between schizophrenia and rs4680 (Shifman, et al., 2004). But Lajin and colleagues, showed lack of association of rs4680 with schizophrenia (Lajin, et al., 2011). Also Shifman showed no association between rs4680 and BPD (Shifman, et al., 2004). While results of Mynett-Johnson demonstrated an association between rs4680 and BPD (Mynett-Johnson, et al., 1998).

Obviously, our study is not able to prove or reject the complexity of glutamate and dopamine neurotransmission. But it does present more confirmation for the association of this neurotransmissions with presence of schizophrenia and bipolar disorder in southwest Iran. It is noteworthy that available treatments for psychotic disorders, have had partial success, because for around forty years, most of the work on psychotic disorders was only focused on dopamine. While glutamate is the most abundant excitatory neurotransmitter in the nervous system and it plays a key role in most aspects of normal brain functions, including cognition, memory and learning. Also, it is necessary to be described that detection of the association between schizophrenia and bipolar disorder with DAOA and COMT genes recreate the glutamate and dopamine hypothesis. Genetic linkage analysis has identified numerous overlapping regions in these diseases, consist of chromosome 6p, 13q, 18q and 22q (Badner and Gershon, 2002). In addition, based upon many genetic observations, first or second familial degrees of schizophrenia or bipolar disorder patients are at high risk for these two diseases (Arajarvi, et al., 2006). Although the schizophrenia and bipolar disorder are defined as separate and exclusive diagnostics, they show an overlap of symptoms. Hence, the etiologic segregation of these diseases into homogenous subtypes is currently under debate. In conclusion, our finding prepares more document for a positive dependency among the DAOA and COMT genes with schizophrenia and bipolar disorder. These conclusions may provide more validation for existence of genetic overlap in the common genes of schizophrenia and bipolar disorder.

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Authors' contributions

Leila Ahmadi and Parisima Behbahani had equal role in sample collecting, experimental studies, design, work, statistical analysis and manuscript writing. Seyed Reza Kazemi Nezhad was the supervisor and edited the manuscript. Nilofar Khajeddin collected samples. Mehdi Pormehdi Borojeni executed the statistical analysis. All authors studied and authorized the ultimate manuscript.

Conflicts of Interest

The authors declared no conflict of interests.

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Table 1. Description of genotyped markers.

Genes	SNPs	Chr Position (bp)	Alleles
DAOA gene **(13q34)**	rs947267	**105487313**	**A/C**
	rs3918342	**105533400**	**C/T**
COMT gene **(22q11)**	rs165599	**19969258**	**A/G**
	rs4680	**19963748**	**A/G**

Table 2. Primer sequences of the rs947267, rs3918342, rs165599 and rs4680 SNPs.

Genes	SNPs	Alleles	Primers	
DAOA gene				
	rs947267	A/C	**Forward**:	5'-GGGAAAAGGTATCAGGGAGAG-3'
			Reverse:	5'-TTGCACACGAACCAAATCAG-3'
	rs3918342	C/T	**Forward**:	5'-GGAAACCAGAAGGTGAAA-3'
			Reverse:	5'-GAATCAGAAAGGAAAAGTGT-3'
COMT gene				
	rs165599	A/G	**Forward**:	5'-CACAGTGGTGCAGAGGTCAG-3'
			Reverse:	5'-CTGGCTGACTCCTCTTCGTTT-3'
	rs4680	A/G	**Forward**:	5'-TCATCACCATCGAGATCAACC-3'
			Reverse:	5'- CCCTTTTTCCAGGTCTGACA-3'

Table 3. Genotypic distribution of the SNPs in case and control groups.

SNPs	Genotypic distribution	Schizophrenia	Bipolar I disorder	Controls
rs947267				
	AA	39%	41%	30/7%
	CC	12%	2%	22.85%
	AC	49%	57%	46.45%
rs3918342				
	TT	46%	23%	52.755%
	CC	17%	22%	4.725%
	TC	37%	55%	42.52%
rs165599				
	AA	41%	60%	16.5%
	GG	8%	3%	44.9%
	AG	51%	37%	38.6%
rs4680				
	AA	94%	67%	74%
	GG	0%	10%	1%
	AG	6%	23%	25%

Table 4. Allelic frequency and P-Values of the SNPs in case and control groups.

SNPs	Case and control	Allelic frequency		P-Value
rs947267				
	Schizophrenia	**C**	0.365	**P=0.09**
		A	0.635	
	Bipolar I disorder	**C**	0.305	**P<0.001**
		A	0.695	
	controls	**C**	0.54	
		A	0.46	
rs3918342				
	Schizophrenia	**C**	0.355	**P=0.001**
		T	0.645	
	Bipolar I disorder	**C**	0.495	**P<0.001**
		T	0.505	
	controls	**C**	0.26	
		T	0.74	
rs165599				
	Schizophrenia	**A**	0.665	**P<0.001**
		G	0.335	
	Bipolar I disorder	**A**	0.785	**P <0.001**
		G	0.215	
	controls	**A**	0.358	
		G	0.642	
rs4680				
	Schizophrenia	**A**	0.97	**P<0.001**
		G	0.03	
	Bipolar I disorder	**A**	0.785	**P=0.02**
		G	0.215	
	controls	**A**	0.865	
		G	0.135	

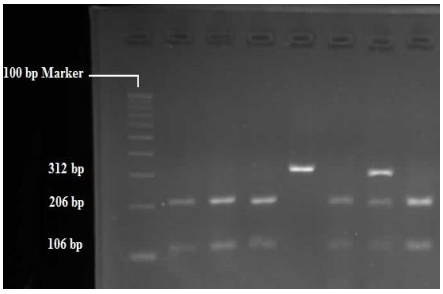

Figure 1. RFLP of rs947267 by HaeIII restriction enzyme: AA (312 bp), CC (206 bp/ 106 bp), AC (312 bp/ 206 bp/ 106 bp)

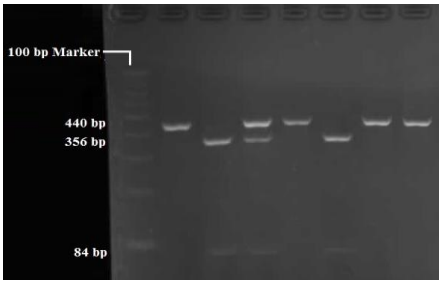

Figure 2. RFLP of rs3918342 by BsaAI restriction enzyme: TT (440 bp), CC (356 bp/ 84 bp), TC (440bp/ 356bp/ 84 bp)

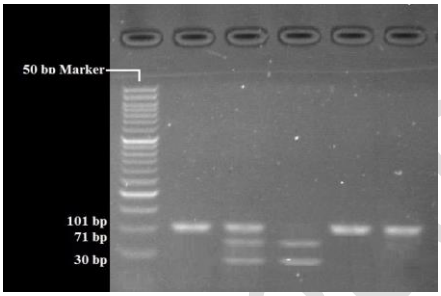

Figure 3. RFLP of rs165599 by MspI restriction enzyme: AA (101 bp), GG (71 bp/ 30 bp), AG (101bp/ 71bp/ 30 bp)

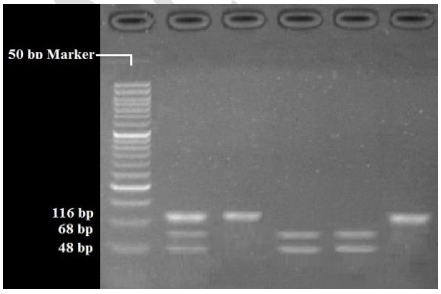

Figure 4. RFLP of rs4680 by Hin1II restriction enzyme: GG (116 bp), AA (68 bp/ 48 bp), AG (116bp/ 686bp/ 48 bp)