Monoamine Oxidase A Gene Polymorphisms and Bipolar Disorder in Iranian Population

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Background: Bipolar disorder (BPD) is a common and severe mood disorder. Although genetic factors have important roles in the etiology of bipolar disorder, no specific gene has been identified in relation to this disorder. Monoamine oxidase gene is suggested to be associated with bipolar disorder in many studies.

Objectives: This study aimed to investigate the role of *MAOA* gene polymorphisms in the etiology of bipolar disorder in Iranian population.

Patients and Methods: This study is a case-control study, with convenient sampling. Three common polymorphisms, a CA microsatellite, a VNTR, and a RFLP were typed in 156 bipolar patients and 173 healthy controls. Patients were chosen from Imam Hossein General Hospital, Psychiatry Ward (Tehran/Iran). Control samples for this study consisted of 173 healthy individuals recruited by convenient sampling. Allelic distributions of these polymorphisms were analyzed in bipolar and control groups to investigate any association with *MAOA* gene.

Results: Significant associations were observed regarding *MAOA-CA* (P = 0.016) and *MAOA-VNTR* (P = 0.004) polymorphisms in the bipolar females. There was no association between *MAOA-RFLP* and bipolar disorder.

Conclusions: The obtained results confirm some previous studies regarding a gender specific association of *MAOA* gene with the bipolar disorder.

Keywords: Bipolar Disorder; Genetic Association Studies; Genetics; Monoamine Oxidase; Iran

1. Background

Bipolar disorder (BPD) is a common and severe mood disorder characterized by manic and depressive episodes. Approximately 1.0% of the general population (ranging from 0.1% in Nigeria to 3.3% in the United States) meet lifetime criteria for BP type I (BP-I) (1). The familial aggregation of bipolar disorder is well established (2, 3). Both adoption (4) and twin (5) studies show that a substantial proportion of the variance in the etiology of bipolar disorder may be attributed to genetic factors. Although much data support a genetic component in the etiology of bipolar affective disorder, the mode(s) of inheritance are unclear and the specific genes associated with predisposition to this condition have not been well-characterized (6).

One candidate gene on the X chromosome that is of particular interest for bipolar disorder is the gene for monoamine oxidase A. Monoamine oxidase is an enzyme expressed in the outer mitochondrial membrane; it catalyzes the degradation of biological amines (7). The first association of the *MAOA* gene with psychiatric disease

was reported in a study of a large family in Holland (8).

In many studies, *MAOA* gene had been identified as a significant issue related to psychological disease such as BPD (9), depression (10-13) and antidepressant response (12), sleep quality (11), alcoholism combined with BPD (14), impulsive behaviors and alcohol dependence (15), alcoholism (13), and paranoid schizophrenia (16, 17).

The association of *MAOA* polymorphisms with BPD has been contradictory in different populations. For example, while studies conducted in Germany (18), the U.S.A. (19), Canada (20, 21), and China (22, 23) have shown no associations in their overall samples, positive correlations have been reported from studies conducted in Japan (24), Canada (25), China (26, 27), South Africa (28), and the UK (29). Furthermore, studies done in France, Switzerland (30), and the UK (31) have reported positive correlations in female subjects only. Recently, in a meta-analysis, the association between *MAOA* and mood disorders was confirmed. Their results demonstrated significant association

between uVNTR and MDD in the Asian group or male Asian group, CA polymorphism, and BPD in all Caucasians for the overall alleles. They concluded the *MAOA* gene can be associated with mood disorders by sex and ethnicity (10).

Studies of the association between bipolar disorder and *MAOA* have mainly focused on 3 markers in this gene: 1) a dinucleotide repeat polymorphism referred to as *MAOA*-CA (32), 2) a dinucleotide repeat directly adjacent to the imperfectly duplicated 23-base-pair variable number of tandem repeats (VNTR) motif (33), and 3) a restriction fragment length polymorphism (RFLP) resulting from single-base-pair substitution in the third base of a triplet codon (34).

As differences across studies may be attributable to either undetected population stratification or real genetic differences between study populations, conducting similar studies with different populations are crucial. Thus, in the present study, we examined all 3 polymorphisms in the Iranian population, and observed some significant associations similar to some other populations and different from others.

2. Objectives

This study aimed to investigate the role of *MAOA* gene polymorphisms in the etiology of bipolar disorder in Iranian population.

3. Patients and Methods

This study is a case-control research. To determine the sample size, the following equation (35) was used.

$$n = \frac{T^2 pq}{d^2}$$

Where T = 1.96, p = 0.5, q = 0.5, and d = 0.11. The minimum sample size needed to obtain statistically valid results was 79, and we added 80 more to the sample to obtain higher validity.

The participants of the present study were recruited by convenient sampling method, from the department of psychiatry at Imam Hossein Hospital in Tehran, Iran from January 2013 to February 2014. Bipolar disorder type I was diagnosed based on the DSM-IV-TR criteria using a psychiatric interview Structured Clinical Interview for DSM-IV (SCID-I) (36) by 2 psychiatrists. Subjects with a history of, or a current neurological disease, or mental retardation were excluded. Samples for this study consisted of 156 unrelated bipolar patients and 173 healthy controls. The control samples had no evidence of psychiatric disorders in their family histories confirmed by a psychiatrist. The patients were in the range of 18-60 years old, and normal samples were matched to patient samples in terms of age, sex, and ethnicity.

This study was approved by Ethics Committee of Behavioral Sciences Research Center of ShahidBeheshti University of Medical Sciences. All participants provided written consent.

Genomic DNA was extracted from 2 mL of peripheral blood using salting-out method: 50 ng of each of the primers, 0.2 mMdNTP, 1.5 mM magnesium chloride, and I unit of Taq polymerase in total reaction volume of 20 μL. The PCR primer used for amplifying 3 polymorphisms is presented below.

1) *MAOA*-CA
F: 5´ AGAGACTAGACAAGTTGCAC 3´
R: 5´ CACTATCTTGTTAGCTCACT 3´
30 cycles, 94˚C denaturation, 56.5˚C annealing, and 72˚C extension

2) *MAOA*-VNTR
F: 5´ GGTAGACTCCTTTAAGAAAA 3´
R: 5´ CAATAAATGTCCTACACCTT 3´
30 cycles, 94˚C denaturation, 55.5˚C annealing, and 72˚C extension

3) *MAOA*-RFLP
F: 5´ GACCTTGACTGCCAAGAT 3´
R: 5´ CTTCTTCTTCCAGAAGGCC 3´
30 cycles, 94˚C denaturation, 60.5˚C annealing, and 72˚C extension

The PCR product of allele detection CA was performed by Sanger Sequencing method. The PCR product of the VNTR polymorphism was separated on 8% polyacrylamide gel and alleles were detected. The restriction enzyme used for digestion in the RFL-PCR reaction was Fnu4HI. The digestion products of RFLP polymorphism were separated on 1% agarose gel. Types and sizes of different alleles for each polymorphism are described in Table 1.

Table 1. Several Alleles of CA and VNTR Polymorphisms of *MAOA* Gene

Gene, Allele	Length, bp
MAOA-CA	
a0	128
a1	128
a2	124
a3	122
a4	120
a5	118
a6	116
a7	114
a8	112
a9	110
a10	108
MAOA-VNTR	
V1	375–388
V2	355–365
V3	335–345
V4	315–325
V5	295–305
RFLP	
R_1	180
R_1	65

We usedOpenEpisoftwarefor the statistical analysis of the data (http://www.openepi.com/menu/OPENEpi:Menuhtm). Chi-square and Fisher exact Test were used to compare frequency of the genotypes.

4. Results

4.1. Genotyping and Association Analysis

Three polymorphisms of *MAOA* gene, CA, VNTR and RFLP were genotyped in our case-control groups (Table 2). Regarding *MAOA*-CA polymorphism, 11 alleles for males and 10 alleles for females were found in patients group. In the control group, 10 alleles were found for each male and female participants. There was no female participant with a10 or 108bp allele in our study group. Regarding*MAOA*-VNTR polymorphism, we identified 4 alleles in both males and females in patient samples, and in control group, 4 and 5 alleles for males and females, respectively. For this polymorphism, no V5 or 295-305 bp allele was identified. Regarding *MAOA*-RFLP, there are 2alleles for both males and females consisting of R1 an uncut allele and R2 the cut allele.

Allele frequencies of 3 analyzed polymorphisms were compared between patient and control groups and also between males and females of study population, using $\chi2$ test and SPSS software.

According to the obtained information, for *MAOA*-CA polymorphism, no difference was observed in distribution of alleles between patient and control groups. But, comparing males and females, there was an association between the *MAOA*-CA polymorphism and bipolar disorder among females ($\chi2 = 20.245$, df = 9, P = 0.016) (Table 3).

Regarding *MAOA*-VNTR polymorphism, a significant deviation from the normal allele distribution was observed between patients and controls for V4 allelic subgroup ($\chi2 = 10.04$, df = 1, P = 0.002). However, association in this subgroup was just observed in females. On the whole, counting all VNTR allelic subgroups, another significant difference in allelic distribution was found in female patients compared to controls, but there was no such a result for males (Table 4).

Similarly, no deviation was observed comparing total patient and control groups. Regarding *MAOA*-RFLP, statistical analysis showed no significant association between the marker and bipolar disorder (Table 5).

Table 2. Distribution of CA, VNTR and RFLP Alleles in Patients and Controls [a]

Allele	Patients			Controls		
	Male	**Female**	**Total**	**Male**	**Female**	**Total**
CA						
Total	50	106	156	53	120	173
VNTR						
V1	2 (4)	17 (16)		7 (13.2)	24 (21.4)	
V2	27 (54)	48 (45.3)		30 (56.6)	51 (45.5)	
V3	6 (12)	7 (6.6)		5 (9.4)	19 (17)	
V4	15 (30)	34 (32.1)		11 (20.8)	16 (14.3)	
V5	0 (0)	0 (0)		0 (0)	2 (1.8)	
Total	50	106	156	53	120	173
RFLP						
R1	27 (54)	71 (71)		24 (48)	75 (75)	
R2	23 (46)	29 (29)		26 (52)	25 (25)	
Total	50	106	156	60	113	173

[a] Data are presented as No. or No. (%).

Table 3. Statistical Analysis of *MAOA*-CA in Males and Females

	$\chi2$	df	P Value [a]
Gender			
Male	8.401	10	0.590[a]
Female	20.245	9	0.016
Total	10.286	10	0.416

[a] P < 0.05.

Table 4. *MAOA*-VNTR V4Allele and Total Allelic Distribution

Allele	χ2	df	P Value [a]
V4			
Male	1.165	1	0.280
Female	9.651	1	0.002
Total	10.046	1	0.002
Total			
Male	3.558	3	0.313
Female	15.151	4	0.004
Total	14.509	4	0.006

[a] P < 0.05.

Table 5. RFLP Allele and Total Allelic Distribution

RFLP	χ2	df	P Value [a]
Gender			
Male	0.360	1	0.548
Female	0.406	1	0.524
Total	0.015	1	0.903

[a] P < 0.05.

5. Discussion

Like other psychiatric disorders, BPD has a complex etiology, including multiple affecting genes and several non-genetic risk factors (37). Each of these factors plays a small role in the etiology of disorders and association studies help us identifying these small risk factors in complex diseases (38). Many studies have attributed the MAOA gene to several human behavioral disorders, including aggression (39), anxiety (13), and depression (10-13); all belong to the same spectrum of phenotypes. Therefore, *MAOA* is involved in this spectrum of disorders with abnormalities of CNS. We included all 3 common polymorphisms of *MAOA* gene in our study to be more precise.

In our sample group, we replicated some previous results of *MAOA*-CA, *MAOA*-VNTR and *MAOA*-RFLP association studies with the bipolar disorder. Our results were similar to that of Preisig et al. (30), regarding the difference between the allelic distributions of female patient and control groups. However, we found no difference in the overall sample, and no particular allele in association with the disease, as they showed an association of a6 allele with the disease in female group. Similarly, Lim et al. (31) reported the allelic distribution differences in overall sample and control groups and also association of a2 and a5 alleles with the disorder in females group. Unlike the mentioned studies, Craddock et al. (40) found no relationship between *MAOA*-CA marker with bipolar disorder.

Regarding *MAOA*-VNTR, there is discrepancy in reported results of different studies. We found a significant association between VNTR marker and bipolar disorder in overall sample and another obvious association between V4 allele and the disorder, both just in females. Some studies (10, 14, 15, 31) have shown the association of this marker to bipolar disorder and specifically in females while other studies (23, 40) found no association. It is stated that the long alleles of this polymorphism are transcriptional and more active than the shorter alleles (41) that matches our findings.

Regarding *MAOA*-RFLP marker, we replicated the results of most previous studies; similar to our results, Parsian and Todd (19), and Craddock et al. (40) found no association between this marker and bipolar disorder. However, two studies (29, 31) have found an association for this marker in females and also in general comparison between overall sample and control group.

According to many studies mentioned above, those have reported significant gender-specific associations of *MAOA* gene with psychiatric disorders; it seems that *MAOA* based etiology of BPD may be different in males and females.

Our different results compared to other studies, show that the allele frequencies of such polymorphisms are population and ethnic specific, and some factors like genetic background and founder effects may affect the allele frequencies in different populations. The allele typing difficulties due to close allele sizes in polymorphic markers may be another affecting factor, as well. Thus, more studies using larger sample groups and also association analysis of these polymorphisms with BPD subtypes (with different symptoms) are suggested to achieve more reliable results. Overall, our results show that CA and VNTR polymorphisms are associated with bipolar disorder in Iranian population, and could be used as risk factors in the diagnosis of at risk people.

It was the first time in Iran that a study was conducted on the role of *MAOA* gene polymorphisms (CA microsatellite, VNTR and RFLP) in the etiology of bipolar disorder in Iranian population. A larger sample size is required to find more valid results.

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