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A Study on the Association of Interleukin-1 Cluster with Genetic Risk in Bipolar I Disorder in Iranian Patients: A Case-control Study

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ABSTRACT

The pathogenesis of Bipolar I Disorder (BP-I) involves immune-mediated mechanisms, especially an imbalance in pro-inflammatory/anti-inflammatory cytokines in plasma or cerebrospinal fluid. Interleukin-1 (IL-1) gene cluster, coding some of these pro-inflammatory cytokines, might play a role in various neuropathologies related to neuron inflammation.

The aim of the present study was to investigate the possible role of IL-1 gene cluster polymorphisms in determining the susceptibility to BP-I in Iranian population.

48 patients with BP-I in Mashhad (in north-eastern Iran), diagnosed by two psychiatrists using SCID (structured clinical interview for DSM disorders) were selected through convenient sampling and were compared with 47 healthy controls, voluntarily enrolled in the study. Patients with non-Persian ethnicity, history of immunoallergic disorders, endocrinopathies, neurologic disorders, and substance-induced mood disorders were excluded from both case and control groups. Genotyping of IL-1 gene cluster polymorphisms, including IL-1α-889, IL-1β +3954, IL-1β-511, and IL-1RN (VNTR) were carried out using Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and compared by SPSS using Fisher's exact and chi-square tests.

The frequency of IL-1β-511 CC genotype and C/T allelic frequency were significantly different between BMD patients and healthy controls (p=0.04 and p=0.02, respectively). Among patients, -511 T allele was significantly more frequent in those with a positive history of major depression. Moreover, +3954 T allele was significantly more frequent in early onset BMD patients.

The results suggest a positive association between IL-1 gene cluster variation and BP-I. This polymorphism may contribute to genetic vulnerability through its possible role in neuron inflammation.

Keywords: Bipolar disorder; Interleukin 1 gene cluster; Iran; Polymorphism

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INTRODUCTION

Bipolar I disorder (BP-I) is one of the major psychiatric disorders with a lifetime prevalence of 1% in the general population, which is seen in all societies.[1] In this disorder the patient experiences one or more manic or mixed episodes with or without depressive episodes, which causes a marked impairment of functioning in different aspects of the person's life. World health organization (WHO) considers it to be the 7th worldwide cause of disability.[2] Etiology of BP-I is multifactorial and complex.[3] Studies on families, monozygotic and dizygotic twins, and adoption studies have shown heritability as a major contributing factor. [4,5] However, the exact genes involved in bipolar disorder have not been identified, yet.

One of the areas under study in this regard is alterations in genes related to the immune system, mainly cytokines. Many studies have highlighted the role of immune system and systemic inflammatory responses in BP-I.[6,7] Dysregulation of inflammatory responses is associated with the pathophysiology of several psychiatric disorders.[8] Many studies on the serum levels of immune system components have observed an imbalance between pro-inflammatory and anti-inflammatory cytokines in the plasma or cerebrospinal fluid of bipolar and schizophrenia patients.[9-11] In particular, increased concentrations of the three pro-inflammatory cytokines including TNF-α, IL-1, and IL-6 in the serum of bipolar patients have been reported.[7] Furthermore, IL-6, the soluble form of TNF-α receptor1 (sTNF-R1), and IL-1 receptor antagonist (IL-1RN) have been shown to be correlated with affective states and severity of the disorder, especially depression.[7,12,13] These inflammatory markers are correlated with severity (including psychotic symptoms) and also poorer course of illness (including more episodes and earlier age of onset) in bipolar and schizophrenia patients.[7] Increased level of cytokines is proposed to be related to acute episodes of the disorder or considered by some researchers as a trait marker influenced by genetic factors.[14]

The IL-1 family is a group of 11 cytokines including IL-1α, IL-1β, and IL-1RN. IL-1α and IL-1β activate their respective receptors while IL-1RN inhibits them. The genes encoding the IL-1 family are mostly located on chromosome 2q13. Some polymorphic variants of these genes have been observed to be associated with autoimmune disorders.[16] It has been demonstrated that single-nucleotide polymorphisms (SNPs) within cytokine genes can influence the transcription and function of cytokines which might lead to pathological outcomes.[17] Regulatory processes of IL-1 activity may alter to some extent under the influence of genetic variance; therefore synthesis of IL-1 subtypes such as IL-1α, IL-1β, and IL-1RN might be affected by genetic polymorphisms in IL-1 family.[16] In several psychiatric disorders, including BP-I, variations have been seen in serum levels of IL-1 and other cytokines.[18] During neurodevelopment of brain, IL-1β affects proliferation, leading to production of some trophic factors such as nerve growth factor in astrocytes as well as inhibition of brain-derived neurotrophic factor production.[16] Therefore it seems that some variants of IL-1 β gene could play a role in the pathogenesis of heritable neurodevelopmental heritable psychiatric disorders. Regarding the heritability hypotheses in bipolar disorder, we can assume that one of the factors contributing to the pathogenesis of BP-I is cytokine gene polymorphisms, including polymorphisms in genes encoding IL-1 family members.

Although some studies have been carried out regarding the association between BP-I and serum levels of some of these immunologic markers, the relationship of this disorder with alterations in genes encoding these cytokines have not been studied adequately. In addition, some studies on the gene polymorphism of different cytokines have shown contradictory results [1, 16, 19]. In a study by Papiol et al. in 2004 on Spanish patients, polymorphism in the promoter region (-511) of the IL-1β gene and an 86 base pair) bp) variable number of tandem repeat (VNTR) in intron 2 of the IL-1RN gene were studied in schizophrenic and bipolar patients and in healthy controls. The results showed that the haplotypic combination -511 allele*1/VNTR allele*2 was 2 to 3 times more prevalent in schizophrenic and bipolar patients, compared to the control group.[16] In a more recent study by Rafiei et al., VNTR polymorphism in IL-1Ra has been shown to be present in BMD.[1] According to these studies, different variants of IL-1 gene clusters are probable contributing factors in the pathogenesis of BP-I; however, previous researches have not been conclusive and whether the findings in different ethnic groups (such as Korean [19] or Spanish people) could be generalized to Persian population, still

remains unknown.

This research was carried out in Iranian population for the first time to investigate the IL-1 gene cluster polymorphisms including IL-1α (-899 C/T), IL-1β (+3954 C/T), IL-1β (-511 C/T), and IL-1RN 86 bp tandem repeats in BP-I patients and control group. This study aims to elucidate the heritability of this disorder to improve ways to diagnose individuals who are at risk in the future.

MATERIALS AND METHODS

Study Population

This is a case-control study on 48 patients from 18 to 45 years of age, referring to Ibn-e-Sina psychiatric hospital in Mashhad (the second largest city of Iran, located in northeast) and 47 healthy controls. BP-I was diagnosed by two psychiatrists according to diagnostic and statistical manual of mental disorders, fourth edition, text revision (DSM-IV-TR) criteria and through structured interviews using structured clinical interview for DSM disorders (SCID-I). SCID-I is a diagnostic tool used to determine DSM-IV-TR axis I disorders. The SCID-I was translated into Persian language and found to be cross-culturally equivalent.[20] The control group was selected from a group of non-relative volunteers without a positive family history of mood disorders, BP-I was ruled out with mood disorder questionnaire (MDQ). MDQ has been designed as a screening self-report inventory to screen bipolar mood disorders in clinical practice. It is translated and validated (with Cronbach's alpha coefficient of 0.773) in Iranian population by Ghoreishizadeh et al. in 2011.[21] Patients with known history of immunoallergic disorders (autoimmune diseases, immune deficiencies, allergy, and other conditions related to inflammation and immune system), endocrinopathies (e.g. impaired thyroid function tests, history of other endocrinopathies such as Cushing disease or hyperprolactinoma), neurologic disorders, and substance-induced mood disorders (ruled out by taking a thorough medical history) were excluded from both case and control groups.

This research was approved by research vice chancellery, Iran as a residency thesis (No. 85504). After taking written informed consent based on Declaration of Helsinki, 10 mL venous blood was drawn from each participant. Half of each blood sample was kept with EDTA as anticoagulant and stored at -70°C to be transferred to immuno-genetic department of Bou-Ali research center in Mashhad. The other half was sent to laboratory for thyroid function tests in dry tubes. Genomic DNA was isolated using the "salting out" method Biogene kit (BioGene, Mashhad, Iran). The purity of DNA samples were assessed by spectrophotometry.

IL-1α -889 Gene Polymorphism

The region that contains the *Nco*I polymorphic site in the 59-flanking region of the IL-1α gene was amplified by PCR using the sense and antisense primers (Faza Pajooh Co., Tehran, Iran), described previously by Katila et al. (Table 1).[22] The following PCR parameters were used: 95°C for 5 min, followed by 35 cycles of 95°C for 1 min, 60°C for 1 min and 72°C for 1 min, and a final incubation at 72°C for 5 min followed by a cooling to 4°C. The PCR products were analyzed by electrophoresis on 1.5% agarose gels containing 0.1% ethidium bromide.

The 99 bp fragments were then digested overnight at 37°C with final concentration of 1 U/µl *Nco*I (Fermentas, Tehran, Iran) resulting in fragments that either remained intact (allele 2 or variant allele, 99bp) or were cut into two fragments of 83 and 16 bp, respectively (allele 1 or wild-type allele). The point of effect for the enzyme was located in S′…CCATG↓CT…3′. These fragments were analyzed by electrophoresis on a 17% polyacrylamide gel stained with silver nitrate to assess the genotypes 1.1 (CC), 1.2 (CT), and 2.2 (TT).

IL-1β +3954 Gene Polymorphism

Single nucleotide gene polymorphism at IL-1β +3954 were also assessed. The region that contains the *Taq*I polymorphic site within exon 5 of the IL-1β gene was amplified by PCR (Corbett, UK) using the sense and antisense primers, as described previously (Table 1). The following parameters were used: 95°C for 5 min, followed by 35 cycles of 95°C for 1 min, 60°C for 1 min and 72°C for 1 min, and a final incubation at 72°C for 5 min followed by cooling to 4°C. The PCR products were analysed by electrophoresis on a 2% agarose gel stained with 0.1% ethidium bromide.

The 250 bp fragments were digested overnight at 65°C with final concentration of 1 U/µl *Taq*I (Fermentas, Karaj, Iran) resulting in fragments that either remained intact (allele 2 or variant allele) or were cut into two

Table 1. Primers used for PCR quantification of bipolar I disorder and normal control groups in Mashhad, Iran

Genotype	Primer Sequence:
IL-1α -889	Forward: 5′-TGT TCT ACC ACC TGA ACT AGG C-3′
	Reverse: 5′-TTA CAT ATG AGC CTT CAA TG- 3′
IL-1β +3954	Forward: 5′-GTT GTC ATC AGA CTT TGA CC- 3′
	Reverse: 5′- TTC AGT TCA TAT CGA CCA CCA GA- 3′
IL-1β -511	Forward: 5′- GCC TGA ACC CTG GAT ACC GT- 3′
	Reverse: 5′- GCC AAT AGC CCT CCC TGT CT - 3′
IL-1RN (VNTR)	Forward: 5′- CTC AGC AAC ACT CCT AT-3′
	Reverse: 5′- TCC TGG TCT GCA GGT AA- 3′

fragments of 138 and 112 bp, respectively (allele 1 or wild-type allele). The point of effect for the enzyme was located in *S′...c↓catgg…3′*. These fragments were analyzed by electrophoresis on 4% agarose gels containing 0.1% ethidium bromide to assess the genotypes 1.1 (CC), 1.2 (CT), and 2.2 (TT).

IL-1β -511 Gene Polymorphism

The distribution of the biallelic polymorphism (The C→T substitution) at position -511 in the promoter region of the IL-1β gene was assessed. This region was amplified by PCR, using the sense and antisense primers (Table 1). The following parameters were used: 3 cycles at 95°C for 30 s, 65°C for 30 s, and 72°C at 30 s, 15 cycles at 94°C for 30 s, 60°C at 30 s, and 72°C at 30 s, 15 cycles at 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s, 5 cycles at 94°C for 30 s, 56°C for 30 s, and 72°C for 30 s, 5 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and a final incubation at 72°C for 10 min followed by cooling at 4°C. The PCR products were analyzed by electrophoresis on a 2% agarose gel stained with 0.1% ethidium bromide.

The 155 bp fragments were digested overnight at 37°C with 1 U/μl *Ava I* (Fermentas, Karaj, Iran) resulting in fragments that either remained intact (allele 2 or variant allele) or were cut into two fragments of 88 and 67 bp (allele 1 or wild-type allele), respectively. The point of effect for the enzyme was located in S′…C↓PyCGPuG…3′. These fragments were analyzed by electrophoresis on a 17% Polyacrylamide gel stained with silver nitrate to assess the genotypes 1.1 (CC), 1.2 (CT), and 2.2 (TT).

IL-1RN Gene Polymorphism

The polymorphic region within the second intron of the IL-1RN gene, which contains a VNTR of 86 bp, was amplified by PCR using the sense and antisense primers (Table 1). The parameters were with initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 60°C for 1 min, and elongation at 72°C for 1 min. The final elongation was at 72°C for 5 min followed by cooling at 4°C. The PCR products of 410 bp (allele 1 = four repeats of the 86 bp region), 240 bp (allele 2 = two repeats), 500 bp (allele 3 = five repeats), 325 bp (allele 4 = three repeats), and 595 bp (allele 5 = six repeats) were analyzed by electrophoresis on a standard 2% agarose gel stained with 0.1% ethidium bromide.

Statistical Analysis

At first, demographic data were calculated and compared between the two groups. To analyze the difference between genotype and allele frequencies in two groups, Fisher's exact test and chi-square (χ^2) test were used by SPSS software (Version 15, SPSS, Inc, Chicago, IL, USA). Regarding the fact that frequencies in some tables were less than expected, making chi square test invalid, the tables were merged to acquire expected greater values and Fisher's exact test was used to analyze the data. *P-values* less than 0.05 were considered statistically significant.

RESULTS

The case group consisted of 48 individuals with an average age of 29.40 years (±8.12) and included 24 men and 24 women. The control group consisted of 47 individuals, with average age of 29.72 years (±8.94), and included 19 men and 28 women. The two groups had no significant difference regarding age and sex

(p=0.85 df=93 and p=0.35 df=1, respectively). The clinical characteristics of the patients are presented in Table 2.

IL-1α -889 Genotype

Table 3 presents the frequency distribution of IL-1α -889 genotypes in the case and control groups. The genotypes are C/C (1.1), C/T (1.2), T/T (2.2), wild type allele C (1), and mutant allele T (2). Despite the increased frequency in 1.2 genotype in the control group and 1.1 genotype in the case group, there was no significant difference between the two groups regarding the IL-1α -889 polymorphism (df=2, p=0.26).

IL-1β +3954 Genotype

Table 4 presents the frequency distribution of IL-1β +3954 genotypes in the case and control groups. Wild type allele is defined as 1 (C) and mutant allele as 2 (T). Chi-square test showed no significant difference between the two groups (df=2, p=0.83)

IL-1β -511 Genotype

Table 5 presents the frequency distribution of IL-1β -511 genotypes in the case and control groups. The genotypes include 1.1 (C/C), 1.2 (C/T), 2.2 (T/T), wild type allele 1 (C), and mutant allele 2 (T). Despite the difference of 1.1 and 2.2 genotypes in the case and control groups, no statistically significant difference was seen in chi-square test (df=2, p=0.06). Each of these genotypes was further compared separately with other variants using Fisher's exact test and their odds ratio was calculated. A significant difference was seen in the frequency distribution of 1.1 genotype with other variants of IL-1β -511 between the case and control groups (p=0.04) and an odds ratio of 0.34 was reported which is considered significant regarding the 95% confidence interval (0.11–0.97).

IL-1RN (VNTR) Genotype

In this locus, various genotypes can be assumed regarding the 5 alleles A1–A5, but in the study of the case and control groups, only 8 genotypes were detected. As some of the boxes in the table were reported as zero, chi-square test could not be performed. Therefore, the two more frequent IL-1RN genotypes A1A1 and A1A2 were compared separately with other variants using Fisher's exact test and their odds ratio was calculated. No significant difference was seen between the case and control groups (p=1.00 and p=0.52, respectively) and their odds ratios were not significant, either.

Table 2. Characteristics of bipolar I disorder patients in Mashhad, Iran

	History of depressive episode		Age of Onset		History of Psychosis		Type of First Episode			History of suicide	
	+	-	<20yr	≥20yr	+	-	euphoric	dysphoric	depressive	Pos.	Neg.
N	12	36	16	32	32	16	20	23	5	3	45
%	25	75	33.3	66.6	66.6	33.3	41.7	47.9	10.4	6.3	93.7

Table 3. Genotype and Alleles frequencies of IL-1α -889 in patients with bipolar I disorder and normal controls in Iran

IL-1α -889	Patients		Controls		*p* value	OR	Confidence interval 95%
	Frequency	Percent	Frequency	Percent			
Genotypes:							
1.1 (CC)	29	60.4	23	48.9	0.30	1.59	0.70-3.59
1.2 (CT)	11	22.9	18	38.3	0.12	0.48	0.20-1.17
2.2 (TT)	8	16.7	6	12.8	0.77	1.37	0.43-4.29
Alleles:							
1 (C)	69	71.9	64	68.1	0.75	1.04	0.87-1.26
2 (T)	27	28.1	30	31.9		0.90	0.581-1.40

OR: odd ratio

Table 4. Genotype and Alleles frequencies of IL-1β +3954 in patients with bipolar I disorder and normal controls in Iran

IL-1β +3954	Patients		Controls		*p* value	OR	Confidence interval 95%
	Frequency	Percent	Frequency	Percent			
Genotypes:							
1.1 (CC)	31	64.5	28	59.6	0.67	1.24	054-2.84
1.2 (CT)	15	31.3	16	34.0	0.83	0.88	0.38-2.08
2.2 (TT)	2	4.2	3	6.4	0.68	0.64	0.10-4.00
Alleles:							
1 (C)	77	80.2	72	76.6	0.60	1.05	0.90-1.22
2 (T)	19	19.8	22	23.4		0.84	0.49-1.46

OR: odd ratio

Table 5. Genotype and Alleles frequencies of IL-1β -511 in patients with bipolar I disorder and normal controls in Iran

IL-1β -511	Patients		Controls		*p* value	OR	Confidence interval 95%
	Frequency	Percent	Frequency	Percent			
Genotypes:							
1.1 (CC)	6	12.5	14	29.8	0.04*	0.34	0.12-0.97*
1.2 (CT)	28	58.3	26	55.3	0.83	1.13	0.50-2.55
2.2 (TT)	14	29.2	7	14.9	0.14	2.35	0.85-6.50
Alleles:							
1 (C)	38	39.6	54	57.4	0.02*	0.69	0.51-0.93*
2 (T)	58	60.4	40	42.6		1.42	1.07-1.89*

OR: odd ratio; *Significantly different

Allelic Distribution Frequency of the Case and Control Groups in Different Loci

In addition to assessing the genotype frequency, frequency distribution of alleles in different loci was evaluated and compared between the case and control groups with the Fisher's exact Test. A statistically significant difference was seen between the case and control groups in the frequency of alleles 1 and 2 in the IL-1β -511 locus (p=0.02). Allele 1 (C) was reported more frequent in the control group and allele 2 (T) was more frequent in the case group (Table 5). This difference was not significant in the other loci. Odds ratio of Alleles 1 and 2 in the case group was significant in the control group, considering the confidence interval of 95%. (OR=0.69, CI 95%=0.51–0.93), and allele 2 (T) is a risk factor (OR=1.42, CI 95%=1.07–1.89).

Allelic Distribution Frequency of the Case Group in the Four Loci According to Clinical Features

Assessment of allelic distribution frequencies in the four loci based on the presence or absence of psychotic features in the recent episode was done through Fisher's exact Test which showed no significant difference in any of the gene loci.

Allelic distribution frequency of the four loci was also compared in patients with or without history of major depressive episodes. Distribution frequency of alleles C and T in IL-1β (-511) locus was evaluated in the case group and the two subgroups (the ones with and without history of a major depressive episode) by Fisher's exact Test (χ^2=4.70 and p=0.03). The difference in the distribution frequency of C and T alleles was significant in this locus. Odds ratio was 3.21, which is significant (CI=1.08–9.55). Distribution frequency of the other loci was not significantly different in the two subgroups (Table 6).

Allelic distribution frequency in the four loci in the case group, considering the family history of a major psychiatric disorder in first degree relatives, was evaluated by calculating odds ratio with Fisher's exact Test. It showed no significant difference (OR=1.53, p=0.45, χ^2= 0.74).

Table 6. Allele frequencies of the four SNPs based on the history of major depression in patients with bipolar I disorder in Iran

Genotype	Allele	History of major depression		X^2	OR	CI	*p*
		-	+				
IL-1α -889	C (%)	54 (75.0)	15 (62.5)	1.39	1.8	0.67-4.81	0.30
	T (%)	18 (25.0)	9 (37.5)				
IL-1β +3954	C (%)	57 (72.9)	20 (83.3)	0.20	0.76	0.23-2.56	0.77
	T (%)	15 (20.8)	4 (16.7)				
IL-1β -511	C (%)	33 (45.8)	5 (20. 8)	4.70	3.21	1.08-9.55	0.03*
	T (%)	39 (54.2)	19 (72.9)				
IL-1RN**	A1 (%)	87.5 (63)	20 (83.3)				
	A2 (%)	4 (5.6)	2 (8.3)				
	Others (%)	5 (6.9)	2 (8.4)				

CI: Confidence Interval; SNP: single-nucleotide polymorphism; * Significantly different; ** Chi square was not applicable

DISCUSSION

Over the past decades, several studies among other ethnic groups have examined the role of cytokines, including IL-1 cluster, in the pathogenesis of bipolar disorder. In these studies, cytokine serum levels have shown changes that implicate the role of immune system in the pathophysiology of bipolar disorder. For instance, Liu et al. showed increased IL-1RN serum levels in manic patients prior to treatment compared to control group, which was also significantly higher after the treatment.[12] In another study by Söderlund et al. in 2011, increased IL-1β levels in the cerebrospinal fluid of the bipolar patients was seen in comparison with healthy controls, which indicates an altered brain cytokine profile.[23] IL-1β plays a part in neurogenesis and development of central nervous system through regulation of nerve growth factor, apart from its role in inflammatory processes.[24] Considering the influence of genetic polymorphisms on the modulation of IL-1 level and activity,[24] and regarding the altered levels of IL-1 in bipolar patients, we can consider the genes involved in the synthesis of IL-1 as possible factors in the etiopathogenesis of bipolar disorder.

The present study determined the frequency of genetic variations in IL-1 cluster in four loci. We found out that IL-1β -511 C/C genotype was less frequent than other genotypes in bipolar patients compared to normal controls (OR=0.34, p=0.04). Furthermore, the frequency of alleles T and C in this locus was significantly different in the case and control groups (p=0.02).

Previous studies on the association between cytokine gene polymorphisms and psychiatric disorders have yielded conflicting results. In a study by Kim et al. in 2004, polymorphism of IL-1Ra gene was compared in schizophrenia and bipolar patients with healthy controls. Genotype distribution and allele frequencies were significantly different in schizophrenia patients and the control group, but this difference was not seen in bipolar patients.[19] In another study by Papiol et al. in 2004 on schizophrenic and bipolar patients, no significant difference was seen between bipolar patients and the control group in allele frequency and genotype distribution of IL-1β -511 polymorphism, but the prevalence of haplotypic combination -511 allele*1/VNTR allele*2 was found significantly higher (2 to 3 times) both in schizophrenic and bipolar patients compared to the control group.[16] This association was also absent in major depressive disorder.[25,27]

Our study presents new evidence regarding the possible association between gene polymorphism of IL-1β for the promoter region of -511 with BP-I for the first time. Since, there are some common points in the clinical features and genetic risk factors of both schizophrenia and bipolar I disorder; it seems that the pathogenesis of the two disorders is related to each other.[16,27] There is some evidence supporting the relationship between cytokine gene polymorphisms and schizophrenia. In a meta-analysis by Xu et al. in 2010, the association between IL-1 gene cluster (IL-1α, IL-1β, and IL-1Ra) and susceptibility for schizophrenia has been confirmed.[28] One haplotype in the promoter region of TNF-α gene has been associated with schizophrenia.[29] Polymorphism of neuroglin 1 gene, as

one of the genes associated with schizophrenia is synergistically correlated to polymorphism of IL-1β gene leading to increased risk of schizophrenia.[30] Presence of allele 2 polymorphism of IL-1β -511, similar to our study, was associated with the risk of schizophrenia, and development of depressive symptoms in patients with schizophrenia spectrum disorders.[31] In this regard, a systemic inflammation exists during the active episodes of both disorders.[7] The present study, in line with previous studies, indicates genetic variants responsible for the synthesis of IL-1 as a possible genetic risk factor in the pathogenesis of these two disorders.

The functional role of polymorphisms of IL-1β and IL-1RN genes in the pathogenesis of schizophrenia and bipolar disorder has not been defined well. Meisenzhal et al. have shown that carriers of allele 2 (genotypes T/T or C/T) in the (-511) locus had more gray matter defects in the bifronto-temporal area and decreased white matter of the brain.[32] In another study by Papiol et al. in 2008, the subgroup of bipolar patients carrying allele T of IL-1β (-511) had experienced longer episodes compared with the non-carrier subgroup. Further study showed that defects in total brain gray matter and also left dorso-lateral prefrontal cortex were significantly more frequent in carriers of allele T (-511) than in non-carriers.[5] Therefore, previous studies suggest that genetic individual differences in interleukin-1β (especially in –511 allele 2 carriers) may affect brain morphology in bipolar disorder and schizophrenia as two distinct but with shared psychopathological dimensions.[5,32] This is especially true for a subgroup of bipolar patients with similar morphologic aberrancies in schizophrenia. It should be noted that decreases in dorsolateral prefrontal cortex is related not only to having a diagnosis of BP-I disorder but is also an endophenotype for people with the genetic risk of BP-I.[33] Functional imaging has also proposed that decreased activity in the dorsolateral prefrontal cortex regions is a feature of both mania and depression in bipolar patients, consistent with findings that cognitive functions related to prefrontal activity, such as working memory and executive function, are altered in bipolar disorder patients.[34] These findings, together with the results of the present study, suggest this allele as the common genetic risk factor for schizophrenia and bipolar I disorder.

IL-1β has been suggested to play a role in the differentiation of dopaminergic neuronal progenitors leading to the development of dopaminergic neurons.[35] In line with the neuro-developmental hypothesis in the pathogenesis of major psychiatric disorders, imbalance in pro-inflammatory (such as IL-1β) and anti-inflammatory cytokines can affect neuronal development in fetal brain.[16] We can therefore assume that polymorphic loci mentioned in the present study can affect IL-1β expression and disrupt the equilibrium toward the dominance of pro-inflammatory cytokines, which can affect neuronal development in fetal brain and also neurodegeneration, considering the fact that IL-1 is a key mediator in ischemic brain damage, apparent in at least some bipolar patients.[5] However, to better understand the relationship between the IL-1 gene cluster variants with clinical manifestations and neuroimaging of BP-I, we need more studies on biochemical signaling pathways.

In the present study, no association was found between other loci such as IL-1α (-889 C/T), IL-1β (+3954 C/T), and IL-1RN tandem repeats and BP-I. To the best of our knowledge, no other studies have been conducted on the first two loci in bipolar disorder. But as for the IL-1RN VNTR, in Rafiei et al. study, IL-1RN *1/2 was more frequent in bipolar patients than in the control group, which is in contrast with the results of a former study by Papiol et al.[1] Considering the small sample size as a major limitation in our study, further studies employing larger or family-based samples are needed to confirm our results.

In the study by Papiol et al., compared to other combinations, haplotypic combination -511 allele*1/VNTR allele*2 was more frequent in schizophrenic and bipolar patients (especially the bipolar patients with a positive family history) than the control group.[16] Considering the evidence regarding the strong association between IL-1 cluster loci, future studies can focus on haplotypic frequency distribution in different loci.

In summary, the results of our study add to the evidence concerning the association between IL-1 gene cluster variations and BP-I. We have shown that the presence of allele 2 (T) in the IL-1β -511 locus can be in part a risk factor of genetic vulnerability to the disorder. However, the principal role and function of these genetic variations remain unknown and further studies are needed to shed light on the underlying mechanisms through which these cytokine alterations lead to pathogenesis of the disorder. Regarding the strong linkage disequilibrium between the different loci

of IL-1 cluster,[5] analysis of haplotypes would be a more appropriate way to investigate the involvement of this region in BP-I.

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