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Association between IFN-γ +874 T/A (Rs2430561) Polymorphisms and Bipolar 1 Disorder: A Study in an Ethnic Iranian Population

Mahdieh Fatemi Nayeri[1], Ali Talaei*[1], Jalil Tavakkol Afshari[2], Amin Reza Nikpoor[3,2], Andisheh Talaei[4], Rashin Ganjali[2]

Abstract

Background: The pathophysiology of bipolar 1 disorder (B1D), a major psychiatric disorder with inflammatory origins and structural changes in the brain, is of great interest to researchers. Pro-inflammatory biomarkers and specific gene expression play pivotal roles in B1D development, and IFN-γ has emerged as an important inflammatory marker. The aim of this research was to determine whether the INF-γ +874 T/A polymorphism is associated with B1D susceptibility in an ethnic Iranian population.

Methods: The IFN-γ +874 T/A (rs2430561) gene polymorphism was studied in 106 B1D patients and 109 control subjects using sequence specific primers (SSPs) and amplification refractory mutation system-polymerase chain reaction (ARMS-PCR).

Results: Significant statistical differences in IFN-γ +874 T/A polymorphism genotype distribution were found between the patients and control subjects (P = 0.0006). Decreased risk of B1D was detected in the codominant model (T/T vs T/A and A/A, OR = 0.19, 95% CI = 0.07-0.49 for T/A, OR = 0.38, 95% CI = 0.12-1.24 for A/A, P value=0.0006), and in the dominant model (T/T vs T/A-A/A, OR = 0.21, 95% CI = 0.08-0.54, P = 0.0005). However, no significant difference in the IFN-γ polymorphism allele distribution was found between the two groups (P = 0.25).

Conclusions: The IFN-γ +874 T/A polymorphism may have a significant role in BID development.

Keywords: Bipolar 1 Disorder, Gene Polymorphism, Interferon gamma.

Introduction

Bipolar 1 disorder (B1D) is considered as one of the most important psychiatric disorders, regarding morbidity, symptom severity, chronic and relapsing courses, and cognitive and social impairment. Moreover, 0.5% of total proportion of Disability Adjusted Life Years (DALYs) is related to B1D, and its lifetime prevalence is 2.4% (1). As a consequence, the pathophysiology and treatment of B1D are of great interest to researchers. Previous studies illustrated various biological aspects including immune dysregulation, inflammation, and genetic factors, which cause dysregulation in brain regions, as contributors to B1D pathophysiology (1-4). Family, twin, and adoption studies demonstrated the significant role of genetic factors, indicating the concordance rates for various mood disorders, particularly in monozygotic twins, of 70 to 90% (5, 6). B1D is a complex genetic disorder with variable expressivity and gene interaction and the specific gene(s) involved in B1D induction has (have) not yet been identified; however, an interferon gamma (IFN-γ) polymorphism susceptibility to B1D has

1: Psychiatry and Behavioral Sciences Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
2: Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
3: Department of Immunology, Faculty of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.
4: Department of Biotechnology, University College of Science, University of Tehran, Tehran, Iran.
***Corresponding author: Ali Talaei; Tel: +98 51 7112540, Fax: +98 51 7124184, E-mail: talaeia@mums.ac.ir.**
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recently been identified (7, 8).

Previous studies demonstrated that B1D patients produce more pro-inflammatory cytokines than normal individuals. These include tumor necrosis factor-α (TNF-α), interleukin-2 (IL-2), IL-4, IL-6, IL-10, soluble interleukin-2 receptor (sIL-2R), sIL-6, and IL-1 receptor antagonist (9-12). Studies of immunologic variables, especially T helper 1 and T cytotoxic IFN-γ-secreting lymphocytes, detected some immune modulation functions in B1D (4, 13, 14). IFN-γ, a member of the type 2 class of interferons, produced by natural killer (NK), CD4+ Th1, and CD8+ cytotoxic T cells, has recently been recognized as an important factor in B1D development. Among its many roles, IFN-γ activates major histocompatibility complex (MHC) class II molecules, increases antigen presentation and macrophage and NK cell activity, promotes leukocyte migration, and stimulates IgG2 and IgG3 production (14-18). In conclusion IFN-γ has immunoregulatory effects.

The IFN-γ-encoding gene is located on chromosome 12q14. A single nucleotide polymorphism (SNP) in the first intron of the human IFN-γ gene containing a nuclear factor-KB (NFKB) -binding region is associated with several autoimmune and inflammatory diseases (19-22). The T allele is seen in high, while the A allele is seen in low, plasma IFN-γ. Although prior studies illustrated the dysregulation of serum IFN-γ in B1D, few studies have addressed IFN-γ polymorphisms, and their conclusions have been contradictory (7, 8). Moreover, no studies have addressed a potential association between IFN-γ polymorphisms and B1D in in an Iranian population. The aim of this study was to investigate the potential association between the +874 T/A IFN-γ polymorphism and B1D in an ethnic Iranian population.

Materials and methods

Study population

This was a case-control study in ethnic Iranian patients. All the B1P patients were referred to Ibn-e-Sina psychiatric hospital in Mashhad, Iran's second-most populous city, located in northeast Iran. B1D was diagnosed by two psychiatrists by structured interviews using Structured Clinical Interview for DSM Disorders (SCID-I) according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) criteria. The SCID-I used to determine DSM-IV-TR disorders was translated into Persian and found to be cross-culturally equivalent (24). The control group was selected from unrelated volunteers in whom B1D was ruled out with a Mood Disorder Questionnaire (MDQ). Members of the control group had no family histories of mood disorders. The MDQ is a self-reported questionnaire designed to screen bipolar disorders. It was validated in the Iranian population by Ghoreishizadeh et al. in 2011 with a Cronbach's alpha coefficient of 0.773 (25). Individuals with significant histories of allergies, autoimmune diseases, immune deficiencies, endocrinopathies, or substance abuse were excluded from both groups.

One hundred and six randomly-selected ethnic Iranian euthyroid BID patients aged 18-70, and 109 Iranian healthy control subjects were enrolled in the study. The inclusion criteria for control group were: age 18-70, negative medical history of psychotic or mood disorders, thyroid dysfunction, or autoimmune disease, and negative family history of psychotic or mood disorders in first and second relatives. Written informed consent was obtained from all participants and the research protocol was approved by the Ethnic Committee of Mashhad University of Medical Sciences (MUMS), for Psychiatry and Behavioral Sciences Research Center (PBSRC) (code: IR.MUMS.REC.1395.609).

Genomic DNA extraction

Ten ml of peripheral venous blood were obtained from each subject. Six ml were aliquoted into an EDTA tube, the samples were centrifuged at 500 relative centrifugal force (RCF) for 15 minutes, and plasma collected and stored at -70 °C until analysis. The other 4 ml of blood was used to analyze thyroid function. Genomic DNA was extracted from the 6 ml samples by the salting-out method using a DNA extraction kit (BioGene, Mashhad, Iran). Sample purity was assessed by spectrophotometry (23).

Genotyping for the IFN-γ polymorphism

The IFN-γ +874 T/A polymorphism in the first intron (access number NT029419) was analyzed by polymerase chain reaction (PCR) with sequence-specific primers (SSPs) according to Jing

Ping et al. (21). These SSPs distinguish between the allelic variants. The internal control and primer sequences were verified by primer blast program, and product sizes were detected with gene runner software. The polymorphisms, product sizes, and primer sequences are shown in Table 1.

Table 1. IFN-γ polymorphisms, PCR product sizes, and primer sequences

Polymorphism	Product size (bp)	Primer sequence
IFN-γ +874 T/A (Reverse)	Consensus primer	5`-CAA CAA AGC TGA TAC TCC A-3`
+874 T (Forward 1)	262	5`-TTC TTA CAA CAC AAA ATC AAA TCA-3`
+874 A (Forward 2)	262	5`-TTC TTA CAA CAC AAA ATC AAA TCT-3`
Internal control (forward)	612	5`-ACT TCG TTG CTC ACT GGG ATT TT-3`
(reverse)	612	5`-TTT CCT TTT CAA CTC TTC TGC TT-3`

The amplification refractory mutation system–polymerase chain reaction (ARMS-PCR)

The ARMS-PCR genotyping of the +874 T/A polymorphism was performed as follows: the 20 µL PCR reaction mixture contained 0.2 µM of the five primers shown in Table 1, 25 mM KCl, 1.2 µL of MgCl2, 200 µM dNTPs, 0.5 U of Taq polymerase, and 200 ng of genomic DNA. Thermal condition was as follows: 3 min at 94 °C followed by 10 cycles of 60 sec at 94 °C, 60 sec at 62 °C, 60 sec at 72 °C, and 25 cycles of 60 sec at 94 °C, 60 sec at 58 °C, and 60 sec at 72 °C, with a final extension for 5 min at 72 °C.

The amplified products were electrophoresed on a 2% agarose gel containing ethidium bromide. The presence of a control band with an allele-specific band of the expected size in a lane was considered as evidence for the allele, whereas the presence of a control band without an allele-specific band indicated the absence of that allele.

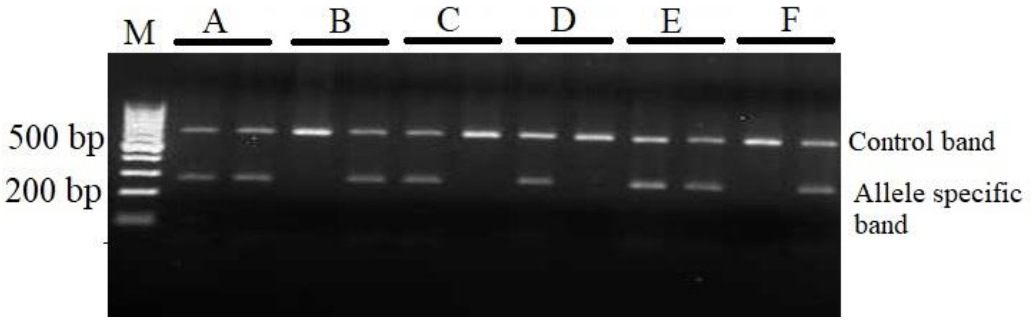

Fig. 1. Agarose gel electrophoresis of ARMS-PCR products. Genotyping for the IFN-γ +874 T/A polymorphism. Lane M: DNA marker; lanes A and E: +874 A/T genotype; lanes B and F: +874 T/T genotype; lanes C and D: +874 A/A genotype.

Statistical analysis

The results were analyzed by descriptive statistics, logistic regression test, independent sample t-test, one-way ANOVA, Pearson's Chi-squared test, and Fisher's exact test using SPSS software version 17. P values < 0.05 were considered statistically significant.

Results

The mean patient age was 38 ±10.6 years and the mean control age was 30.06 ± 8.7 years. In the control and patients' groups 39 (35.8%) and 34 (32.1%) subjects were male, while 70 (64.2%) and 72 (67.9%) were female, respectively (P = 0.33) (Table 2).

Table 2. Characteristics of normal controls and bipolar 1 disorder patients

	Normal controls (n= 109)	Bipolar patients (n=106)
Sex (male/female)	39/70	34/72
Age (years)	30.06±8.7	36.38±10.6
Family history of mood disorder (positive)		70 (66.1%)
History of suicide (positive)		74 (69.8%)
History of psychotic feature (positive)		88 (83%)

The IFN-γ polymorphism genotypes and their frequencies were the following: TA in 92 patients (84.4%), AA in 11 patients (10.1%), and TT in six patients (5.5%) (Fig. 1). Regarding codominant inheritance model analysis, a statistically significant difference in the genotype distribution of the +874 T/A polymorphism was seen between the patients and control subjects (p = 0.0006). The T/A and A/A genotype patients had less B1D risk than T/T patients compared with control subjects in a codominant model (T/T vs T/A and A/A, OR = 0.19, 95% CI = 0.07-0.49 for T/A, OR = 0.38, 95% CI = 0.12-1.24 for A/A, P = 0.0006). Regarding dominant inheritance model analysis, the T/A-A/A genotype patients were at a lower risk than T/T patients for B1D compared with controls (T/T vs T/A-A/A, OR = 0.21, 95% CI = 0.08-0.54, P = 0.0005). Furthermore, the overdominant inheritance model analysis indicated that T/A genotype patients were at lower risk than T/T-A/A patients for B1D compared with controls (T/T-A/A vs T/A, OR = 0.32, 95% CI = 0.17-0.61, P = 0.0004). In conclusion the A allele is a protective factor. Additionally, the T allele frequency was greater in B1D patients and the A allele frequency was greater in control subjects, however, no significant difference in allele distribution was found between the two groups (P = 0.25) (Table 3).

No significant association was found between family histories for mood disorders, suicides, or psychotic features and the IFN-γ +874 T/A polymorphism (Table 4).

Table 3. Genotype and allele frequencies of IFN +874T/A (rs2430561) gene polymorphism in controls and bipolar 1 disorder patients

IFN +874T/A (rs2430561)		Control N=109 (%)	Patients N=106 (%)	P value	Odds ratio (95% CI)
Codominant					
	T/T	23 (21.7%)	6 (5.5%)		1.0 (reference)
	T/A	67 (63.2%)	92 (84.4%)	0.0006	0.19 (0.07-0.49)
	A/A	16 (15.1%)	11 (10.1%)		0.38 (0.12-1.24)
Dominant					
	T/T	23 (21.7%)	6 (5.5%)	0.0005	1.0 (reference)
	T/A-A/A	83 (78.3%)	103 (94.5%)		0.21 (0.08-0.54)
Recessive					
	T/T-T/A	90 (84.9%)	98 (89.9%)	0.27	1.0 (reference)
	A/A	16 (15.1%)	11 (10.1%)		1.58 (0.70-3.59)
Overdominant					
	T/T-A/A	39 (36.8%)	17 (15.6%)	0.0004	1.0 (reference)
	T/A	67 (63.2%)	92 (84.4%)		0.32 (0.17-0.61)
Alleles					
	T	104 (48%)	113(53%)	0.25	1.0 (reference)
	A	114(52%)	99(47%)		0.79 (0.54 to 1.16)

Table 4. Association of clinical histories and B1P patient IFN +874T/A (rs2430561) gene polymorphisms

Clinical histories	Total	IFN +874T/A (rs2430561)			P value
		TT	TA	AA	
Negative family history	36 (33.9%)	6 (16.7%)	25 (69.4%)	5 (13.9%)	0.59
Positive family history	70 (66.1%)	17 (24.3%)	42 (60%)	11 (15.7%)	
Negative patient's history of suicide	32 (30.2)	6 (18.8%)	20 (62.5%)	6 (18.8%)	0.74
Positive patient's history of suicide	74 (69.8%)	17 (23%)	47 (63.5%)	10 (13.5%)	
Negative patient's history of psychosis	18(17%)	2 (11.1%)	13 (72.2%)	3 (16.7%)	0.48
Positive patient's history of psychosis	88 (83%)	21 (23.9%)	54 (61.4%)	13 (14.8%)	

Discussion

Due to the cognitive impairment and social and financial consequences caused by B1D, its pathophysiology and treatment are of great interest to researchers. Brain structure changes

C and specific gene expression. The aim of this research was to identify a potential association between the INF-γ +874 T/A polymorphism and B1D susceptibility in an ethnic Iranian population. We found that inheritance of the IFN-γ +874 T/A polymorphism A allele is protective in B1D pathogenesis.

Although previous studies have shown IFN-γ dysregulation in BD patients (4, 14), to our knowledge this is the first study of to examine a possible link between the IFN-γ +874 T/A polymorphism and B1D susceptibility in an Iranian population.

Several other proinflammatory markers have also been associated with B1D; these include C-reactive protein (CRP), interleukin (IL) -2 receptor, IL-6, and tumor necrosis factor-alpha (TNF-α) (1-4, 24-26).

Although IFN-γ levels have been shown to differ between B1D patients and healthy controls (5, 15), it is not yet known whether inflammatory dysregulation leads to B1D, or vice versa. Our goal was to investigate the IFN-γ +874 T/A polymorphism, which contributes to both inflammation and B1D. In this situation B1D might promote the inflammatory process. IFN-γ, which is secreted by innate and adaptive immune cells, is a T helper 1 immunomodulatory cytokine and a member of the type 2 interferon class. IFN-γ activates and induces MHC expression in microglial cells and macrophages, release of TNF-α and IL-1 from macrophages, and stimulates leukocyte adhesion (27). In this regard, Zheng et al in their study reported that IFN-γ had a significant role in the development of Guillain-Barre syndrome (GBS); however, IFN-γ also has a regulatory T cell function that can be used to treat GBS (28). Therefore, IFN-γ seems to be not only an etiological factor for neural cell damage, but also a protective marker that modulates the inflammatory process.

Structural changes in the brain including enlargement of the third and lateral ventricles (29, 30) and amygdala (31), and changes in the frontal lobe, cerebellum, hippocampus, and pituitary (32, 33) have all been reported. These findings suggest that the genetic alterations in B1D affect brain function and morphology. Our finding of the protective effect of the A allele on B1D is consistent with previous research in an ethnic Chinese population, which indicated that the IFN-γ T allele was associated with an increased risk of developing B1D (7). In contrast, a study of B2D patients found a lower percentage of TT genotype and T allele in patients than in controls (8). Genetic analyses of IFN-γ in other disorders were consistent with our results. Wu et al. examined leukemia and the IFN-γ +874 T/A polymorphism and reported decreased chronic lymphocytic leukemia (CLL) risk in the allelic, codominant, and dominant models, and increased chronic myelogenous leukemia (CML) risk in the dominant model (34).

A meta-analysis in 2014 suggested that the IFN-γ +874 T/A polymorphism was associated with hepatitis B infection (35), while a study of chronic hepatitis patients reported that those with the IFN-γ +874 T/A polymorphism T allele had higher cirrhosis rates than healthy controls (36). Another study in a Taiwanese population showed a role for IFN-γ +874 T/A polymorphism in susceptibility to tuberculosis (37). A case-control study of IFN-γ +874 T/A polymorphisms reported that IFN-γ SNPs and their haplotypes are associated with pneumonia-induced sepsis, however sepsis in these patients was unlikely to progress to severe form (38).

The T allele is reported to be a protective factor in breast and cervical cancers and leprosy (39-42). Furthermore, the A allele was associated with acute respiratory syndrome, especially the severe type of the illness (43). It is evident that the T allele, which is associated with high IFN-γ production, is related to inflammatory disorders including bipolar disorders. Additionally, it regulates the immune response and sometimes decreases illness severity and helps to control the inflammatory process.

In this study the clinical manifestations of B1D, including history of suicide and psychotic events, and also the family histories for mood disorders, were analyzed. No significant differences in these factors were found between the three genotypes. In conclusion, the IFN-γ +874 T/A polymorphism may affect B1D development; moreover, the A allele may be protective and the TT genotype frequency seems to be greater in BID patients than in controls.

More studies with larger sample sizes, various ethnic populations, and more clinical

manifestation parameters are recommended. As our understanding of B1D pathophysiology increases, treatment and management of the disorder will improve. We also believe future studies should focus on IFN-γ haplotypic frequency distribution in different loci.

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