TNF-α-308 G/A variant may be associated with bipolar disorder in a Turkish population

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Abstract

Background: Tumor necrosis factor alpha (TNF-α) is a proinflammatory multifunctional cytokine produced by macrophages. A dysregulation of the immune system contribute to the pathogenesis of bipolar disorder (BD). In this study, we aimed to investigate the relationship between the TNF-α gene -308G/A promoter variant and the risk of BD. Methods: A total of 104 BD patients and 94 healthy controls were enrolled in the study. Genomic DNA was isolated and TNF-α -308G/A variant was analyzed using PCR-RFLP method.

Results: TNF-α -308G/A variant GG genotype and G allele were more prevalent in BD patients compared to the controls (p = 0.002 and p = 0.017, respectively). The patients carrying GG genotype had a 5.927-fold higher risk of developing BD. Then, we divided patients into two groups as smokers and non-smokers. TNF-α -308G/A variant GA genotype was higher in non-smoker BD patients than smoker patients (p = 0.027). We found that TNF-α -308G/A AA genotype and A allele increased in smoker patients compared to non-smoker patients (p = 0.008, p = 0.002, respectively). Discussion: Our results provided evidence that TNF-α -308G/A variant may contribute to development of BD in a Turkish cohort. In addition, this variant plays a relevant role in the smoker status of BD.


Key words: Bipolar disorder, tumor necrosis factor alpha, variant, PCR-RFLP.

Introduction

Bipolar disorder (BD) is among the major psychiatric disorders, in terms of morbidity, symptom severity, a chronic and relapsing course, along with cognitive and social impairment. The lifetime prevalence is approximately 2.4%. Previous studies suggested several biological factors such as immune dysregulation, inflammation, and genetic background, which cause dysregulation in brain regions, as contributors to BD pathophysiology. Evidence obtained from manual studies has indicated increased circulating levels of proinflammatory cytokines, suggesting that immune-mediated mechanisms could be linked with the neurobiology of BD and its neuroprogression. Family, twin, and adoption studies showed that genetic factors play a significant role, implying the concordance rates for several mood disorders, especially in monozygotic twins, of 70 to 90%.

Tumor necrosis factor-alpha (TNF-α) is a pleiotropic inflammatory cytokine that is involved in numerous inflammatory conditions, such as growth inhibition and promotion, inflammation, angiogenesis, cytotoxicity, and immunomodulation. The TNF-α gene is found within the class III region of the major histocompatibility complex (MHC) on the small arm of chromosome 6 (6p21.1-21.3). Various polymorphisms have been described in the TNF-α gene promoter region. Among these, the polymorphism located at nucleotide position -308 (rs1800629) has been shown to have a direct impact on TNF-α expression. This is a well-defined biallelic base exchange polymorphism, that contains a common variant with a guanine (G) at position -308 (TNF-α), and an uncommon variant with an adenine (A) at -308 TNF-α. The TNF-α-308 A allele has been strongly related with higher TNF-α generation and in some cases with high morbidity and mortality in numerous infectious (sepsis, malaria, leishmaniasis), autoimmune (type 1 autoimmune hepatitis, SLE) and other immune-mediated disorders (asthma, contact dermatitis).

It has been suggested that an impairment of the immune system due to chronically activated macrophages and T cells could be involved in the pathogenesis of BD. Therefore, we conducted a study in a Turkish population, to investigate the relationship between the TNF-α-308G/A promoter variant and the risk of BD.
Methods

The subjects in this study included 198 Turkish individuals, consisting of 104 patients with BD and 94 healthy blood donors (controls) of similar age, ethnicity and gender. BD patients were gathered from Department of Psychiatry, Bakirkoy Research and Training Hospital for Psychiatry, Neurology and Neurosurgery, Istanbul, Turkey and the diagnosis of BD was based on accepted clinical criteria. Control subjects had a negative family or past history of any psychiatric disorders and had no family relationship to the present study patients. All participants, patients and healthy controls, were of Turkish origin. Patients who smoked were active smokers. These subjects were defined as those who had previously smoked more than one cigarette per day but had quit smoking for more than one year. The non-smoker patients were defined as those who had smoked less than one cigarette per day for no more than 1 year during their lifetime. The study protocol was approved by the Local Ethics Committee of Istanbul University, Faculty of Medicine, and written informed consent was obtained from the study participants.

Genotyping

Genomic DNA was extracted from whole venous blood samples using salting out method. The TNF-α -308G/A genotyping was performed by the polymerase chain reaction sequence-specific primer method (PCR-SSP), using the Cytokine Genotyping Tray kit according to the manufacturer’s instructions. TNF-α -308G/A were analyzed previously described method by Karaoglan et al. Statistical analysis

All data were analyzed using SPSS software version 18.0 for Windows (SPSS Inc., Chicago, IL, USA). The statistical significance of the differences between the patient and the control groups was estimated by Pearson’s χ² analysis. Odds ratio (OR) and 95% confidence interval (CI) were also calculated. All analyses were two-tailed, and differences were interpreted as statistically significant when \( p < 0.05 \).

Results

One hundred and four patients and 94 healthy subjects participated in the study, as the patients and the control groups, respectively. The demographic characteristics of the patients are summarized in Table 1.

Allelic and genotypic distributions of the TNF-α -308G/A variant in patients and controls were shown in Table 2. The genotype and allele frequencies of this variant showed statistically significant difference between the BD patients and the controls. GG genotype (16.2% versus 3.2%, respectively) and G allele (19.2% versus 10.6%, respectively) frequencies of patient group were significantly higher than the control group (\( p = 0.002 \), OR: 5.927, 95% CI: 1.677-20.946; \( p = 0.017 \), OR: 0.500, 95% CI: 0.280-0.891, respectively).

Then, we subdivided patients as smokers and non-smokers. The genotype distribution and allele frequencies of TNF-α -308G/A variant in smoker and non-smoker patients were shown in Table 3. TNF-α -308G/A GA genotype increased non-smoker BD patients compared to smoker BD patients while AA genotype increased smoker BD patients than non-smoker patients (\( p = 0.008 \), OR: 2.517, 95% CI: 0.814-7.775 and \( p = 0.027 \), OR: 0.296, 95% CI: 0.135-0.642, respectively).

Discussion

This study examined the effect of -308G/A variant of TNF-α gene susceptibility BD. In the last decade, the significance of cytokines in neuronal survival was documented, along with the well organized action of neurotransmitters, hormones, and neurotrophins. Cytokines are the essential signaling molecules in inflammation, exerting a regulatory effect in both the innate and the adaptive immunological response. They are generated by immune cells as well as non-immune cells and exert their effects beyond the immune system. In addition to this conventional role, they can directly influence neuronal activity, triggering neuronal excitability and plastic changes. Furthermore, cytokines can affect the hypothalamic-pituitary-axis (HPA) via actions on the HPA feedback regulation and on the glucocorticoid receptor function. Cytokines activate the HPA axis, enhancing the levels of corticotrophin releasing hormone (CRH), adrenocorticotrophic hormone (ACTH), and cortisol and reducing the expression, translocation, and downstream effects of glucocorticoid receptors. Multiple lines of studies indicate that BD is a systemic disease. A
meta-analysis of 30 studies that included 1351 individuals with BD and 1248 controls found that plasma/serum levels were increased for Interleukin-6 (IL-6), TNF-α, soluble IL-2 receptor, IL-4, IL-10, IL-1 receptor antagonist, and soluble TNF receptor-1. Kunz et al. studied three cytokines (IL-6, IL-10 and TNF-α) in a chronic sample and found higher IL-10 in schizophrenia, but not in BD, whereas both groups had elevated IL-10 compared to the controls, and there were no significant differences between the groups for TNF-α. Another meta-analysis of 18 schizophrenia, 16 BD, and 12 major depressive disorder studies demonstrated significant increase of IL-6, TNF-α, IL-1 receptor antagonist, and soluble IL-2 receptor in acute episodes of all groups compared to controls. Besides, in chronic patients, IL-6, soluble IL-2 receptor, and IL-1β were similarly increased in schizophrenia and BP patients compared to controls.

TNF-α is a cytokine that is a member of the TNF superfamily of 19 different protein ligands. These cytokines mediate their cellular responses through 29 receptors of TNF-receptor (TNFR) super family. It was reported that TNF-α expression was upregulated in the brain of patients with neurodegenerative diseases such as Alzheimer’s and Parkinson’s disease suggesting a causative role of TNF-α in neurodegenerative disorders. Inhibition of peripheral TNF-α action particularly hinders structural changes at the synaptic level. The peripherally synthesized cytokines are also capable of acting on the brain via deficient blood-brain-barrier, active transport via saturable transport molecules, activating endothelial cells to produce second messengers, and binding to receptors onafferent nerve fibers. Recently, it was found that TNF-α was increased level significance in BD patients compared to healthy controls, and was negatively associated with global cognition, processing speed, and working memory in these patients. In a preclinical mouse model, Yang et al. have shown that peripheral TNF-α can influence the brain structure via dendritic elimination independent of central inflammatory activity.

TNF-α -308G/A promoter variant is among the best described single-nucleotide polymorphisms at the nucleotide position -308, which affects a consensus sequence for a binding site of the transcription factor activator protein 2. The functional polymorphisms in cytokine genes may lead to imbalances in the pro- and anti-inflammatory cytokine generation. Several studies have reported genetic polymorphism in mood disorders. Kadosh et al. reported that TNF-α -308G/A and TNF-β +252G/A variants may increase the susceptibility to schizophrenia in Saudi patients. Zhang et al. found that TNF-α gene -1051C/T variant is associated with onset age but not with risk of schizophrenia in a Chinese population. In a meta-analysis, no significant association between TNF-α -308G/A and depression was found. Cerri et al. found a significantly higher percentage of the TNF-α -308G/A GG genotype and G allele in depressed subjects. Czerski et al. observed an association of the -308G allele with both schizophrenia (p = 0.008) and BD (p = 0.039), and also with a positive family history in patients with schizophrenia (p = 0.048) and BD (p = 0.027). Pae et al. conducted a association study between TNF-α -308G/A variant and BD in Korean population. They found significant difference in genotype distributions and allele frequencies. Clerici et al. showed bipolar II patients were characterized by an absence of adenine (A) high producer allele of TNF-α.

In the present study, we investigated for the first time the association between the human TNF-α -308G/A functional variant and BD in the Turkish population. We found a significantly higher percentage of the GG genotype in BD patients compared to healthy controls (p = 0.002) (Table 2). Moreover, this genotype significantly raised the risk of developing BD (OR: 5.927). Also, G allele confers a significant risk for developing BD in Turkish patients (p = 0.017). Then we subdivided the patients as smokers and non-smokers. In smokers group, TNF-α -308G/A AA genotype and A allele increased than non-smokers group (p = 0.008 and p = 0.002) (Table 3). TNF-α -308G/AGA genotype was higher in non-smokers group (p = 0.027). In a study analyzing TNF-α blood levels in smoker and non-smoker subjects, it was observed that TNF-α serum levels were significantly higher for the group of smokers compared to the group of non-smokers. We found that the A allele providing higher TNF-α production was higher in the smoker group, suggesting that smoking status had an effect on TNF blood level.

This present study has some limitations. First, the relatively small sample size may influence the applicability of these results. Second, only one variant of the TNF-α gene was analyzed in the present study, which does not encompass all of the genetic variations located on the TNF-α gene. Besides, TNF-α expression was not measured and this is another limitation.

Conclusion
To summarize, in our study, we showed an association of the TNF-α-308 G/A variant with BD in a Turkish population. Considering the polygenic effect on BD, further case-control studies with large number of subjects and with the information on other related genetic polymorphisms could provide reliable evidence for the role of TNF-α -308G/A variant in terms of susceptibility to BD.

Disclosure
The authors declare that they have no conflict of interest.

References