Investigation of inflammation related gene polymorphism of the mannose-binding lectin 2 in schizophrenia and bipolar disorder

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ABSTRACT

(MBL2) gene polymorphism distributions in SCZ or BD patients were significantly different from the control group. The heterozygous genotype percentages were significantly higher in the control group than in the SCZ or BD patients (OR: 0.450; 95% CI: 0.243-0.830; p=0.010; OR: 0.532; 95%CI: 0.284-0.995; p=0.047, respectively), and there were statistically significant differences in the MBL2 polymorphism distributions between treatment-responsive SCZ or BD patients and treatment-resistant patients diagnosed with SCZ or BD. The heterozygous genotype percentages were also significantly higher in the treatment-responsive group than in the treatment-resistant group in SCZ or BD patients (OR: 7.857; 95% CI: 1.006-61.363; p=0.023; OR: 8.782; 95% CI: 1.114-69.197; p=0.016, respectively).

Conclusion: The presence of a heterozygous MBL2 genotype seems to be favorable both in terms of the absence of SCZ and BD in the healthy population and treatment response for Turkish patients.

Methods: One hundred and eighteen patients with SCZ, 100 patients with BD, and 100 healthy volunteers were included in the case-control study. The patients consecutively admitted to the outpatient clinic in December 2017-May 2018 and were evaluated with some scales for clinical parameters. Polymerase chain reaction and RFLP were used to determine MBL2 polymorphism in DNA material.

Objectives: To investigate the association between mannose-binding lectin 2 (MBL2) codon 54 polymorphism and clinical features of patients diagnosed with schizophrenia (SCZ) or bipolar disorder (BD).

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Schizophrenia (SCZ) and bipolar disorder (BD) are chronic psychiatric disorders that cause substantial disruptions in psychosocial capacity and occur in approximately 1% of the world population.\textsuperscript{1} Genome-wide association studies (GWAS) of Psychiatric Genomics Consortium for SCZ recognized more than a hundred common single nucleotide polymorphisms (SNPs) with minor individual effects presenting susceptibility to the SCZ.\textsuperscript{2} A similar mega-analysis for BD, although including a more moderate sample, identified common risk variants specific to BD.\textsuperscript{3} The long-lasting alterations in gene expression patterns after environmental exposures imply that epigenetic mechanisms might also play a critical role in chronic psychiatric disorders.\textsuperscript{9} Again, previous researches have constantly reported shared genetic etiology between SCZ and BD. Researches showing the genetic overlap between SCZ and BD have improved from studying family and twin inheritance to determine genetic correlation and performing polygenic risk score analysis from GWAS data in large case-control samples.\textsuperscript{5} Despite these researches with large sample size, the cause of both disorders is still relatively unknown; recent studies have shown that uncontrolled activity of microglia and excessive inflammatory responses caused by pro-inflammatory cytokines are among the factors that play a role in the development of SCZ and BD based on genetic susceptibility.\textsuperscript{1,6} A relationship between SCZ and many autoimmune diseases and an increase in the prevalence of an autoimmune disease occurrence by about 45% have been found. Moreover, infections of embryonic and early childhood periods lead to delays in fetal brain development and excessive synaptic pruning during adolescence, are among the possible risk factors of psychosis.\textsuperscript{7,8} Systemic inflammation and central inflammation are thought to be associated with episodes, remission, and prognosis of BD. Neuroendocrine irregularities, neurotransmitter abnormalities, and glial cell dysfunctions cause plastic alterations in the mood-regulating brain areas. The high rate of comorbid autoimmune diseases also supports this claim.\textsuperscript{9} 

Mannose-binding lectin (MBL) has a vital function in the innate immune system by stimulating the complement system's lectin pathway. Therefore, it is the only collectin that binds to microorganisms, serves as an opsonin, promotes phagocytosis, and stimulates macrophages. MBL2 gene, which consists of 4 exons in the q11.2-q21 region of the long arm of chromosome 10, encoded MBL. Mutation at codon 54 follows in a replacement of glycine to aspartic acid (allele B), and the normal MBL2 allele is defined by allele A.\textsuperscript{10,12} In heterozygous mutants, serum MBL decreases almost 10-fold, whereas, in homozygous mutants, the level decreases to an undetectable level. MBL deficiency is the most common immune defect in humans, affecting approximately 5-7% of individuals.\textsuperscript{13} A decrease in serum MBL level can cause recurrent spontaneous miscarriage,\textsuperscript{14} premature birth,\textsuperscript{15} and exacerbation of chronic diseases such as ischemic heart disease,\textsuperscript{16} and severe infections such as sepsis and systemic inflammatory response syndrome (SIRS).\textsuperscript{17} Besides, previous studies suggested that MBL plays an essential role in the pathogenesis of autoimmune diseases.\textsuperscript{12,18} We believe that this is the first case-control study comparing MBL2 genotype distributions in patients with SCZ or BD according to treatment resistance, clinical characteristics, and scale scores in detail.

\textbf{Aims of the study.} We aimed to examine whether MBL2 codon 54 polymorphism was involved in the etiopathogenesis and treatment response of SCZ and BD compared with healthy controls.

\textbf{Methods. Patient selection.} The patients diagnosed with SCZ (n=118) or BD (n=100) were consecutively admitted to the outpatient clinic of the Bilkirkoy Mazhar Osman Mental Health and Neurology Training and Research Hospital in December 2017-May 2018 for 6 months; additionally, 100 gender-, age- and ethnicity-matched healthy participants were included in our case-control study. This study was according to the ethical standards on human experimentation confirmed by the Helsinki Declaration and approved by the Clinical Research Ethics Committee of the Istanbul Faculty of Medicine (21/21.12.2017).\textsuperscript{19}

\textbf{Diagnoses and symptom measurement.} We instructed the participants concerning the study’s aim, materials, and methods and acquired their written informed consent. In addition, we applied the researchers’ detailed interview data form about clinical information. Then, the Structured Clinical Interview for DSM-IV Axis-I Disorders (SCID-I) was administered to verify the patients’ diagnosis.\textsuperscript{20,21}

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Symptoms Scale (PANSS)\textsuperscript{22,23} was applied for patients with SCZ, while the Hamilton Depression Rating Scale (HAM-D)\textsuperscript{24,25} and the Young Mania Rating Scale\textsuperscript{26,27} were for evaluating BD patients concerning the severity of symptoms. We assessed the impairment of insight in SCZ with the Schedule for Assessing the Three Components of Insight (SATCI).\textsuperscript{28,29} Finally, we investigated the severity and treatment response of the psychiatric disorder with the Clinical Global Impression Scale (CGI).\textsuperscript{30}
Criteria for treatment-resistant in schizophrenia and bipolar disorder. Conley and Kelly’s criteria were applied to define the resistance of treatment in SCZ patients. It was described as a lack of response to treatment and the permanence of moderate to severe symptoms despite 2 separate antipsychotics in the proper dose (400-600 mg/day chlorpromazine) and duration (4-6 weeks). For treatment resistance in BD, Sachs suggested that mood episodes must be handled separately and defined the resistance as being no improvement in the mania for 6 weeks despite using at least 2 antimanic drugs with adequate dose and duration of treatment (provided that no drug caused an increase in the mood). Inclusion and exclusion criteria: Participants of either gender, literate, of 18 to 65 years of age, agreed to be involved in the study, diagnosed with SCZ or BD according to the SCID-I interview, had no other systemic/neurological disease that may affect cognitive functions (dementia, epilepsy, Parkinson disease, head trauma accompanied by loss of consciousness) included in the study. In addition, we had excluded subjects who refused participation or had mental retardation, neurodevelopmental disorders, a diagnosis of axis-1 disorder other than SCZ or BD due to SCID-I interview, SCZ, or BD secondary to a general medical condition.

### Table 4 - Comparison of MBL2 genotype distributions of schizophrenia or bipolar disorder patients with the same gender control group.

| Genotype | Schizophrenia | Control | OR (95% CI) | P-value |
|----------|---------------|---------|-------------|---------|
| **Male** |               |         |             |         |
| AA       | 68 (78.2)     | 31 (67.4) | 1.732* (0.779-3.851*) | 0.176* |
| AB       | 17 (19.5)     | 15 (32.6) | 0.450* (0.243-0.830*) | 0.094* |
| BB       | 2 (2.3)       | 0 (0)    | 1.541* (1.359-1.748*) | 0.544* |
| **Female** |             |         |             |         |
| AA       | 19 (61.3)     | 33 (61.1) | 1.008* (0.407-2.494*) | 0.987* |
| AB       | 6 (19.4)      | 20 (37)  | 0.408* (0.143-1.164*) | 0.089* |
| BB       | 6 (19.4)      | 1 (1.9)  | 12.720* (1.453-111.378*) | **0.009** |
| **Allele** |          |         |             |         |
| A        | 44 (71)       | 86 (79.6) | 0.625* (0.304-1.286*) | 0.200* |
| B        | 18 (29)       | 22 (20.4) |             |         |
| **Bipolar Dis.** | |         |             |         |
| **Male** |               |         |             |         |
| AA       | 31 (73.8)     | 31 (67.4) | 1.364* (0.541-3.434*) | 0.510* |
| AB       | 8 (19)        | 15 (32.6) | 0.486* (0.181-1.304*) | 0.148* |
| BB       | 3 (7.1)       | 0 (0)    | 2.179* (1.730-2.746*) | 0.105* |
| **Female** |             |         |             |         |
| AA       | 42 (72.4)     | 33 (61.1) | 1.670* (0.755-3.696*) | 0.204* |
| AB       | 14 (24.1)     | 20 (37)  | 0.541* (0.239-1.224*) | 0.138* |
| BB       | 2 (3.4)       | 1 (1.9)  | 1.893* (0.167-21.494*) | 1.000* |
| **Allele** |          |         |             |         |
| A        | 98 (84.5)     | 86 (79.6) | 0.754* (0.701-2.768*) | 0.343* |
| B        | 18 (15.5)     | 22 (20.4) |             |         |

*Criteria for treatment-resistant schizophrenia and bipolar disorder.*
**DNA isolation and genotyping analyses.** We used the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) method to analyze the *MBL2* codon 54 polymorphism. Therefore a three-step pathway was used to detect gene polymorphisms as genomic DNA isolation, amplification of target gene region by PCR, restriction enzyme digestion, and application of agarose gel electrophoresis method for visualization of the obtained product. We collected 2 cc blood samples in ethylene diamine tetraacetic acid (EDTA) tubes to obtain DNA from participants. After the genomic DNA was isolated with the Plus Blood Genomic DNA Purification Kit (GeneMark), reproduction of the desired region of DNA was carried out by PCR using artificially prepared oligonucleotide primers. For all these processes, the GeneAmp PCR System 9700 brand PCR device was used. At the end of the procedure, 10 μl of the PCR product was removed, and electrophoresis was carried out using 2% agarose gel. DNA strands were stained with ethidium bromide and observed in ultraviolet light. The DNA allele cut by restriction enzyme was noted as A allele (Normal: 260+89 bp), and the non-cut DNA was noted as B allele (Mutant: 349 bp) (BanI restriction endonuclease enzyme from New England Biolabs). If the 349 bp PCR product from the *MBL2* gene was cut into 2 different products of 260 bp and 89 bp, then the genotype was identified as AA; if three specific products were formed as 349 bp, 260 bp, and 89 bp, then the genotype was identified as AB; and if the product was 349 bp, then the genotype was identified as BB.

**Statistical analyses.** The statistical analysis was performed using IBM SPSS version 21.0 (IBM Corp. released 2012; Armonk, NY, USA). Quantitative data (clinical characteristics and scale scores) represented as descriptive statistics included median, minimum, maximum values, and the associations between MBL genotype or alleles frequency distributions and clinical variables (gender, treatment response, and clinical specifiers) were analyzed by Fisher’s exact test or Pearson chi-square.

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**Table 5 -** Comparison of *MBL2* genotype distributions of patients due to the resistance to treatment and clinical specifiers.

| Treatment Resist. | No | Yes | OR     | 95% CI       | P-value |
|-------------------|----|-----|--------|--------------|---------|
| **Schizophrenia** |    |     |        |              |         |
| *MBL2*            | n= (%) | n= (%) |        |              |         |
| AA                | 66 (71.7) | 21 (80.8) | 0.604* | 0.206-1.772* | 0.356*  |
| AB                | 22 (23.9) | 1 (3.8)  | 7.857* | 1.006-61.363*| 0.023*  |
| BB                | 4 (4.4)  | 4 (14.8) | 0.284* | 0.066-1.218* | 0.093*  |
| **Allele**        |    |     |        |              |         |
| A                 | 154 (84.1) | 43 (81.5) | 1.074* | 0.474-2.435* | 0.863*  |
| B                 | 30 (15.9) | 9 (18.5)  | 1.074* | 0.474-2.435* | 0.863*  |
| **Bipolar Dis.**  |    |     |        |              |         |
| *MBL2*            | n= (%) | n= (%) |        |              |         |
| AA                | 51 (67.1) | 22 (91.7) | 0.185* | 0.040-0.852* | 0.018*  |
| AB                | 21 (27.6) | 1 (4.2)  | 8.782* | 1.114-69.197*| 0.016*  |
| BB                | 4 (5.3)  | 4 (14.2) | 0.284* | 0.066-1.218* | 0.093*  |
| **Allele**        |    |     |        |              |         |
| A                 | 123 (80.9) | 45 (93.8) | 0.283* | 0.082-0.974* | 0.035*  |
| B                 | 29 (19.1) | 3 (6.3)  | 0.283* | 0.082-0.974* | 0.035*  |
| **Atypical Depres./ Bipolar Dis.** |    |     |        |              |         |
| *MBL2*            | n= (%) | n= (%) |        |              |         |
| AA                | 55 (68.8) | 18 (90)  | 0.244* | 0.053-1.135* | 0.056*  |
| AB                | 20 (25)  | 2 (10)   | 3.000* | 0.639-14.079*| 0.028*  |
| BB                | 5 (6.3)  | 0 (0)    | 1.267* | 1.142-1.405* | 0.580*  |
| **Allele**        |    |     |        |              |         |
| A                 | 130 (81.2) | 38 (95)  | 0.228* | 0.052-0.998* | 0.034*  |
| B                 | 30 (18.8) | 2 (5)    | 0.228* | 0.052-0.998* | 0.034*  |

* n= 92, *n= 76, *n= 80, Resist-Resistance, OR- odds ratio, CI - confidence interval, Depres - depression, *Pearson chi-square, *Fisher's exact test, Dis - disorder
chi-square test. The allele and genotype frequencies determined according to Hardy-Weinberg equilibrium (HWE). The Shapiro-Wilk test of normality was carried out on continuous variables to verify the fit of the data to a normal distribution. Comparison of scale scores and clinical parameters according to the MBL2 genotype were performed by Mann Whitney U testing since the variables did not have a normal distribution. We had to group the genotypes by considering the presence of mutation allele (AA, AB/BB) to compare scale scores of 2 groups containing a statistically significant number of patients. We accepted the statistical significance as $p<0.05$ for the outcomes of all analyses. The power analysis was performed with the “G*power” software (version 3.0.5, http://wwwpsycho.uni-duesseldorf.de/abteilungen/aap/gpower3/), post hoc goodness of fit $\chi^2$ test, with an “error” probability of 0.05. The potential assumable impacts of population stratification bias in the studied population were estimated with the formulas of Lee and Wang,\textsuperscript{36} regarding MBL2 SNP frequencies at codon 54 reported for white populations,\textsuperscript{37-39} and incidence rates of BD and SCZ in Turkey, as reported by Erol et al.\textsuperscript{40}

**Results.** MBL2 genotyping. 118 SCZ (31 female/ 87 male) and 100 BD (58 female/ 42 male) patients were evaluated according to clinical characteristics and scale scores, as shown in Table 1 and Table 2. The MBL2 genotype distribution of SCZ was significantly different from the control group. The healthy control participants had a higher frequency of MBL2 AB genotype than in the SCZ patients (OR: 0.450, 95%CI: 0.243–0.830; $p=0.010$). In addition, the percentage of participants with the BB genotype was significantly higher in the SCZ group than in the control group (OR:1.689, 95% CI: 1.297–2.200; $p=0.041$). The BD group’s MBL2 genotype distribution was also significantly different from the control group. The MBL2 AB genotype was significantly higher in the control group than the BD group (OR: 0.532; 95%CI: 0.284–0.995; $p=0.047$) (Table 3). The MBL2 genotype distribution in the female SCZ group was significantly different from the female control group. The frequency of the BB genotype was significantly higher in female SCZ patients than in the same gender control group (OR: 12.720; 95%CI: 1.453–111.378; $p=0.009$). The MBL2 genotype and the male or female BD group’s allele frequency distributions were not significantly different from the same gender control group (Table 4).

**Comparison of genotype distributions of patients based on the treatment resistance.** The MBL2 genotype distribution was significantly different between the treatment-resistant and the treatment-responsive SCZ group. The frequency of the AB genotype was significantly higher in patients with treatment-responsive SCZ than in patients with treatment-resistant SCZ (OR: 7.857; 95%CI: 1.006–61.363; $p=0.023$). While the frequency of the AB genotype was significantly higher in the treatment-responsive BD group compared to the treatment-resistance BD

### Table 6 - Comparison of scale scores and clinical parameters according to MBL2 genotype in patients with bipolar disorder.

| AA          | Median (min-max) | AB/BB | Median (min-max) | **P-value** |
|-------------|------------------|-------|------------------|-------------|
| HAM-D score | 10(0-34)         | 9(2-25)| 0.479           |
| YMRS score  | 4(0-38)          | 7(0-28)| 0.207           |
| CGI sev.    | 5(2-7)           | 5(3-7)| 0.698           |
| CGI imp.    | 2(1-4)           | 2(1-4)| 0.847           |
| Manic episode | 2(0-21)     | 3(1-25)| 0.512           |
| Dep. episode | 1(0-12)          | 0(0-2)| 0.042           |
| Total episode | 4(1-23)       | 3(1-27)| 0.329           |
| Duration of disease | 15(1-40) | 14(5-40)| 0.721           |
| Age of onset | 24(13-44)       | 25(10-52)| 0.457           |
| Number of hospit. | 2(0-21) | 2(0-21)| 0.576           |

**Mann Whitney U test, min - minimum, max - maximum, CGI - clinical global impression scale, HAM-D - hamilton depression rating scale, YMRS - young mania rating scale, sev. - severity, imp - improvement, dep - depressive; hospt - hospitalization
When comparing the MBL2 AA genotype against the treatment-resistant BD patient group, it was found that the MBL2 AB genotype was significantly higher in the treatment-resistant BD patient group than in the treatment-responsive BD group (OR: 8.782; 95%CI: 1.114–69.197; p=0.016). Additionally, the MBL2 AA genotype was significantly higher in the treatment-resistant BD patient group than in the treatment-responsive BD patient group (OR: 0.185; 95%CI: 0.040–0.852; p=0.018). Also, the frequency of the A allele was significantly higher in the treatment-resistant BD patient group than in the treatment-responsive BD patient group (OR: 0.283; 95%CI: 0.082–0.974; p=0.035) (Table 5).

**Comparison of MBL2 genotype distributions of patients according to the presence of clinical specifiers.** Comparing the MBL2 genotype and allele frequency distributions of the BD patients according to the presence of clinical specifiers (psychotic features, atypical features, seasonal pattern, mixed features, rapid cycling history, peripartum onset) showed that allele frequency distribution was significantly different between the groups of patients with BD in terms of atypical depression history. The frequency of the A allele was significantly higher in the group with a history of atypical depression (OR: 0.228; 95%CI: 0.052–0.998; p=0.034) (Table 5). However, there was no significant difference between the groups’ other clinical specifiers (psychotic features, seasonal pattern, mixed features, rapid cycling history, peripartum onset) according to the MBL2 genotype distributions (p>0.05).

**Comparison of age of onset, duration of disease, number of hospitalizations, and scale scores according to MBL2 genotypes (AA, AB/BB).** When comparing the age of onset, duration of the disorder, a number of hospitalizations, and scale scores (SATCI, CGI sev., CGI imp., PANSS total, PANSS neg., PANSS pos., PANSS psycho.), regarding the MBL2 genotype (AA, AB/BB) groups (grouping by considering the presence of mutation allele) in SCZ patients, there was not found a significant difference between groups of MBL2 genotype (p>0.05). However, comparing scale scores (CGI sev., CGI imp., HAM-D, YMRS) and clinical parameters (number of manic episodes, depressive episodes, total episodes, number of hospitalizations, age of onset, and duration of disorder) due to the MBL2 genotype (AA, AB/BB) group in patients with BD, the number of depressive episodes were significantly different between the groups of the MBL2 genotype (p=0.042). The number of depressive episodes was significantly higher in patients with the AA genotype (Table 6).

**Discussion.** Our study analyzed data from 318 participants (118 SCZ, 100 BD patients, and 100 healthy volunteers) and revealed that the participants carrying the MBL2 BB genotype had a significantly higher risk of developing SCZ; the MBL2 AB genotype had a protective effect on the risk of SCZ. Mayilyan et al. found MBL-induced mannose-binding protein-associated serine protease 2 (MASP-2) activity and, therefore, MBL and MASP-2-mediated complement activation capacity was higher level in SCZ patients than in the control group. They thought that the high activity of MASP-2-mediated complexes could promote extreme complement activation and excretion of inflammatory mediators by any activator. Again, in the SCZ patient group, the significantly higher rate of the homozygous genotype of the mutation allele (BB) than the control group was consistent with the literature. In a study by Foldager et al., although the MBL concentration was high in the SCZ group, they showed that the genotype percentage with the mutation allele was higher in this group than in the healthy participants. Recently, we reported that MBL2 polymorphism is related to attempted suicide in SCZ, and macrophage migration inhibitory factor (MIF) polymorphism is associated with attempted suicide in BD. Again, it was found that MIF −173 G/C polymorphism is related to the impairment of insight and age of illness onset in SCZ, in another research of ours. When the complement proteins and SCZ association researches were reviewed, a recent study determined that as C4 complement protein expression increases, the risk of SCZ increases; thus, neuron-microglia interactions through the complement system have a part in the pathogenesis of SCZ.

Additionally, in our study, the MBL2 AB genotype had a protective effect on the risk of BD, the participants carrying the BB genotype were non-significantly higher percentages in the BD group. Accordingly, we can speculate that the heterozygous advantage for MBL2 gene polymorphism is reported in terms of the absence of SCZ and BD in the healthy Turkish population. Although, in the literature, few studies on this subject have examined the role of the lectin pathway of the complement system in BD, a study by Foldager et al. on the MBL2 genotype of patients with BD reported that the genotype with mutation allele was higher in the BD group than the controls similar to our study. Therefore, it was reported that MASP-2 levels were lower in BD patients than in the healthy participants. Furthermore, in a study examining the peripheral blood concentrations of C3a, C5a, C5b-9 complement cascades in 30 patients diagnosed with BD for at least ten years, the blood concentration of all values was higher than healthy controls.

In our study, the percentage of patients with the MBL2 BB genotype was significantly higher in women.
with SCZ than in the same gender participants. In the literature, recent gene polymorphism studies have shown that some dopaminergic genes (Catechol-O-methyltransferase (COMT), monoamine oxidase (MAO)) have a gender-specific relationship with SCZ and symptom types. In a study examining the connection between negative symptoms and polymorphisms in the COMT gene in SCZ adults, it was found that the rs740603 and rs740603 (G) -rs4818 (G) haplotypes of the COMT gene may be related to the negative symptoms of SCZ, especially in women. In our study, the significantly higher detection of BB genotype in codon 54 compared to the control group in female SCZ patients may reveal that inflammation may take part in women with SCZ. If other inflammation-related gene polymorphisms supported our findings, SNPs associated with inflammation might suggest that inflammation has gender-specific effects in psychiatric disorders.

The SCZ patients carrying the MBL2 AB genotype had a significantly lower risk of treatment resistance in our study. Previous literature on inflammation-related genes has reported that the predominance of the human leukocyte antigen-A1 (HLA-A1) allele in treatment-resistant SCZ was distinctive from treatment-responsive SCZ, but another study reported no significant difference between the groups HLA antigens. Another GWAS examining the response to a single antipsychotic found an association with the rhotekin 2 (RTKN2) gene, which may affect both the innate and adaptive immune response by regulating the nuclear factor kappa B (NF-κB) pathway. Again, our published recent research investigating the connection between the SCZ and inflammation-related gene polymorphism in tumor necrosis factor-alpha (TNF-α) -238 G/A, -308 G/A have found that TNF-α-238 G/A polymorphism is associated with treatment resistance in SCZ patients.

Our study also showed statistically significant differences in the MBL2 genotype and allele frequency distributions between the treatment-resistant and the treatment-responsive BD group. Our findings showed that while the percentage of patients with the AB genotype was higher in patients with treatment-responsive BD than in the treatment-resistant BD group, the BD patients carrying the AA genotype or A allele had a significantly higher risk of treatment resistance. When we reviewed the linkage studies on the response to treatment, the inositol pathway, the circadian signaling system, the neurotransmitter system, and brain-derived neurotrophic factor (BDNF)-related gene polymorphisms have found to be associated with the mechanism of action of lithium. Although we did not find any publications in the literature regarding the relationship between inflammation-related gene polymorphism and treatment response, several studies in the literature provide evidence to support the anti-inflammatory outcomes of mood stabilizers through several mechanisms. It was suggested that lithium decreases the synthesis of pro-inflammatory enzymes and molecules (interleukin-1 (IL-1), TNF-α, prostaglandins (PG), nitric oxide (NO), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and phospholipase A2 (PLA2)) and regulates microglial activity during treatment. Similar to lithium, valproic acid also inhibits the synthesis of COX-1 and COX-2 in the rat brain and down-regulates the arachidonic acid signal cascade. Thus, valproate and other antiepileptic mood stabilizers (lamotrigine, topiramate, carbamazepine, and oxcarbazepine) were also found to reduce the synthesis of many cytokines in vitro. Although our study is the first in this field, it is not surprising that our study reported a significant difference between MBL2 variants and treatment resistance in BD patients considering the above anti-inflammatory effects of mood stabilizers.

Comparison of MBL2 genotype distributions in the patient group diagnosed with BD according to scale scores and clinical characteristics revealed that the number of depressive episodes was significantly higher in patients with the AA genotype. In a clinical study of 22 chronic and 24 first-episode BD patients, a negative correlation was found between serum C4 complement levels and disease duration in BD patients, while no correlation was found between factor B and sC5b-9 levels and duration of disease. YMRS scores showed a statistically significant relationship only with blood sC5b-9 levels, while no significant connection was shown between Montgomery and Asberg Depression Scale (MADRS) scores and any complement levels in serum. Nevertheless, any complement factor in serum was not related to age and the number of manic-depressive episodes. In another study conducted in patients with SCZ and BD, a comparison of gene expression and the effect to Global Assessment of Functioning (GAF), CGI, PANSS, YMRS, and Calgary Depression Scale for Schizophrenia (CDSS) scores showed that AKT serine/threonine kinase 1 (AKT1) and double-stranded RNA-specific endoribonuclease (DICER1) gene expression levels were higher in patients diagnosed with BD compared to healthy participants and patients with SCZ. However, no relationship was found between AKT1, DICER1 gene expression and scale scores, the severity of manic-depressive symptoms, and dysfunction in BD patients. It has been proposed that the expression of AKT1 and DICER1 gene, which
play a critical role in many signaling pathways, might be more specifically related to manic properties.\textsuperscript{57} On the contrary to these 2 studies, neopterin levels caused by cellular immune system activation were reported to be higher in patients with major depressive episodes than those with a single depressive episode and the control group.\textsuperscript{58} Moylan et al\textsuperscript{59} showed that inflammation had an essential role in the chronicization of depression, increased susceptibility to subsequent episodes, and accelerated the disease’s progressive course. Similar to our findings, changes in inflammatory markers were associated with the number of depressive episodes.

In the present study, when we compared the \textit{MBL2} genotypes and allele frequency distributions of patients in the 2 groups regarding clinical specifiers, we found a significant difference between the groups of patients with BD in terms of their history of atypical depression. Previous literature has demonstrated a potential relationship between inflammation and atypical features of depression. Increased appetite is the only atypical feature related to both inflammatory and metabolic markers in the context of depressive episodes, and this symptom may be a vital characteristic of the immuno-metabolic model of depression.\textsuperscript{60} In one study, atypical features were associated with decreased IL-4 and increased IL-2, while in another study, IL-2 levels were observed to be lower in patients with atypical depression. In a study comparing atypical depression and melancholic type depression, although serum CRP, IL-6, and TNF-\alpha levels were not significantly different between the groups, serum CRP level was increased in atypical depression compared with the control group.\textsuperscript{66-62}

In our study, the higher percentage of B allele in the BD group without a history of atypical depression than the BD group with a history of atypical depression supports a relationship between an atypical feature and the increase of inflammation in the depressive episode.

Several limitations should be considered in our study. If we consider the multi-genetic nature of SCZ and BD and gene-gene interaction or biological pathways in the etiology, our study’s first limitation is that it was about single inflammation-related \textit{MBL2} gene polymorphism. The second limitation of the study was the small sample size, although it was calculated by considering the frequencies of \textit{MBL2} polymorphism in the white population. Finally, only polymorphism in codon 54 was investigated, but it was impossible to determine whether other \textit{MBL2} mutations in codon 52 and codon 57 precipitated the disorder.

In conclusion, in our study, having the heterozygosity for \textit{MBL2} can be advantageous for the response to treatment and the prevention of disease occurrence in both BD and SCZ patients. Especially in female patients with SCZ, there may be evidence that inflammation may play a role in the disease. Besides, these findings showed that there are relationships between inflammation-related gene polymorphism and the number of depressive episodes along with the history of atypical depression in patients diagnosed with BD. Confirming the present results with other inflammation-related gene polymorphisms in other populations covering larger regions will better explain the role of inflammation in the pathogenesis of SCZ and BD.

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