Rs6454674, Rs806368 and Rs1049353 CNR1 Gene Polymorphisms in Turkish Bipolar Disorder Patients: A Preliminary Study

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Abstract

Bipolar disorder (BD) is one of the most prevalent psychiatric disorders in clinical practice. The etiology of the BD is not thoroughly understood. Endocannabinoid system, which is involved in regulation of emotion, stress, memory, and cognition, may have an important role in the pathophysiology of BD. Mutations on the cannabinoid-1 receptor (CNR1) gene are associated with several psychiatric disorders. The main cannabinoid (CB) receptor is CB1 and its activation inhibits neuronal depolarization. One previous study showed rs1049353 polymorphism of CNR1 gene is associated with major depression but not with BD. In this study, we aimed to investigate the rs6454674, rs806368 and rs1049353 CNR1 gene polymorphisms in Turkish BD patients. A total of 96 patients and 58 healthy controls were included in the current case-control study. Blood samples of study participants were collected into sterile tubes and processed to obtain genomic DNA. Restriction Fragment Length Polymorphism analysis were done by digesting the PCR products with HpyCH4III and BseGI enzymes for the rs6454674 and rs806368 restriction sites, respectively. Single-Strand Conformation Polymorphism (SSCP) analysis was also performed. Among three polymorphisms investigated in this study, only rs6454674 polymorphism was significantly different between BD patients and controls (rs6454674 T/G; p=0.042, rs806368 T/C; p>0.05, rs1049353 A/G; p>0.05). Furthermore, we found that the mean of the yearly manic attacks was statistically higher in patients who have heterozygote (0.91±0.67) rs6454674 T/G polymorphism compared to those with homozygote (p=0.043) polymorphism. The post-hoc analysis showed that the main differences were between the heterozygotes genotype and non-mutant (GG) homozygotes (0.42±0.31; p=0.037) but not in homozygote mutant genotype (0.74±0.74; p=0.149). When patients were compared with the other clinical parameters, and mutated alleles and genotypes for each polymorphism, we did not find any significant difference. These results suggest that a heterozygote advantage exists for the CNR1 gene rs6454674 polymorphism in manic episode frequency in Turkish BD patients. The results of our study should be confirmed in future studies with larger sample size.

Introduction

Bipolar disorder (BD) is one of the most prevalent psychiatric disorders in clinical practice. BD is classified among affective disorders because the main issue about the illness is affective component. Biochemical substances, neurotransmitters, genetics, environmental circumstances, oxidative stress... etc. were associated with the etiology of the BD (1). Although there are...
many reports about the etiology of BD, it is not thoroughly understood. The main accepted theory of affective disorders is the dysregulation of monoamine neurotransmitters (1). Besides this in the human brain there are several modulatory systems that may also play an important role in the pathophysiology of BD such as endocannabinoid system, which is involved in regulation of emotion, stress, memory, and cognition (2). On the other hand, dysfunction of this system may lead to various psychiatric disorders such as schizophrenia, BD, anxiety disorders, and depression (3).

Mutations on the cannabinoid-1 receptor (CNR1) gene that codes for cannabinoid 1 (CB1) receptors are associated with several psychiatric disorders. CB1 receptors are highly expressed in olfactory receptor system, hippocampal formation, basal ganglion, cerebellum, and neocortex in the brain (4). The main cannabinoid receptor is CB1 and by the activation of them leads an inhibition on neuronal depolarization; diminish in the production of action potential, and releasing of excitatory and inhibitory neurotransmitters. Thus causes a decrease in impulse propagation. Receptor agonists for CB1 act like antidepressants by the activation of serotonin neurotransmission. Moreover, antagonism of CB1 receptors may lead depression like clinical pictures in some patients (5,6). Cannabinoid usage may lead the early onset of BD, and sometimes induce BD in susceptible individuals (7).

There was only one study that investigated the possible relationship of CNR1 gene polymorphisms and affective disorders. The results of the study showed that there might be an association between the CNR1 gene (rs1049353) polymorphisms and major depression but not in BD (8). Taken all together, we aimed to research the possible association between the additional CNR1 gene polymorphisms and BD.

Materials and Methods

Study Populations

A total of 96 patients and 58 healthy controls were included in the current case-control study. The study approved by the local ethics committee in accordance with the Declaration of Helsinki. All study participants gave informed consent before enrollment.

Blood Samples and DNA Isolation

Blood samples of study participants were collected into sterile Vacutainer tubes with disodium ethylenediaminetetraacetic acid and processed to obtain genomic DNA. To obtain genomic DNA proteinase K digestion and salt-chloroform method was utilized (9). Genomic DNA samples were stored at -20 °C for the next applications.

Polymerase Chain Reaction and Restriction Fragment Length Polymorphism Analysis

Amplification of DNA samples was achieved in AB Thermal Cycler (ABI Inc. CA, USA). Amplified PCR products were verified by running through 2% agarose gel and visualized using ethidium bromide stain. Restriction Fragment Length Polymorphism analysis were analyzed digesting the PCR products with HpyCH4III and BseGI enzymes for the rs6454674 and rs806368 restriction sites, respectively. Restriction fragments were assessed after resolving at 3% agarose gel and ethidium bromide visualization. Studied CNR1 gene polymorphisms, primer sequences, sizes of the amplicons, and annealing temperatures are presented in Table 1.

Single-Strand Conformation Polymorphism

Single-Strand Conformation Polymorphism analyses were performed on 7% acrylamide/bisacrylamide gel with a ratio of 49:1 as previously described (10). All SSCP gels were neutralized with low-concentrated acetic acid and visualized by silver staining. Further direct sequencing analysis was performed for the some of the samples to confirm banding patterns.

Statistical Analysis

Overall statistical analysis was performed using GraphPad Prism (v6.02, GraphPad Software Inc., San Diego, CA, USA) and SPSS (v16.0) programs. For the Statistical analysis of more than two groups Student-Newman-Keuls test, for the significance in the genotype and allele frequency differences the χ2 test (with Yate’s correction) or Fisher’s exact tests. Haplotypes under 3% population frequency were excluded. All applied tests were two-tailed and p <0.05 was considered as significant.

Results

The mean ages (F=5.587, p=0.923) and the gender distribution (x2=0.06; df = 1, p=0.806) of the study groups were similar.

The distribution of the CNR1 rs6454674 (T/G), and rs806368 (T/C) genotypes did not deviate from the Har-
Table 1. Studied CNR1 gene polymorphisms, primer sequences, sizes of the amplicons, and annealing temperatures.

| Reference SNP no | Primer Sequences (5'→3') | Expected size of PCR product (bp) | Annealing temperature (°C) |
|------------------|---------------------------|----------------------------------|---------------------------|
| rs6454674        | F: ATGCAACTGAGCTAACATGGAAT  R: ACGGGGAAATTTAGCAGGCTT | 510                             | 58                        |
| rs806368         | F: GTTTCCCGCTGAACATGGGA    R: GAAATGGCCCAACACCAAGA | 510                             | 59                        |
| rs1049353        | F: GATCATGTTCAATACACCTTTC  R: TGCCACCTCTCTGCATAAC | 300                             | 57                        |

Table 2. The comparisons of the polymorphisms and alleles according to the study groups.

| Genotype/Allele | Healthy Control (n=58) % | Patient (n=96) % | p       | OR (95% CI) |
|-----------------|--------------------------|-----------------|---------|-------------|
| rs6454674       |                          |                 |         |             |
| TT              | 22 (37,9)                | 52 (54,2)       |         |             |
| TG              | 27 (46,6)                | 32 (33,3)       | 0.042   | 2.03 (1.026-4.009) |
| GG              | 9 (15,5)                 | 12 (12,5)       | 0.103   | 0.57 (0.294-1.119) |
| T               | 71 (61,2)                | 136 (70,8)      |         |             |
| G               | 45 (38,8)                | 56 (29,2)       | 0.082   | 1.53 (0.946-2.502) |
| HWE (x², p)     | Yes (0.02, 0.988)        | Yes (3.58, 0.166) |         |             |
| rs806368        |                          |                 |         |             |
| TT              | 28 (50)                  | 46 (50)         |         |             |
| TC              | 26 (46,4)                | 44 (47,8)       | 1.000   | 1.00 (0.514-1.943) |
| CC              | 2 (3,6)                  | 2 (2,2)         | 0.868   | 1.06 (0.543-2.058) |
| T               | 82 (73,2)                | 136 (73,9)      |         |             |
| C               | 30 (26,8)                | 48 (26,1)       | 0.894   | 1.04 (0.608-1.764) |
| HWE (x², p)     | Yes (0.53, 0.765)        | Yes (5.30, 0.070) |         |             |
| rs1049353       |                          |                 |         |             |
| AA              | 0                        | 0                |         |             |
| AG              | 57 (100)                 | 88 (100)        | 1.000   |             |
| GG              | 0                        | 0                |         |             |
| A               | 57 (50)                  | 88 (50)         |         |             |
| G               | 57 (50)                  | 88 (50)         | 1.000   | 1.00 (0.624-1.602) |
| HWE (x², p)     | No (57, <0.001)          | No (88, <0.001) |         |             |

d–Weinberg equilibrium in both healthy controls and BD patients, but rs1049353 (G/A) genotype deviated in both study groups. The comparison of the patients and control groups according to the three polymorphisms showed that any significance except in the rs6454674 polymorphism (rs6454674 T/G; p=0.042, rs806368 T/C; p>0.05, rs1049353 A/G; p>0.05). The comparisons of the polymorphisms and alleles according to the study groups are presented in Table 2.

The mean of the yearly manic attacks were statistically higher in patients with heterozygote (0.91±0.67) rs6454674 T/G polymorphism compared to those with homozygote polymorphism (p=0.043). The post-hoc analysis showed that the main differences were between the heterozygotes and non-mutant (GG) homozygotes (0.42±0.3; p=0.037) but not in homozygote mutant genotype (0.74±0.74; p=0.149). When patients were compared with the other clinical parameters such as; presence of past suicide attempts, total number of episodes, onset age of BD, duration of the illness, and mutated alleles and genotypes for each polymorphisms we did not find any significant difference.

Discussion

The main findings of the study were; a relationship between the rs6454674 polymorphism and BD patients,
and mean yearly manic attacks higher in heterozygotes than non-mutant homozygotes. In recent years, BD and major depressive patients were enrolled in a study that found the parallel results about the rs1049353 polymorphism with the present study (8). In recent years there were several studies that found a possible association polymorphism of the CNR1 gene with affective symptoms and mainly with depression (11-14). However we studied two more CNR1 polymorphisms and found that a statistically significant difference in rs6454674. The mutant allele in rs6454674 was detected as T. The distribution of the genotypes showed that TT genotype was seen more in patient groups.

Another important finding of the study was relationship between the heterozygote genotype of rs6454674 polymorphism with yearly manic episodes. One may think that the difference should be more apparent between the mutant and non-mutant homozygotes. These results suggest that a heterozygote advantage exists for the CNR1 gene rs6454674 polymorphism in manic episode frequency in Turkish BD patients.

The small number of subjects enrolled in the groups might be accepted as the first limitation of the study. Although the limited number of samples the Hardy–Weinberg equilibrium showed that the distribution of the variables were normal. A second limitation for the present study was the discordant number of the groups.

The results of the present study suggested that there might be an association between CNR1 gene mutation, and clinical picture of BD and prevalence of BD. There is a need for replication of these findings by large sample size studies.

Conflict of Interest: None
Acknowledgments: None

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