INTRODUCTION

Bipolar disorder-I (BD-I) is a highly prevalent and often chronic mood disorder with a lifetime prevalence of 1% to 5% (Akiskal et al. 2000), which is frequently characterized by episodic recurrent mania or hypomania and major depression (Belmaker 2004). Historically, BD-I has been perceived primarily as a brain disorder; however, recent evidence indicates that BD-I is a systemic disease, with widespread biochemical alterations occurring in and beyond the central nervous system (Kapczinski et al. 2008). In addition, BD-I is associated with higher rates of general medical comorbidities including premature mortality from cardiovascular, respiratory, and endocrine causes (Roshanaei-Moghaddam et al. 2009, Carney & Jones 2006, Krishnan 2005, Tavcar 2015). BD-I is a complex illness, and multiple genes and environmental factors determine its pathogenesis. Family, twin, and adoption studies strongly indicate a genetic basis for BD-I: with heritability of 0.8 (Craddock & Forty 2006). Several studies have established that BD-I and inflammation are linked through shared genetic polymorphisms and gene expression, as well as altered cytokine levels. BD-I (Cunha et al. 2008). Many studies have reported increased levels of inflammatory cytokines, which indicates activation of inflammatory pathways in BD-I (Drexhage et al. 2010). Pro-inflammatory cytokines IL-6 and TNF-α are elevated during the early and late stage, and the anti-inflammatory IL-10 is increased only in the early phase of BD-I (Berk et al. 2011).

Cyclooxygenases (COX) catalyze the rate-limiting step in the conversion of arachidonic acid to prostaglandins and thromboxanes, and lipid mediators involved in several physiologic and pathologic processes in the brain (Bosetti 2007). Inflammation is associated with an increased expression of COX and elevated levels of prostaglandins. COX-derived prostaglandins promote the migration of monocyte-derived cells (Legler et al. 2006) as well as breakdown of the blood–brain barrier (Schmidley et al. 1992). There are two COX isoforms, COX-1 and COX-2, which share 60% homology in their amino acid sequences and have comparable kinetics (Smith et al. 2000); however, the two isoforms differ in regulatory mechanisms through preferential coupling to upstream and downstream enzymes (Murakami and Kudo 2004). It has been reported that the promoter polymorphisms COX-2-765G→C and COX-2-1195A→G have been shown to modify COX-2 transcription and mRNA levels (Zhang et al. 2005). As discussed, inflammation is related to BD-I, so COX-2 gene polymorphisms that affect COX-2 levels may be associated with BD-I by altering the inflammatory response.
It was to investigate a possible association between COX-2-765G→C and COX-2-1195A→G polymorphisms with the presence of BD-I. Not only because BD-I is a complex illness but also has distribution with multiple genes and environmental factors.

SUBJECTS AND METHODS

Study population

Patients with BD-I were recruited from the psychiatric department of Istanbul Erenkoy Psychiatric and Neurological Disorders Hospital, which has an inpatient ward for acute psychiatric patients. All patients completed medical reports at their first admission. Assessment for diagnosis of BD-I using the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria were performed by one psychiatrist based on consensus, utilization of cross-sectional interviews, and case records. All patients had active symptoms at the time of the study. Most of the patients were receiving antipsychotic medication at the time of the scan. Our sample consisted of 180 patients with BD-I (mean age: 38.75±11.5 years), and 170 healthy controls (mean age: 36.97±10.5 years). There were no significant differences among the study and control groups in terms of mean age and sex distribution.

Control Population

Control patients were randomly selected from the psychiatric department of Istanbul Erenkoy Psychiatric and Neurological Disorders Hospital, which has an inpatient ward for acute psychiatric patients. All patients completed medical reports at their first admission. Assessment for diagnosis of BD-I using the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria were performed by one psychiatrist based on consensus, utilization of cross-sectional interviews, and case records. All patients had active symptoms at the time of the study. Most of the patients were receiving antipsychotic medication at the time of the scan. Our sample consisted of 180 patients with BD-I (mean age: 38.75±11.5 years), and 170 healthy controls (mean age: 36.97±10.5 years). There were no significant differences among the study and control groups in terms of mean age and sex distribution.

Inclusion and exclusion criteria

Inclusion criteria were a diagnosis of current DSM-IV BD-I. Patients were excluded if their primary diagnosis was not BD-I. Patients were also excluded from the study if they had a history of neurologic or medical disorder that would affect neuropsychologic function (i.e. seizures, head trauma, stroke, brain tumor, meningitis) or if they had a recent history of abusing alcohol or psychoactive drugs. In addition, control subjects were excluded if they had a diagnosis of any DSM-IV axis I and II disorders.

Measurements, protocol and procedure

All subjects were examined according to a standardized interview. Assessment was done on a semi-structured sociodemographic proforma, which required patient information regarding demographic and personal details of the patients and informants, symptoms of the patients, history of present illness, details of medical or surgical interventions, past history, family history, personal history, premorbid personality, details of physical examination, mental status examination, and diagnostic formulation. The potential participants were interviewed by a psychiatrist, and their medical records were reviewed whenever relevant. Secondly, a consultant psychiatrist audited the first-phase results and confirmed or rejected the diagnosis. The diagnosis was made using the Structured Clinical Interview for DSM-IV (SCID-I) (Ozkurkcugil et al. 1999). The patients were then screened on various rating scales like Brief Psychiatric Rating Scale (Overal & Gorhan 1962, Ventura et al. 2000) for patients with BD-I, Scale for the Assessment of the Rating Scale for Mania (Karadag et al. 2002, Young et al. 1978) for BD-I, Controls with BD-I were excluded from the study using the Structured Clinical Interview for DSM-IV (SCID-I) interviews. Normal control participants were recruited from a large medical outpatient clinic. We investigated their demographic data, medical, and psychiatric history. None of the comparison subjects had a history of significant medical illness, head injury, neurologic disorder, psychiatric disorder, or alcohol or substance abuse, and none had a family history of psychiatric disorder.

To minimize the effect of ethnic differences in gene frequencies, the study participants were of Turkish population living in the western region of Turkey. The study was approved by the Medical Ethics Committee of Istanbul Medical Faculty, and all participants (i.e. controls, patients or unaffected family members (on behalf of some patients) gave written informed consents.

Polymorphism Analysis

Blood samples from all study participants were collected in EDTA-containing tubes. Genomic DNA was extracted from peripheral whole blood in accordance with the salting-out technique. Polymerase chain reaction (PCR)/restriction fragment length polymorphism (RFLP) analysis was performed for the detection of the variations in COX-2 gene (Zhang et al. 2005, Shi et al. 2004). The appropriate primers were used to amplify the corresponding gene of the participants by PCR and the reaction products were digested using the appropriate enzyme at 37°C. The digested products were analyzed on 2% agarose gel stained with ethidium bromide and examined under transillumination. Each gel was read by two observers unaware of the subject’s status. If there is any conflict, samples were repeated.
Statistical Analysis

Statistical analyses were performed using the SPSS software package (revision 11.5 SPSS Inc., Chicago, IL, U.S.A.). Differences in the distribution of COX-2-765G→C and 1195A→G genotypes or alleles between patients and controls were tested using the Chi-square test. Linkage disequilibrium between COX-2-765G→C and 1195A→G polymorphisms was assessed using r^2 values obtained through the Haploview program (http://www.broad.mit.edu/mpg/haploview/documentation.php). Values were considered statistically significant.

RESULTS

Controls and patients were matched in age and sex. Table 1 shows the characteristics of patients and control groups. More smokers were represented in the patients group compared with the controls, and more alcohol consumers were represented in the controls compared with the patient group.

There were statistically significant differences in COX-2-1195A→G genotypes and alleles between the controls and patients (χ^2:28.7; p<0.000; χ^2:14.3; p<0.000) (Table 2). Frequencies of COX-2-1195A→G G+ genotype in controls (46.5%) were higher than the patients (22.2%). It seems that there is a protective role of G+ genotype against BD-I (p<0.000, χ^2:22.9, OR:0.32, 95% CI:0.20-0.52).

Frequencies of COX-2-1195A→G AA genotype in patients (77.8%) were higher than the controls (53.5%). The individuals with COX-2-1195A→G AA genotype had seems to be associated for BD-I (p<0.000, χ^2:22.9, OR:3.03, 95% CI:1.91-4.82).

On the other hand, we found no significant differences in COX-2-765C→G genotype and allele frequencies between patients with BD-I and controls (p>0.05) (Table 3).

In addition to SNP analyses, haplotypes were evaluated for association with BD-I (Table 3). Haplotype analysis confirmed the association of gene variants with BD-I and revealed that the frequencies of COX-2-765G:1195A haplotype frequencies were significantly higher in patients compared with those of controls, and COX-2-765G:1195G and COX-2-765C:1195G haplotype frequencies were significantly higher in controls compared with those of patients.

There was a weak linkage disequilibrium between COX-2-765G→C and COX-2-1195A→G polymorphisms (D:0.498, r^2:0.015, LOD:0.74).

Table 1. Characteristics of patients with BD-I and control groups.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Patients</th>
<th>P</th>
<th>χ^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>36.97±10.5</td>
<td>38.75±11.5</td>
<td>0.135</td>
<td></td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>72.4/27.6</td>
<td>63.3/36.7</td>
<td>0.071</td>
<td>3.25</td>
</tr>
<tr>
<td>Smoking (no/yes)</td>
<td>53.9/46.1</td>
<td>41.7/58.3</td>
<td>0.024</td>
<td>5.08</td>
</tr>
<tr>
<td>Alcohol (no/yes)</td>
<td>75.0/25.0</td>
<td>85.1/14.9</td>
<td>0.022</td>
<td>5.42</td>
</tr>
</tbody>
</table>

Table 2. The distribution of COX-2-765 C→G and COX-2-1195A→G genotype frequencies in patient and control groups

<table>
<thead>
<tr>
<th></th>
<th>Controls N:170</th>
<th>Patients N:180</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>7(4.1%)</td>
<td>9(5%)</td>
</tr>
<tr>
<td>GG</td>
<td>102(60%)</td>
<td>112(62.2%)</td>
</tr>
<tr>
<td>CG</td>
<td>61(35.9%)</td>
<td>59(32.8%)</td>
</tr>
<tr>
<td>ALLELES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>75(22.5%)</td>
<td>77(21.3%)</td>
</tr>
<tr>
<td>G</td>
<td>265(77.9%)</td>
<td>283(78.6%)</td>
</tr>
<tr>
<td>COX-2-1195A→G GENOTYPES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>91(53.5%)</td>
<td>140(77.8%)</td>
</tr>
<tr>
<td>GG</td>
<td>2(1.2%)</td>
<td>6(3.3%)</td>
</tr>
<tr>
<td>AG</td>
<td>77(45.3%)</td>
<td>34(18.9%)</td>
</tr>
<tr>
<td>ALLELES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>259(76.1%)</td>
<td>314(87.2%)</td>
</tr>
<tr>
<td>G</td>
<td>81(23.8%)</td>
<td>46(12.7%)</td>
</tr>
</tbody>
</table>

Table 3. The frequencies of haplotypes of COX-2 gene in patients and controls

<table>
<thead>
<tr>
<th>Number of haplotype</th>
<th>Haplotype Associations</th>
<th>Overall Frequency</th>
<th>Control</th>
<th>Chi Square</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>COX-2-765G: 1195A</td>
<td>0.623</td>
<td>0.669</td>
<td>0.575</td>
<td>6.655</td>
</tr>
<tr>
<td>2</td>
<td>COX-2-765C: 1195A</td>
<td>0.197</td>
<td>0.204</td>
<td>0.190</td>
<td>0.212</td>
</tr>
<tr>
<td>3</td>
<td>COX-2-765G: 1195G</td>
<td>0.160</td>
<td>0.118</td>
<td>0.205</td>
<td>9.76</td>
</tr>
<tr>
<td>4</td>
<td>COX-2-765C: 1195G</td>
<td>0.019</td>
<td>0.009</td>
<td>0.031</td>
<td>4.345</td>
</tr>
</tbody>
</table>
DISCUSSION

In the field of mood disorders, the immune system seems to play a particularly important role. Inflammation, associated with an increased expression of COX and elevated levels of prostaglandins, has been implicated in a variety of acute and chronic neurologic and neurodegenerative disorders, including Alzheimer’s disease, Parkinson’s disease, amyotrophic lateral sclerosis, and HIV infection (Griffin et al. 1994, Mahhofner et al. 2003, Teismann et al. 2003, Hoozemans et al. 2008). Multiple studies have shown increased expression of COX-2 in several diseases (Denkert et al. 2007, Esmaeili et al. 2011, Iglesias et al. 2009), but limited data exist about their expression changes in neurodegenerative diseases. It has been demonstrated that the up-regulation of COX-2 in Alzheimer-type dementia and Down’s syndrome may be causally related to neuronal degeneration (Oka & Takashima 1997).

It has been shown that COX-2-1195 G allele has decreased transcriptional activity and mRNA levels compared with the COX-2-1195 A allele, both in vitro and in vivo studies (De Souza Pereira 2009). A previous study showed that the COX-2-765C allele is associated with a significantly lower promoter activity than COX-2-765G allele (Papafili et al. 2002); therefore, 765C allele might reduce the expression of COX-2 enzyme. Consequently, it would be expected that individuals carrying the -1195 G allele and -765C allele would have lower expression of this enzyme and decreased inflammation over their lifetimes. Thus, these individuals may have less risk to BD-I. Supporting this hypothesis, in our study we have detected that COX-2-1195A→G G+ genotype in controls were higher than in the patients, and had a protective effect against BD-I. In addition, the individuals with COX-2-1195A→G AA genotype might have an impact on development for BD-I. In contrast, we did not see this relationship between COX-2-765 G allele and BD-I.

The present study has some potential limitations; the small sample size makes our study under-powered. This could be a reason for some of the results that demonstrated no statistical significance. Big-size studies in different races will help us understand whether COX-2 genotypes effect BD-I.

CONCLUSIONS

Our findings suggest that COX-2 gen variants could facilitate the development of BD-I. Further studies with larger sample groups are necessary to clarify the role of COX-2 gen variants and the development of BD-I.

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Conflict of interest: None to declare.

References


