Brief report

Genetic association between bipolar disorder and 524A>C (Leu133Ile) polymorphism of CNR2 gene, encoding for CB2 cannabinoid receptor


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Introduction:
Several studies provided evidence that the endocannabinoid system (ECS) is involved in psychiatric diseases, like major depression, schizophrenia and bipolar disorder (BD), mainly focusing on CB1 cannabinoid receptor, and FAAH, the fatty acid amide hydrolase involved in endocannabinoid metabolism. In this study we investigated the possible association of BD with three missense SNPs, of the gene CNR2, encoding for CB2 cannabinoid receptor.

Methods:
The possible association between BD and three CNR2 missense SNPs, namely rs2501432 (315A>G; Arg63Gln), rs41311993 (524C>N; Leu133Ile) and rs2229579 (1073C>T; Tyr316His), was investigated through a case–control study. Eighty patients and one hundred and sixty healthy subjects were recruited. Allele Specific Oligonucleotide (ASO)-PCR and restriction fragment length polymorphism (RFLP) methods were used for genotyping.

Results:
A statistically significant association was found between BD and the CNR2 524C>N; Leu133Ile (P(χ²)=0.001; OR=4.74; 95% C.I.=2.52–10.50) while no statistically significant difference between BD and control group was observed for the other two SNPs.

Conclusion:
Though further investigations are necessary to confirm this data, our results suggest that CB2 cannabinoid receptor may play a role in BD.

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1. Introduction

Several studies established that heavy cannabis use decreases the age at onset of both bipolar disorder (BD) and schizophrenia (De Hert et al., 2011), increases the risk for manic symptoms in the general population (Henquet et al., 2006) and is associated to higher levels of illness severity, mania and psychosis among bipolar patients (Van Rossum et al., 2009).

Cannabis contains more than 400 different substances, including tetrahydrocannabinols which are mainly responsible for its psychotropic action. These compounds interact with the central nervous system (CNS), binding to specific membrane receptors, whose discovery in the early 90s opened the way to the detection of a complex endogenous cannabinoid system (ECS). Two specific cannabinoid receptors, belonging to the G-protein coupled receptor superfamily, were until now discovered: CB1, for many years considered to be the sole expressed in the brain, and CB2, greatly expressed by immunocytes, but recently found in CNS, too (Morgan et al., 2009; Patel et al., 2010; Stella, 2010).

Recently, several authors explored the influence of the genetic variability of CNR1 and, to a lesser extent, of CNR2, the genes encoding for the two endocannabinoid receptors, in psychiatric diseases and drug abuse. In this context, polymorphisms of CNR1 were found to be associated with higher vulnerability to cannabis, alcohol and drug addiction (Hartman et al., 2009; Proudnikov et al., 2010; Zuo et al., 2007), to major...
depression (MD) and/or BD (Chavarría-Siles et al., 2008; Monteleone et al., 2010) as well as to neuroticism (Juhasz et al., 2009) and poor response to antidepressant therapy (Domschke et al., 2008).

While CNR1 has been extensively studied, only few authors explored the role of CNR2 in psychiatric disorders. A significant association between CNR2 Arg63Gln polymorphism and eating disorder, depression and drug addiction and between low CB2 receptor activity and increased risk for schizophrenia has been reported in the Japanese population (Ishiguro et al., 2007, 2010; Onaivi, 2006; Onaivi et al., 2008).

Although these and other evidences (Leweke and Koethe, 2008; Monteleone et al., 2010) suggest that the ECS might be also involved in the onset of BD, until now the role of CNR2 polymorphisms has not been examined.

In this study we tested the hypothesis that genetic variants of CNR2 might be associated with BD. In particular, by means of a case–control study the frequencies of three missense SNPs, namely rs2501432 (315A>G; Arg63Gln), rs41311993 (524C>N; Leu133Ile) and rs2229579 (1073C>T; Tyr316His) (Fig. 1) have been assessed in a sample of 80 BD patients and 160 healthy control.

2. Materials and methods

2.1. Sample

BD patients were recruited between September 2009 and November 2010 from adult outpatients in treatment at the Psychiatric Clinic of the University of Pisa. The objectives and the protocol of the study were thoroughly explained to potential participants and 80 subjects signed a written consent and were submitted to a clinical interview, for diagnostic assessment, evaluated by the clinicians, using the Structured Clinical Interview for DSM-IV axis-I disorders (SCID-I/P) (First et al., 1995). Socio-demographic information and biological samples for DNA analysis were also collected.

A total of 160 age and gender matched subjects (in a ratio 1:2 to BD patients) were selected as control group within a population sample of 366 subjects recruited among healthy donors. They gave their written consent as well. Study protocol was approved by the Ethical Committee of the Azienda Ospedaliero-Universitaria di Pisa, according to the code of Ethics of the World Medical Association (Declaration of Helsinki).

2.2. DNA sample analyses

DNA was extracted from blood or buccal cell samples obtained from all participants with JETquick Blood & Cell Culture DNA Spin (Genomed, Löhne, Germany). The CNR2 315A−G (Arg63Leu) genotype was determined by restriction fragment length polymorphisms (RFLP) method after PCR amplification of the CNR2 region containing the SNP, with the following primers: Fw 5′ CAC AGA GGC TGT GAA GGT CA 3′, Rev 5′ GGA GGA ATG CTG GGT GAC 3′ (amplicon length 359). Amplification conditions were: 95 °C 10 min for pre-denaturation, followed by 35 cycles of 95 °C 30 s, 64 °C 30 s and 72 °C 30 s and a single cycle of 72 °C for 10 min in a GeneAmp PCR System 2400 (Perkin Elmer, Massachusetts, USA); reaction contained 10 μl genomic DNA (2 ng/μl), 1 μl Primer F (10 μM), 1 μl Primer R (10 μM) (Sigma Aldrich, Milan, Italy), 2 μl Buffer BD 10×, 1.2 μl MgCl2 (25 mM), 2 μl dNTP 2 mM, 2.5 μl H2O and 0.3 μl Taq HOT FIREPol DNA Polymerase (Solis BioDyne, Tartu, Estonia). PCR amplicons were digested with Ple I (Fermentas International Inc., Burlington, Canada) overnight and then separated by 2% agarose (SIGMA Aldrich, Milan, Italy) gel electrophoresis.

The genotypes for the CNR2 524C−A (Leu133Ile) and 1073C−T (Tyr316His) were determined by Allelic Specific Oligonucleotide-PCR (ASO-PCR) protocols with the following

Fig. 1. Amminocid changes in the CB2 sequence corresponding to the three polymorphisms under investigation: rs2501432 (315A−G; Arg63Gln), rs41311993 (524C−A; Leu133Ile) and rs2229579 (103C−T; Tyr316His).
primers: for 524C>A (Leu133Ile) Fw 5′ CCT AGG ACT GGT GGC TG 3′, Rev1 5′ CCC TCA CAT ACT TCT TCC AGT G 3′, Rev2 5′ CCC TCA CAT ACT TCT TCC AGT A3′ (amplicon length 201); for 1073C>T (Tyr316His) Fw 5′ GAT TCC GGA AAA GAG GAA G 3′, Rev1 5′ ACC GCC ATT GAC CGA TAC C 3′, Rev2 5′ ACC GCC ATT GAC CGA TAC A 3′ (amplicon length 263). Amplification conditions were the same as above, except for the annealing temperature which was 63 °C for 1073C>T and 55 °C for 524C>A.

All primers used in this study were designed with Primer Blast Program (http://www.ncbi.nlm.nih.gov/tools/primer-blast).

2.3. Statistical and computer analysis

Deviation from the mean was reported as standard deviation. Deviation of the observed allele and genotype distribution from Hardy–Weinberg equilibrium were tested by Chi squared Test and the differences in allele frequencies distribution from Hardy–Weinberg equilibrium were tested by Fisher’s Exact test on 2×2 contingency tables. Odds Ratios (OR) and 95% Confidence Intervals (95% C.I.) were calculated by logistic regression analysis. All analyses were performed with STATA software (StataCorpLP, College Station, Texas, USA). P<0.05 was considered the limit of significance.

3. Results

Clinical and socio-demographic characteristics of the study samples are summarized in Table 1 (top of table). The two groups did not significantly differ as regards age and gender distribution.

Allele frequencies for CNR2 1073C>T resulted p(C)=0.79 and p(T)=0.21, and these values are comparable to those reported in the NCBI database, referring to HapMapCEU and p(T)=0.21, and these values are comparable to those reported in the NCBI database, referring to HapMapCEU. For CNR2 315A>G p(G)=0.56 and p(A)=0.44 are in accordance with Onaivi et al. (2008). The frequencies for CNR2 524C>A were p(C)=0.96 and p(A)=0.04, but no comparison could be made with literature or DNA sequence databases.

None of the genotype distributions deviates from those predicted by Hardy–Weinberg equilibrium in healthy controls (p=0.91 for CNR2 1073C>T; p=0.42 for CNR2 315A>G and p=0.62 for CNR2 524C>A) and in BD patients (p=0.23 for CNR2 1073C>T; p=0.55 for CNR2 315A>G and p=0.11 for CNR2 524C>A). The comparison between patients and healthy group shows that allele frequencies were significantly different for CNR2 524C>A (p(χ²)=0.001) while there was no significant difference for CNR2 315A>G (p(χ²)=0.15) or 1073C>T (p(χ²)=0.21). OR values and their 95% C.I. are shown in Table 1 (bottom of table).

4. Discussion

In the present case-control study, we assessed the presence of three CNR2 polymorphisms in an Italian population sample, where at least two of them show relatively high frequencies of the less common allele (~17%). In addition, for the first time a significant association was observed between BD and the CNR2 524C>A, supporting the hypothesis that CB2 is involved in the pathogenetic mechanism underlying this affective disorder.

Our results, taken together with those reported for FAAH by Monteleone et al. (2010), provide an interesting evidence of a role for the ECS in mood regulation and dysfunction.

No statistically significant difference was observed between BD and control groups for the other two SNPs, despite that the CNR2 315A>G had been reported to be associated with major depression (MD) in Japanese population by Onaivi et al. (2008). A similar discrepancy was reported by Monteleone et al. (2010) about the CNR1 1359G>A which resulted significantly associated to MD, but not to BD. These findings suggest that the

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Sex (F/M)</th>
<th>Age ± S.D.</th>
<th>Age at onset ± S.D.</th>
<th>N of episodes mean ± S.D.</th>
<th>First episode</th>
<th>Bipolar disorder (I/II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>54/106</td>
<td>38.0 ± 7.5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>BD patients</td>
<td>27/53</td>
<td>42.5 ± 12.15</td>
<td>25.3 ± 6.6</td>
<td>3.32 ± 2.11</td>
<td>Maniacal 26</td>
<td>BD I 69</td>
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<td>Depression 22</td>
<td>BD II 11</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mixed 26</td>
<td>Ipomaniacal 6</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype</th>
<th>Control</th>
<th>Case</th>
<th>Allele frequencies</th>
<th>OR (95% C.I.)</th>
<th>P (χ²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1073C&gt;T</td>
<td>rs2229579</td>
<td>99</td>
<td>56</td>
<td>Control P(C)=0.79</td>
<td>0.70 (0.39–1.24)</td>
<td>0.21</td>
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<td></td>
<td></td>
<td>54</td>
<td>20</td>
<td>Case P(T)=0.21</td>
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<td>7</td>
<td>4</td>
<td>Control P(T)=0.18</td>
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<td>160</td>
<td>80</td>
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<tr>
<td>315A&gt;G</td>
<td>rs2501432</td>
<td>52</td>
<td>30</td>
<td>Control P(G)=0.56</td>
<td>0.80 (0.46–1.41)</td>
<td>0.15</td>
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<td></td>
<td></td>
<td>74</td>
<td>40</td>
<td>Case P(A)=0.44</td>
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<td></td>
<td></td>
<td>34</td>
<td>10</td>
<td>Control P(G)=0.62</td>
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<td></td>
<td></td>
<td>160</td>
<td>80</td>
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<tr>
<td>524C&gt;A</td>
<td>rs41311993</td>
<td>148</td>
<td>55</td>
<td>Control P(C)=0.96</td>
<td>4.74 (2.30–9.78)</td>
<td>0.001</td>
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<tr>
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<td>12</td>
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<td>Case P(A)=0.04</td>
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<tr>
<td></td>
<td></td>
<td>0</td>
<td>5</td>
<td>Control P(A)=0.81</td>
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<td>160</td>
<td>80</td>
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S.D., standard deviation; OR, odds ratio; C.I., confidence interval.
components of the ECS might play different roles in the two mood disorders, and this merits deeper investigation because it might reveal interesting specificities of these nosological entities, being potentially helpful for a more accurate diagnosis as well as for a better therapeutic choice.

The CNR2 524C–A substitution leads to the aminoacid change Leu133Ile which has been suggested to influence the stability and/or functionality of the CB2 receptor. In fact, Leu133 provides a hydrophobic residue involved in an intra-chain bond in the transmembrane domains and its presence is thought to be crucial for the stability of the receptor and may influence the receptor-G protein coupling (Xie et al., 2003).

Even though CB2 receptor is also present on the neuron surface, its highest level of expression in the CNS was found in microglial cells (Patel et al., 2010). The involvement of these cells in a number of neurodegenerative and psychiatric disorders has been related to the release of pro-inflammatory cytokines (Shie et al., 2011). Given that a neuroimmunological and inflammatory basis has been proposed also for BD (Goldstein et al., 2009), the role played by CB2 in modulating immune response in the brain might be relevant, as suggested by the observation that its stimulation by endogenous mediators or by agonist drugs decreases microglial activation and inflammation (Rivers and Ashton, 2010). A stimulation of CB2 receptors and decrease of microglial activation might be expected also by medicinal and food plant lipids (Gertsch, 2008) with a potential intriguing link between CB2 cannabinoid receptor, nutrition and psychiatric diseases.

Finally, one would assume that a reduced stability and/or functionality of CB2 receptor, related to Leu133Ile substitution, would act in the opposite direction, by determining a defective microglial activation control, sustaining inflammatory processes in the CNS and promoting the onset of BD. If this hypothesis will be confirmed, CB2 receptor could represent an interesting target for new drugs to be developed for the treatment of BD and other psychiatric disorders.

5. Conclusion

In summary, we found a significant association between CNR2 524C–A and BD, supporting the hypothesis that ECS plays a role in this affective disorder and suggesting that the CB2 cannabinoid receptor could be a potential target for developing new therapeutic options in mood disorders.

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Conflict of interest

All authors declare no conflict of interest.

References


