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# Investigation of *CNR1* and *FAAH* endocannabinoid gene polymorphisms in bipolar disorder and major depression

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ABSTRACT

Experimental data suggest that the endogenous cannabinoid system is involved in mood regulation, but no study has been performed so far to investigate the role of endocannabinoid genes in the susceptibility to major depression (MD) and/or bipolar disorder (BD). We assessed the CB1 receptor gene (*CNR1*) single nucleotide polymorphism (SNP) rs1049353 (1359 G/A) and the *fatty acid amide hydrolase* (*FAAH*) gene rs324420 SNP (cDNA 385C to A) for their associations with MD and/or BD in 83 Caucasian patients with recurrent MD, 134 Caucasian individuals with BD, and 117 Caucasian healthy subjects. The distribution of the *CNR1* 1359 G/A genotypes and alleles significantly differed among the groups ($\chi^2 = 12.595$; df = 4, $P = 0.01$ for genotypes; $\chi^2 = 13.773$; df = 2, $P = 0.001$ for alleles) with MD patients showing a higher frequency of both AG, GG genotypes and A allele as compared to healthy controls. The distribution of the *FAAH* cDNA 385C to A genotypes, according to the CC dominant model (AA + AC vs. CC), significantly differed among the groups ($\chi^2 = 6.626$; df = 2, $P = 0.04$), with both BD patients and MD patients showing a non-significant slightly higher frequency of the AC genotype. These findings, although preliminary, suggest that the *CNR1* 1359 G/A and the *FAAH* cDNA 385C to A gene variants may contribute to the susceptibility to mood disorders.



## 1. Introduction

The endocannabinoid system, consisting of two cannabinoid receptors ($CB_1$ and $CB_2$), the endogenous ligands anandamide (arachidonoylethanolamide, AEA) and 2-arachidonoylglycerol (2-AG), and the endocannabinoid degrading enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase, is involved in several physiological functions, including mood regulation [1–3].

Experimental data suggest that blocking the endocannabinoid system induces depressive-like behaviours in animals [4,5]. To the contrary, administration of $CB_1$ receptor agonists or endogenous cannabinoid uptake inhibitors or inhibitors of the FAAH enzyme results in antidepressant-like effects and augments the efficacy of the antidepressant fluoxetine in experimental animals [6–8]. $CB_1$ receptors are highly expressed in limbic and frontal brain structures, brain areas implicated in the pathophysiology of depression, and regulate serotonin and norepinephrine release [9]. Moreover, it has been shown that different chronic antidepressant treatments potentiate $CB_1$ receptor activity in several brain areas including the prefrontal cortex, the hippocampus and the hypothalamus [10]. Furthermore, the $CB_1$ receptor gene, termed *CNR1*, is under negative regulation by glucocorticoids [11], supporting the view that the endogenous cannabinoid system may become blunted in individuals who suffer from hypercortisolemia, such as that seen in major depression (MD). All these findings suggest that the endogenous cannabinoid system regulates emotional behaviour to some degree through activation of the $CB_1$ receptor, and that enhancement of this system may be effective in reducing symptoms of depression. However, it should be noted that some studies have demonstrated an antidepressant-like effect of a $CB_1$ receptor antagonist, but these experiments were carried out with elevated doses of the anatgonist [12–14]. Finally, in bipolar disorders (BD), possible therapeutic effects of cannabinoids have been suggested although not proved yet [15].

*Abbreviations:* AEA, arachidonoylethanolamide; 2-AG, 2-arachidonoylglycerol; ANOVA, analysis of variance; BD, bipolar disorder; BMI, body mass index; FAAH, fatty acid amide hydrolase; MD, major depression; SCID-IP, Structured Clinical Interview for DSM IV-Patient Edition; SNP, single nucleotide polymorphism.

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Several genetic variants of the *CNR1* gene and of the *FAAH* gene have been identified [16]. The *CNR1* rs1049353 single nucleotide polymorphism (SNP) (1359 G/A), although synonymous (i.e. not changing the amino acid sequence of the mature protein) could affect the mRNA stability or translation, which might result in an alteration of CB1 receptor function. The rs324420 SNP (cDNA 385C to A) of the *FAAH* gene has been shown to reduce the activity of the FAAH enzyme [17]. Based on the above evidence of endocannabinoid involvement in mood regulation, the possibility exists that those gene variants might have a role in the susceptibility to mood disorders. Therefore, we investigated the CNR1 rs1049353 SNP (1359 G/A) and the FAAH gene rs324420 SNP (cDNA 385C to A) for their associations with MD and/or BD.

## 2. Materials and methods

### 2.1. Subjects

The study was carried out with patients consecutively attending the outpatients units of the Departments of Psychiatry of the Universities of Naples and Turin (Italy).

A total of 217 Caucasian patients (142 women and 75 men) were enrolled into the study: 153 were recruited in Naples (South Italy) and 64 in Turin (North Italy). Eighty-three (65 women and 18 men) of them met the DSM-IV diagnosis of recurrent MD and 134 (77 women and 57 men) the diagnosis of BD (79 had a type I BD and 55 had a type II BD), as confirmed by the Structured Clinical Interview for DSM IV-Patient Edition (SCID-IP) [18]. Six MD patients had a lifetime comorbid anxiety disorder (3 had a panic disorder, 1 had a post-traumatic stress disorder and 2 had a simple phobia) and 1 had a lifetime substance abuse disorder; 9 BD patients had a lifetime comorbid anxiety disorder (6 had a panic disorder, 1 had an obsessive-compulsive disorder and 2 had a simple phobia), 4 had a lifetime eating disorder and 12 had a lifetime substance abuse disorder.

A group of 117 Caucasian healthy subjects (91 women and 26 men) were also recruited: 108 were enrolled in Naples and 9 in Turin. They were mentally healthy as assessed by the SCID-non-patient edition [19]. Both patients and healthy controls were clinically screened to exclude medical and neurological disorders. Twelve patients with MD [body mass index (BMI) range: 25.0–31.08 $kg/m^2$], 15 patients with BD (BMI range: 25.17–42.9 $kg/m^2$) and 3 healthy controls (BMI range: 25.32–28.73 $kg/m^2$) were overweight/obese.

All of the subjects provided written informed consent to participate into the study after a complete description of the study procedure. The study was approved by the Ethics Committee of the Second University of Naples and procedures were in accordance with the Helsinki declaration of 1975.

### 2.2. Clinical and laboratory procedures

Diagnostic assessments were made by investigators (psychiatrists and psychologists) who were formally trained in the use of the SCID-I (Cohen's kappa = 1).

From all subjects, 20 ml of venous blood was drawn into EDTA vacuum tubes and immediately frozen at −20 °C till the extraction of genomic DNA from nucleated white blood cells.

The 1359 (G/A) polymorphism, resulting in the substitution of G to A at nucleotide position 1359 in codon 435 (Thr), was tested by amplification with multiplex-PCR as previously described [20]. We used PCR primers F1-F (5′-AAGACGGTGTTTGCATTCTG-3′) and THAC-R (5′-AAATTCTTTTCCTGTGCTGCCAGGGAG-3′), and allele-specific primers A3-F (5′-AGTGAGAGTTGCATCAAGAGCACA-3′) and T1-R (5′-GACTTGGCAATCTTGACT-3′) or G4-F (5′-AGTGAGAGTTGCATCAAGAGCACG-3′) and C2-R (5′-GACTTGGCAATCTTGACC-3′) to detect the polymorphic A allele or the G allele, respectively.

The polymorphic region of *FAAH* gene was amplified by PCR with 3% dimethyl sulfoxide and 0.75 units of Taq DNA polymerase in a total volume of 15 μl reaction mixture using the following primer sets: 5′-ATG TTG CTG GTT ACC CCT CTC C-3′ and 5′-CAG GGA CGC CAT AGA GCT G-3′. Initial denaturation was performed for 5 min at 95 °C. Then 35 cycles were performed (30 s of denaturing at 95 °C, 30 s of annealing at the appropriate temperature, and 30 s of extension at 72 °C), followed by a final extension at 72 °C for 5 min. The PCR products when then digested with EcoO109I and analyzed on 3.0% agarose gels.

Because of technical drawbacks, not all the subjects had both SNPs characterization (Table 2).

### 2.3. Statistical analyses

One-way analysis of variance (ANOVA) was used to assess differences in the mean age of the subject groups. Association tests were performed by using the Pearson's $\chi^2$ test for comparison of genotype and allele frequencies between patients and healthy controls. For each SNP the odds ratio (OR) (95% CI) for the "at risk allele" (the allele that appeared more frequently in patients than controls) was calculated.

## 3. Results

Clinical and demographic characteristics of the study sample are shown in Table 1. No significant difference emerged among the groups in the mean ages of the subjects ($F_{2,331} = 2.62$, $P = 0.07$). The distribution of male and female subjects significantly differed among the groups ($\chi^2 = 16.04$; df = 2, $P = 0.0003$) with a higher percentage of men in the BD group.

The distribution of the *CNR1* 1359 (G/A) genotypes did not deviate from the Hardy–Weinberg equilibrium in both healthy controls ($\chi^2 = 2.403$; df = 1, $P$ = NS), BD patients ($\chi^2 = 0.057$; df = 1, $P$ = NS) and recurrent MD subjects ($\chi^2 = 1.687$; df = 1, $P$ = NS). Genotype and allele frequencies significantly differed among diagnostic groups ($\chi^2 = 12.595$; df = 4, $P = 0.01$ for genotypes; $\chi^2 = 13.773$; df = 2, $P = 0.001$ for alleles) (Table 2). When each patient group was compared with the control group, genotype and allele frequencies were significantly different from healthy subjects in MD patients ($\chi^2 = 10.321$; df = 2, $P = 0.005$ for genotypes; $\chi^2 = 12.469$; df = 1, $P = 0.0004$ for alleles), but not in BD patients as a whole group ($\chi^2 = 0.926$; df = 2, $P = 0.6$ for genotypes; $\chi^2 = 1.05$; df = 1, $P = 0.3$ for alleles) or in type I BD subjects ($\chi^2 = 0.225$; df = 2, $P = 0.8$ for genotypes; $\chi^2 = 0.016$ df = 1, $P = 0.9$ for alleles) and in type II BD individuals ($\chi^2 = 2.514$; df = 2, $P = 0.2$ for genotypes; $\chi^2 = 3.075$ df = 1, $P = 0.08$ for alleles). Indeed, as compared to healthy controls, MD patients showed a higher frequency of AG and GG genotypes and of the A allele (Table 2); the OR for the A allele was 2.46 with 95% confidence interval (CI) = 1.46–4.137.

The distribution of the *FAAH* cDNA 385C to A genotypes deviated from the Hardy–Weinberg equilibrium in BD patients ($\chi^2 = 5.399$; df = 1, $P < 0.05$) but not in healthy controls ($\chi^2 = 0.36$; df = 1, $P$ = NS) and in MD subjects ($\chi^2 = 3.834$; df = 1, $P$ = NS). Since, the AA genotype of the *FAAH* SNP was present only in one healthy subject, the association analyses were performed according to the CC dominant model (AA + AC vs. CC). Genotype frequencies among diagnostic groups significantly differed ($\chi^2 = 6.626$; df = 2, $P = 0.04$), with both BD patients ($\chi^2 = 3.242$; df = 1, $P = 0.07$) and MD patients ($\chi^2 = 3.748$; df = 1, $P = 0.05$) showing a non-significant slightly higher frequency of the AC genotype (Table 2). Similarly, genotype frequencies did not significantly differ from healthy controls in both type I