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No association of endocannabinoid genes with bipolar disorder or lithium response in a Sardinian sample

Claudia Pisanu [a], Donatella Congiu [a], Marta Costa [a], Massimiliano Sestu [a], Caterina Chillotti [b], Raffaella Ardau [b], Valeria Deiana [b], Mirko Manchia [a,c], Alessio Squassina [a,*], Maria Del Zompo [a,b,d]

[a] Section of Neurosciences and Clinical Pharmacology, Department of Biomedical Sciences, University of Cagliari, Italy
[b] Unit of Clinical Pharmacology, Teaching Hospital, Cagliari, Italy
[c] Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada
[d] Institute of Neuroscience, CNR, Cagliari, Italy

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ABSTRACT

Bipolar disorder (BD) is a chronic and severe psychiatric condition with an underlying component of genetic susceptibility. Mounting evidence suggests a potential role of the endogenous cannabinoid (eCB) system in the pathogenesis of BD. Here we investigated the role of genes encoding for key eCB elements on the risk of developing BD in a sample of 357 BD patients and 422 healthy controls of Sardinian ancestry. Using the HapMap CEU population SNP database, we selected 25 tag Single Nucleotide Polymorphisms (tSNPs) in N-acyl phosphatidylethanolamine phospholipase D (*NAPE-PLD*), cannabinoid receptor 1 (*CNR1*) and fatty acid amide hydrolase (*FAAH*) genes. No significant association was reported for *FAAH* or *CNR1*. SNPs rs11487077 and rs6465903 in *NAPE-PLD* showed nominal association ($p=0.033$ and $p=0.026$, respectively) with BD, not significant after permutation testing. These SNPs were also tested for association with lithium response in 204 BD patients characterized for response to long-term lithium treatment, reporting no significant findings. As a whole, our results do not support a clear role of *FAAH*, *CNR1* and *NAPE-PLD* in BD and lithium response. Additional studies on independent, larger samples are warranted to further explore the involvement of the eCB system in BD.

1. Introduction

Bipolar disorder (BD) is a common and severe psychiatric disorder affecting 0.7–4% of the general population (Merikangas et al., 2007). Several lines of research have suggested the involvement of an altered endogenous cannabinoid (eCB) system in BD (Leweke and Koethe, 2008; van Rossum et al., 2009). This system consists of the receptors CB1 and CB2, their ligands and the enzymes responsible for the biosynthesis (N-acyl phosphatidylethanolamine phospholipase D: NAPE-PLD) and inactivation (fatty acid amide hydrolase: FAAH) of cannabinoids (Di Marzo et al., 2004). In the central nervous system (CNS), CB1 receptors are distributed mainly in the cerebral cortex, hippocampus, basal amygdala and corpus striatum (Di Marzo et al., 2004). These areas appear to be involved in the pathophysiology of BD as shown by neuroimaging studies indicating reduced prefrontal cortical volumes (López-Larson et al., 2002; Phillips et al., 2003) and enlarged amygdala (Strakowski et al., 1999) and caudate volumes (Aylward et al., 1994) in BD patients. BD has the highest rate of substance abuse comorbidity among axis I disorders, with cannabis being the most commonly abused drug in these patients (Leweke and Koethe, 2008). Cannabis derivate cannabidiol (CBD) has been suggested to have potential antipsychotic, antidepressant and anxiolytic properties (Zuardi, 2008). However, heavy cannabis use in BD patients is associated with more severe forms of the disease, such as early onset (Lagerberg et al., 2011) and psychotic symptoms (van Rossum et al., 2009). Aiming to explore the possible involvement of eCB genes in susceptibility to BD we carried out a case-control association study between 25 tag SNPs located in *FAAH*, *CNR1* (the CB1 receptor gene) and *NAPE-PLD* genes and BD in a Sardinian sample of 357 BD patients and 422 controls. These SNPs were also tested for association with response to long-term lithium treatment in a subset of 204 BD patients.

* Corresponding author. Tel.: +39 70 6754323; fax: +39 70 675 4320.
E-mail address: squassina@unica.it (A. Squassina).

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Table 1
Demographic relevant information of the sample.

Variable	Total sample		Lithium response	
	Cases (357)	Controls (422)	FR (60)	Others (144)
Age, mean ± S.D.	44.09 ± 14.35	42 ± 14.21	51,25 ± 15.59	45,71 ± 13.59
Gender (M/F)	134/223	217/205	19/41	46/98
Diagnosis, *n* (%)				
BD I	291 (81.5%)	–	38 (63.3%)	115 (79.9%)
BD II	66 (18.5%)	–	22 (36.7%)	29 (20.1%)
Age at onset, mean ± S.D.	26.29 ± 10.41	–	28.76 ± 12.25	25.90 ± 9.99
Duration of illness	17.62 ± 11.23	–	22.49 ± 10.14	19.81 ± 11.05
Duration of lithium treatment	–	–	8.05 ± 8.15	9.21 ± 6.15
TS, mean ± S.D.	–	–	7.69 ± 0.93	2.66 ± 2.36

Abbreviations: FR—full responders; BD I—bipolar disorder type I; BD II—bipolar disorder type II; S.D.—standard deviation; and TS—total score

2. Material and methods

2.1. Sample

Cases comprised 291 BD type I and 66 BD type II patients (Table 1) diagnosed by trained clinical psychopharmacologists according to DSM-IV criteria and the Schedule for Affective Disorder and Schizophrenia-Lifetime Version (SADS-L) (Endicott and Spitzer, 1978). Of the 357 patients, 204 were characterized for lithium response using the "Retrospective Criteria of Long-Term Treatment Response in Research Subjects with Bipolar Disorder" as described previously (Grof et al., 2002).

Details about the application of the scale to our sample has been previously described (Squassina et al., 2011). Briefly, this validated scale quantifies the degree of improvement in the course of treatment with a score from 0 to 10 (total score, TS). Patients with a TS equal to 7 or higher are considered full responders (FR) (Manchia et al., 2013). Sixty patients were classified as FR and 144 as partial or non-responders (labeled as 'others'). Healthy controls included 422 subjects (Table 1) recruited from blood donors screened for the absence of personal or family history of psychiatric disorders. Cases and controls were of Sardinian ancestry for at least four generations. The research protocol was approved by the local Ethics Committee, and after a detailed description of the study procedures all participants signed informed written consent.

2.2. SNPs selection and genotyping

We selected 25 tag Single Nucleotide Polymorphisms (tSNPs) in *FAAH*, *CNR1* and *NAPE-PLD* genes using information from the HapMap database (http://www.hapmap.org, Release 22/Phase II, CEU population), applying the aggressive tagging 2-marker haplotype algorithm ($r^2 \geq 0.8$), implemented in Haploview, version 4.2 (Barrett et al., 2005). Polymorphisms with a minor allele frequency (MAF) < 0.01 were excluded from the analyses. Genomic DNA was extracted from peripheral blood leukocytes using the salting-out method (Lahiri and Nurnberger, 1991) and all SNPs genotyped with the Kaspar method (www.kbioscience.co.uk).

2.3. Statistical analyses

Differences in demographic variables between cases and controls were evaluated with *t*-test or chi-square (χ^2) test. Deviation from the Hardy-Weinberg equilibrium (HWE) was tested with χ^2 test. As gender distribution significantly differed between cases and controls, the genotypic association analysis was tested using logistic regression under the additive model with sex as a covariate. The association between genotypes and lithium response was assessed with logistic regression comparing FR versus others. All *p* values were adjusted for multiple comparisons by permutation test with 10,000 permutations.

Linkage disequilibrium (LD) between markers was measured by the Lewontin coefficient (D') and Pearson's correlation coefficient (r^2).

All the analyses were carried out with PLINK, version 2.07 (Purcell et al., 2007) and Haploview.

The statistical power of our sample was evaluated using PS-Power and Sample Size Calculation Software, version 2.1.30 (Dupont and Plummer, 1998). At a significance level of 0.05, our sample had 80% power of detecting a genetic variant with an odds ratio (OR) of 1.83, assuming a MAF of 0.1, or with an OR of 1.5 assuming a MAF of 0.4.

Table 2
Genotypic association analysis using logistic regression under the additive model with sex as a covariate in the sample of 357 BD patients and 422 controls.

Gene	SNP	Alleles	MA	OR	β	P
FAAH	rs17361936	T/C	T	1.02	0.18	0.855
	rs6429600	G/A	G	0.84	−1.25	0.210
	rs4141964	A/G	A	0.95	−0.42	0.677
	rs324420	A/C	A	0.83	−1.23	0.218
	rs324419	A/G	A	1.04	0.32	0.749
	rs11576941	T/G	T	1.03	0.26	0.793
CNR1	rs806365	C/T	C	0.99	−0.07	0.946
	rs7766029	C/T	C	0.94	−0.60	0.552
	rs806366	T/C	T	0.98	−0.21	0.833
	rs806368	G/A	G	1.09	0.76	0.447
	rs12720071	G/A	G	1.03	0.23	0.818
	rs1049353	A/G	A	0.89	−0.87	0.385
	rs806374	C/T	C	1.13	1.15	0.250
	rs806375	T/A	T	0.91	−0.90	0.370
	rs806376	C/T	C	0.90	−1.00	0.316
	rs6454672	C/T	C	0.85	−1.10	0.268
	rs9450898	T/C	T	0.83	−1.35	0.176
	rs806380	G/A	G	0.97	−0.31	0.757
NAPE-PLD	rs13232194	T/A	T	1.16	1.19	0.232
	rs17605251	G/A	G	0.87	−0.96	0.337
	rs11487077	C/A	C	1.55	2.13	**0.033***
	rs12540583	C/A	C	0.92	−0.39	0.700
	rs6465903	A/C	A	1.42	2.22	**0.026***
	rs7789727	A/G	A	1.21	0.55	0.581

Abbreviations: MA—minor allele and OR—odds ratio

* Not significant after permutation testing.

3. Results

Call rates were 100% for all SNPs. No significant deviation from HWE was observed in any of the 25 tSNPs. The NAPE-PLD SNP rs1477160 had a MAF lower than 0.01 and was excluded from the analyses. Allele frequencies in our sample were similar to those reported for Caucasians in HapMap.

Logistic regression analysis showed nominal association between two SNPs located in NAPE-PLD and BD (rs11487077, $p=0.033$; and rs6465903, $p=0.026$; Table 2), but not with lithium response.

Separating the samples into two groups of BD I and BD II showed that these two SNPs were nominally associated with BD I but not with BD II. However, odds ratio (OR) was not significantly increased in comparison to the whole sample (SNP rs11487077, OR = 1.55 in the whole sample and 1.63 in the BD I sample; SNP rs6465903, OR = 1.42 in the whole sample and 1.44 in the BD I sample). None of the SNPs of CNR1 or FAAH showed nominal association with BD or lithium response.

Haplotype analyses showed that all the five SNPs of NAPE-PLD laid in one block. Of the 6 possible combinations of NAPE-PLD, only haplotype TACAA (rs11487077, rs6465903 and rs13232194) showed nominal association with BD ($p=0.011$; Table 3) but not