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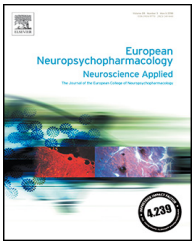

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SHORT COMMUNICATION

Gene-environment interaction between an endocannabinoid system genetic polymorphism and cannabis use in first episode of psychosis

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Abstract
Alterations of the endocannabinoid system (ECS) may play an important role in the development of schizophrenia and other psychotic disorders. Cannabis use is one of the environmental factors more repeatedly related to an increase the risk of developing a psychotic episode, while its use modifies the ECS normal function. In the present study we purposed to examine the gene by environment (GxE) interaction between 15 selected single nucleotide polymorphisms (SNPs) related to the ECS and cannabis use in a cohort of 321 patients with a first episode of psychosis (FEP) and 241 matched healthy controls. We found the fatty-acid amide hydrolase (FAAH) rs2295633 SNP genetic polymorphism was associated with a greater risk of presenting a FEP in subjects with relevant cannabis use, but not in subjects without a history of cannabis use. The probability of presenting a FEP was tenfold higher (OR: 10.69) in cannabis users who were homozygote carriers of the T allele of the FAAH rs2295633 SNP, compared to users of cannabis without this genotype. We also found that a higher a proportion of TT carriers of the FAAH rs2295633 SNP with a positive history of cannabis use was treated with high potency antipsychotic. This study has identified a GxE-environment interaction between a genetic polymorphism from the ECS and cannabis use involved in the risk of presenting a FEP. Although this preliminary data should be replicated with independent samples, our results highlight the importance of the pro-psychotic effects of exogenous cannabis use over the ECS in certain subjects.

1. Introduction

Biological foundations of psychotic disorders include an extensive genetic predisposition, with an estimated heritability around eighty percent (Schizophrenia Working Group of the Psychiatric Genomics, 2014). Genetic studies have linked many genetic variants with schizophrenia related disorders, but each variant is only associated with a small effect (Schizophrenia Working Group of the Psychiatric Genomics, 2014). Therefore, an increasing attention has recently shifted to the weight of environmental risk factors, such as infections during pregnancy, migration, cannabis use or urbanicity, which can carry on a two to four fold increase in risk (van Os et al., 2014). Gene-environment interaction (GxE) has been proposed as a causal mechanism where genetic variants and environmental factors contribute to the causation of a condition, in which the effect of environmental exposure depends on individual's genotype (van Os et al., 2014).

The endocannabinoid system (ECS) may play an important role in FEP physiopathology. Cerebrospinal Fluid (CSF) levels of anandamide (AEA), one of the endogenous ligands of the ECS, were found elevated in subjects with acute schizophrenia (Giuffrida et al., 2004). Higher levels of CSF AEA were also associated with a lower risk of psychotic symptoms following cannabis use in volunteers (Morgan et al., 2013). The remission of psychotic symptoms has been associated with a significant decrease AEA levels and cannabinoid receptor 2 (CB2) mRNA transcripts in peripheral blood mononuclear cells (PBMC) (de Campos-Carli et al., 2017; De Marchi et al., 2003). The antipsychotic effect of cannabidiol has also been related to the inhibition of AEA deactivation (Leweke et al., 2012). Our group described a peripheral ECS dysregulation in subjects with a FEP compared to healthy controls (Bioque et al., 2013), associated to certain cognitive deficits (Bioque et al., 2016). Postmortem studies on brain tissues have shown a genetically predetermined rela-

tionship between lower functioning of CB2 receptors (polymorphism Q63R) and increased risk of schizophrenia when combined with other risk factors (Ishiguro et al., 2010), together with local specific alterations in some endocannabinoids' levels (Muguruza et al., 2013). Finally, neuroimaging studies have showed a reduced CB1 expression and activity in certain brain areas of patients with schizophrenia (Eggan et al., 2008; Wong et al., 2011).

Besides, it is well-known that cannabis use approximately doubles the risk of developing a FEP (McGrath et al., 2010), largely modifying the ECS normal function (Fakhoury, 2016).

The purpose of this study is to examine the interaction between cannabis use and fifteen selected SNPs from the ECS in our cohort of FEPs and matched healthy controls.

2. Experimental procedures

2.1. Subjects

During the recruitment period (2009–2012), 335 subjects who have presented a FEP and 253 matched healthy controls were included in the PEPs Project. Healthy controls were matched by age (± 10%), gender and parental socio-economic status (SES). The inclusion criteria for patients were: age between 7 and 35, presence of a FEP in the last 12 months and speak Spanish correctly. The exclusion criteria for patients were: (1) mental retardation according to DSM-IV criteria, (2) history of head trauma with loss of consciousness and (3) presence of an organic disease with mental repercussions. The exclusion criteria for controls were the same as for patients plus (1) having a history of psychotic and/or major affective disorder and (2) having a first degree relative with psychotic disorder history. The rationale and the complete clinical protocol used were previously published (Bernardo et al., 2013). The study was approved by the investigation ethics committees of all participating centers. Informed consent was obtained from all participants or legal guardians.

From the initial sample, 321 patients with a FEP and 241 healthy controls had the complete genetic and clinical and toxicological data to be included in the present analyses.

2.2. Clinical assessment

The diagnosis was established by the semi-structured diagnostic interviews following the DSM-IV criteria (American Psychiatric Association, 1994). The psychopathological assessment was performed using validated Spanish versions of the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987). Cannabis use was evaluated using the European Adaptation of a Multidimensional Assessment Instrument for Drug and Alcohol Dependence (EuropAsi) (Kokkevi and Hartgers, 1995). The neuropsychological battery used in the PEPs project was conducted as previously reported (Bernardo et al., 2013; Cuesta et al., 2015).

2.3. Blood samples and genotyping methods

Blood samples were collected in K2EDTA BD Vacutainer EDTA tubes (Becton Dickinson, Franklin Lakes, New Jersey), stored at −20 °C and sent to the central laboratory. DNA was extracted with the MagNA Pure LC DNA isolation Kit III and an LC MagNA Pure system (Roche Diagnostics GmbH, Mannheim, Germany). DNA concentration was determined by absorbance (ND1000, NanoDrop, Wilmington, Delaware). 2.5 ug of genomic DNA was sent for genotyping at the Spanish National Genotyping Centre (CeGen).

2.4. SNP selection, genotyping and quality control

15 SNPs (see Table S1) were selected in three candidate gene regions (cannabinoid receptor 1 (CB1), cannabinoid receptor 2 (CB2), fatty acid amide hydrolase (FAAH) gene region (covering target loci and upstream and downstream regions) following one of the following three strategies (Bernardo et al., 2017): (1) tagging analysis (as implemented in Haploview 4.2) at an r2 threshold of 0.8 to capture 98% of the most common HapMap phase II variants based on the CEU panel (minor allele frequency >0.1) (range 91%-100% for individual genes); (2) suspected SNP functionality according to data published in Ensembl (http://www.ensembl.org), dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/), and PupaSuite 3 (http://pupasuite.bioinfo.cipf.es/) databases, with a validated minor allele frequency >0.1 in the Caucasian population; or (3) a previous association reported in the literature. SNPs were genotyped by the GoldenGate assay with the Veracode genotyping system (Illumina, San Diego, USA) at the Madrid Node of the Spanish National Genotyping Center (CeGen). For quality control, 18 samples were genotyped in duplicate for all the SNPs analyzed, with a 100% concordance rate. Two samples were excluded due to problems in DNA isolation or sample preparation.

2.5. Statistical analysis

Sample size and statistical power were calculated using Quanto1.2.4 software (http://hydra.usc.edu/gxe). Given the simple size, and assuming a 5% level of significance and a 25% of cannabis use in our sample, we were able to detect GxE interactions with an odds ratio values of >3.5 with >80% statistical power when polymorphisms with allele frequencies of >0.1were analyzed.

Differences in sociodemographic and clinical characteristics were assessed using two-tailed $\chi 2$ tests on categorical data and *t*-test for continuous variables. A two-tailed non-parametric Mann-Whitney U test was performed when continuous variables did not meet the assumption of normality in the Kolmogorov-Smirnov test. A principal component analysis grouped neuropsychological variables into four cognitive domains (verbal memory, sustained attention, executive function and working memory).

To estimate the independent contribution of each SNP to the disease susceptibility, genotype frequencies were assessed by multivariate methods based on multiple logistic regression analysis and analyzed under codominant, dominant, overdominant, recessive and additive models, adjusted for the variables identified in the univariate analysis, using the SNPassoc R package (Gonzalez et al., 2007). Hardy-Weinberg equilibrium and LD relationships between polymorphisms were evaluated by Haploview software v.3.2 (http://broad.mit.edu/mpg/haploview). The empirical significance of single SNP or genotype tests was defined using the Bonferroni correction and the significance level was adjusted at 3×10^{-3}. As four genetic models were tested (codominant, dominant, recessive and overdominant), we divided the empirical p-value by four, leading a significance threshold of 8×10^{-4}.

Significant genes were further studied, analyzing whether cannabis use mediates on the increase of the risk of psychosis through multiple logistic regression. Clinical and cognitive characterization of groups segmented according to the gene and cannabis use was done by analysis of variance or *t*-test when appropriate.

3. Results

Table 1 shows the general demographic, clinical and cannabis exposure data of the participants.

Table 2 summarizes the results of the association study performed for each individual SNPs of three candidate genes

Table 1 Demographic and clinical characteristics of the sample.

	First episode psychosis subjects	Control subjects	Statistic	*P*-value
Number	321	241	-	-
Age - years [mean (sd)]	23.67 (5.99)	24.16 (6.41)	$t=-0.93$	0.35
Gender - no. (%)			$\chi^2=1.08$	0.32
Female	105 (32.7)	89 (36.9)		
Male	216 (67.3)	152 (63.1)		
Socioeconomic Status - no. (%)			$\chi^2=32.31$	<0.001*
High	60 (18.7)	50 (20.7)		
Medium-High	37 (11.5)	48 (19.9)		
Medium	77 (24)	72 (29.9)		
Medium-Low	93 (29)	62 (25.7)		
Low	46 (14.3)	6 (2.5)		
Unknown	2 (0.6)	2 (0.8)		
Ethnic Group - no. (%)			$\chi^2=11.82$	0.11
Caucasian	273 (85)	220 (91.3)		
Gipsy	5 (1.6)	0 (0)		
Maghrebian	7 (2.2)	2 (0.8)		
Sub-Saharan	4 (1.2)	0 (0)		
Asian	4 (1.2)	1 (0.4)		
Caribbean	8 (2.5)	3 (1.2)		
Hispanic	17 (5.3)	11 (4.6)		
Other	3 (0.9)	4 (0.7)		
Duration of untreated psychosis - days [mean (sd)]	106.56 (148.57)	-	-	-
Diagnosis - no. (%) [a]				
Affective Psychosis	51 (15.9)	-	-	-
Non-affective Psychosis	270 (84.1)			
Cannabis				
Lifetime use - no. (%)	166 (51.7)	66 (27.38)	$\chi^2=33.96$	<0.001*
Lifetime cannabis use disorder - no. (%)[b]	135 (42.05)	25 (10.37)	$\chi^2=68.24$	<0.001*
Years of use disorder [mean (sd)]	2.36 (3.9)	0.62 (2.26)	$t=6.18$	<0.001*
Age of onset [mean (sd)]	16.01 (2.9)	16.45 (1.8)	$t=-1.17$	0.24

* *p*-value <0.05.

[a] Affective psychosis includes DSM-IV diagnosis of unipolar depression or bipolar disorder with psychotic features and schizoaffective disorder.

[b] Lifetime cannabis use disorders include DSM-IV diagnosis of abuse or dependence.

from the ECS, using different inheritance models. The association between the FAAH rs2295633 SNP and the risk of suffering a FEP was stronger also after adjusting for the lifetime presence of a cannabis use disorder or for its duration.

Although the association observed did not reach empirical significance after controlling for multiple testing, the increase in the significance of the test when adjusting by cannabis use could indicate a truly GxE interactions. For this reason, taking into account the exploratory nature of the present study, we performed further analyzes to measure the size of that interaction, to detect the polymorphism of greater risk and to characterize, from a clinical point of view, the subjects with this polymorphism. Tables 3 and 4 show the results of the association study of the FAAH rs2295633 SNP in the subgroup of cases and controls with and without history of cannabis use, respectively. Twenty-three patients with a FEP were cannabis users and also TT carriers of the FAAH rs2295633 SNP, while none of the healthy controls with cannabis use were TT (OR: 10.69, statistical power 0.78), meaning that a subject with a TT genotype of the FAAH rs2295633 SNP has 10 fold more risk to present a FEP if he or she also uses cannabis frequently (Table 3). This association was not present in the TT allele carriers with a negative history of cannabis use (Table 4), meaning that the genotype alone is not a risk factor without the interaction with the environmental factor.

Finally, clinical, neuropsychological and pharmacological characteristics of the patients depending on the presence of the allele TT of the FAAH rs2295633 SNP and cannabis use history are shown inTable 5. The only statistical significant difference that we found in the FEP group was that a higher proportion of TT carriers of the FAAH rs2295633 SNP with a positive history of cannabis use was treated with high potency antipsychotic.

4. Discussion

Our findings suggest that the FAAH rs2295633 SNP genetic polymorphism is associated with a greater risk of presenting a FEP in subjects with relevant cannabis use, but not in subjects without a history of cannabis use. Specifically, we

Table 2 Association study results (*p*-values) in each model used for single nucleotide polymorphisms (SNPs) in candidate genes from the endocannabinoid system.

SNP	Gene	Codo-minant	Domi-nant	Rece-ssive	Overdo-minant	Log-additive	Results adjusted for the lifetime presence of a cannabis use disorder					Results adjusted for duration (years) of cannabis use disorder				
							Codo-minant	Domi-nant	Rece-ssive	Overdo-minant	Log-additive	Codo-minant	Domi-nant	Rece-ssive	Overdo-minant	Log-additive
rs806368	CNR1	0.13198	0.96326	0.05290	0.35736	0.42280	0.09961	0.93212	0.03791	0.34341	0.37496	0.06311	0.65579	0.01941	0.50530	0.20957
rs6454674	CNR1	0.93583	0.84197	0.82109	0.73241	0.96591	0.87682	0.99859	0.62602	0.76152	0.81825	0.81347	0.63054	0.57807	0.89141	0.53549
rs806378	CNR1	0.95090	0.94065	0.75152	0.92438	0.84796	0.75105	0.55115	0.53446	0.78527	0.46355	0.48518	0.26226	0.45075	0.45325	0.22931
rs806380	CNR1	0.60907	0.84197	0.39376	0.44589	0.79178	0.58357	0.85859	0.30616	0.62084	0.53389	0.56150	0.44163	0.33205	0.88681	0.30655
rs806381	CNR1	0.98718	0.94961	0.87283	0.96951	0.90232	0.96503	0.79084	0.95595	0.81828	0.82278	0.72505	0.42961	0.90555	0.47340	0.51822
rs7766029	CNR1	0.31467	0.12950	0.52177	0.37854	0.17817	0.36947	0.16403	0.79134	0.28210	0.29191	0.41832	0.18876	0.73644	0.34661	0.29709
rs1105	CNR2	0.63768	0.96566	0.36267	0.50904	0.59374	0.45026	0.81783	0.21130	0.45824	0.39715	0.76888	0.84992	0.46953	0.70554	0.59917
rs12733278	CNR2	0.99258	0.92424	0.96604	0.90407	0.95323	0.76321	0.50833	0.92368	0.46328	0.62502	0.94834	0.80552	0.89855	0.74928	0.88478
rs2501392	CNR2	0.47679	0.23954	0.99687	0.22554	0.30261	0.67490	0.37721	0.76204	0.42242	0.38536	0.54026	0.27260	0.67147	0.32586	0.27154
rs4285653	CNR2	0.45884	0.56670	0.36026	0.33616	0.85703	0.81913	0.76609	0.64733	0.62469	0.91815	0.72384	0.52853	0.74361	0.43992	0.66230
rs6689530	CNR2	0.88252	0.62352	0.99890	0.61782	0.64860	0.94608	0.74169	0.98772	0.74077	0.75661	0.80376	0.60018	0.77161	0.54056	0.67996
rs2295633	**FAAH**	**0.04474***	**0.02552***	**0.07695**	**0.26455**	**0.01268***	**0.03000***	**0.02506***	**0.03905***	**0.35242**	**0.00845***	**0.01854***	**0.01008***	**0.05369***	**0.18525**	**0.00478***
rs324418	FAAH	0.37702	0.22127	0.31009	0.44169	0.16627	0.24112	0.14653	0.20753	0.37814	0.09597	0.18536	0.07258	0.36906	0.15637	0.06994
rs324419	FAAH	0.09928	0.23656	0.12473	0.10950	0.46076	0.14495	0.27829	0.16133	0.14257	0.50013	0.14568	0.26012	0.17490	0.13095	0.47892
rs324420	**FAAH**	**0.06521**	**0.05934**	**0.05827**	**0.27614**	**0.02498***	**0.03662**	**0.03599**	**0.03913**	**0.22076**	**0.01318***	**0.02413***	**0.01254***	**0.07167**	**0.07709**	**0.00647***

* p-value <0.05. CNR1: Cannabinoid receptor type 1. CNR2: Cannabinoid receptor type 2. FAAH: Fatty acid amide hydrolase.

Table 3 Association study of the SNP rs2295633 in the subgroup of cases and controls with history of cannabis use.

Model	Control - no. (%)	Case - no. (%)	OR	*p*-value	AIC
Codominant					
C/C	37 (56.1)	65 (39.4)	1.00	0.0004755*	264.0
C/T	29 (43.9)	77 (46.7)	1.51 (0.84-2.72)		
T/T	0 (0)	23 (13.9)	10.69 (1.41-80.85)		
Dominant					
C/C	37 (56.1)	65 (39.4)	1.00	0.0214487*	275.1
C/T-T/T	29 (43.9)	100 (60.6)	1.96 (1.10-3.50)		
Recessive					
C/C-C/T	66 (100.0)	142 (86.1)	1.00	0.0003975*	263.9
T/T	0 (0)	23 (13.9)	0		
Overdominant					
C/C-T/T	37 (56.1)	88 (53.3)	1.00	0.7068678	280.3
C/T	29 (43.9)	77 (46.7)	1.12 (0.63-1.98)		
log-Additive					
0,1,2	66 (28.6)	165 (71.4)	2.21 (1.35-3.61)	0.0004755	269.4

* *p*-value <0.05.

Table 4 Association study of the SNP rs2295633 in the subgroup of cases and controls without history of cannabis use.

Model	Control - no. (%)	Case - no. (%)	OR	*p*-value	AIC
Codominant					
C/C	83 (48.0)	64 (42.1)	1.00	0.5665	454.1
C/T	69 (39.9)	68 (44.7)	1.28 (0.80-2.04)		
T/T	21 (12.1)	20 (13.2)	1.24 (0.62-2.47)		
Dominant					
C/C	83 (48.0)	64 (42.1)	1.00	0.2884	452.1
C/T-T/T	90 (52.0)	88 (57.9)	1.27 (0.82-1.97)		
Recessive					
C/C-C/T	152 (87.9)	132 (86.8)	1.00	0.7826	453.1
T/T	21 (12.1)	20 (13.2)	1.10 (0.57-2.11)		
Overdominant					
C/C-T/T	104 (60.1)	84 (55.3)	1.00	0.3768	452.4
C/T	69 (39.9)	68 (44.7)	1.22 (0.78-1.90)		
log-Additive					
0,1,2	173 (53.2)	152 (46.8)	1.16 (0.84-1.59)	0.3670	452.4

found that the probability of presenting a FEP was tenfold higher in cannabis users who were homozygote carriers of the T allele of the FAAH rs2295633 SNP, compared to users of cannabis without this genotype. These results point to a GxE interaction between at least one gene from the ECS and cannabis use in the risk of presenting a FEP.

Taking into account that the ECS has been implicated as a neuroprotective, anti-inflammatory system, the synthesis of endocannabinoids has been proposed as a defense mechanism adopted by the brain in a psychotic state (Bioque et al., 2013; Giuffrida et al., 2004; Leweke et al., 2012). Chronic cannabis users showed lower brain FAAH binding, measured with positron emission tomography and [^{11}C]CURB (Boileau et al., 2016). Thus, continuous cannabis use could accentuate the malfunction of the ECS in certain subjects (Fakhoury, 2016).

To date, the relationship between the presence of the C or T allele in the FAAH rs2295633 SNP and FAAH function is not completely understood. An amphetamine challenge study in animal models linked the C allele to a heightened arousability, which has been associated with a reduced FAAH activity, suggesting possible higher FAAH activity in C allele carriers (Dlugos et al., 2009). Similarly, according to the Genotype-Tissue Expression (GTEx) project (www.gtexportal.org), the T allele carriers show lower mRNA levels in mucosa, muscle, skin and whole blood. FAAH inhibition (and thus increase of AEA levels) has been linked with heightened aversive memory extinction and reduced anxiety levels in animal studies (Varvel et al., 2006). A previous study also suggested that this SNP could serve as a possible marker for increased post-traumatic stress disorder risk in subjects exposed to brain injury and combat experiences (Pardini et al., 2012).

Some evidence suggests that there would be an elevation of AEA levels in response to an acute psychotic episode, as a protective mechanism, and that psychosis remission might be associated to a significant decrease (De Marchi et al., 2003; Giuffrida et al., 2004; Leweke et al., 2012). Thus, a low FAAH activity would lead to a diminished AEA degradation, which would mean an increased AEA availability.

Table 5 Clinical, neuropsychological and pharmacological characteristics of the patients depending on the presence of the TT genotype of the SNP rs2295633 and cannabis use history.

	Group 1	Group 2	Group 3	Group 4	Comparison between all groups		Comparison between group 4 vs. groups 1–3	
					Statistic	*p*-value	Statistic	*p*-value
SNP rs2295633 Homo TT	No	No	Yes	Yes				
History of cannabis use	No	Yes	No	Yes				
Number of subjects	115	122	18	19				
Age – years [mean (sd)]	23.90 (6.67)	23.37 (5.42)	21.11 (6.11)	23.00 (4.52)	$F=0.267$	0.849	$t=0.46$	0.640
DUP – days [mean (sd)]	86.75 (101.82)	123.92 (177.22)	123.76 (186.66)	107.89 (172.48)	$F=1.12$	0.306	$t=0.02$	0.981
PANSS Total score - [mean (sd)]	74.39 (24.23)	74.77 (26.17)	69.33 (18.20)	79.79 (26.14)	$F=0.55$	0.651	$t=0.94$	0.347
PANSS Positive subscale - [mean (sd)]	17.83 (7.05)	18.68 (8.62)	15.67 (6.94)	19.89 (8.92)	$F=1.13$	0.336	$t=0.95$	0.338
PANSS Negative subscale - [mean (sd)]	19.07 (7.95)	18.22 (8.59)	18.78 (7.72)	20.32 (8.71)	$F=0.45$	0.718	$t=0.85$	0.395
PANSS General subscale - [mean (sd)]	37.49 (12.90)	37.87 (13.61)	34.89 (9.85)	39.58 (13.13)	$F=0.44$	0.737	$t=0.67$	0.501
Intelligence Quotient estimation - [mean (sd)]	93.76 (16.29)	91.64 (13.49)	85.56 (15.98)	92.11 (15.83)	$F=1.63$	0.182	$t=0.01$	0.989
Verbal memory - [mean (sd)]	251.98 (80.93)	260.12 (81.64)	231.60 (98.56)	276.30 (79.35)	$F=1.03$	0.377	$t=-1.11$	0.268
Executive function - [mean (sd)]	157.93 (46.71)	158.50 (43.97)	139.93 (45.67)	157. 28 (47.68)	$F=0.76$	0.517	$t=-0.02$	0.986
Attention - [mean (sd)]	88.75 (15.71)	86.98 (14.12)	90.09 (17.43)	90.36 (14.59)	$F=0.49$	0.690	$t=0.63$	0.526
Working memory - [mean (sd)]	68.44 (15.43)	67.72 (13.15)	66.73 (13.02)	63.88 (11.62)	$F=0.59$	0.617	$t=1.22$	0.221
Antipsychotic potency [a]								
Low	59 (50.4)	59 (45.0)	8 (44.4)	10 (50.0)	$\chi^2=12.6$	0.05	X2=8.88	0.012*
Medium	55 (47.0)	72 (54.9)	10 (55.5)	8 (40.0)				
High	3 (2.6)	0	0	2 (10.0)				
Antipsychotic polytherapy – no. (%)	34 (29.0)	34 (25.9)	3 (16.6)	6 (30.0)	$\chi^2=1.4$	0.707	$\chi^2=0.1$	0.748
Use of lithium – no. (%)	10 (8.2)	3 (2.2)	2 (10.0)	3 (13.6)	$\chi^2=7.4$	0.06	$\chi^2=2.4$	0.119
Use of other mood stabilizers - no. (%)	12 (9.9)	12 (8.8)	3 (15.0)	4 (18.8)	$\chi^2=2.3$	0.516	$\chi^2=1.5$	0.212
Use of antidepressants – no. (%)	19 (15.7)	17 (12.5)	1 (5.0)	3 (13.6)	$\chi^2=1.8$	0.600	$\chi^2=0.0$	0.970
Use of benzodiazepines– no. (%)	54 (44.6)	58 (42.6)	8 (40.0)	9 (40.9)	$\chi^2=0.2$	0.970	$\chi^2=0.0$	0.826

DUP: Duration of untreated psychosis; PANSS: Positive and Negative Symptoms of Schizophrenia.

* *p*-value <0.05.

[a] Antipsychotic potency according D2 receptor affinity (High: Haloperidol; Medium: Risperidone, Paliperidone, Amisulpride; Low: Aripiprazole, Olanzapine, Clozapine, Quetiapine).

T allele carriers of the FAAH rs2295633 SNP might present a reduced FAAH activity, so in theory this should be a protective genotype for psychosis. In our study, we did not find differences between the rs2295633 SNP genotypes individually, but only when exogenous cannabis use was present. This finding accentuates the interest in understanding the effects of cannabis consumption over the ECS in psychotic disorders, at least in a subpopulation with a major genetic vulnerability.

Another significant finding of our study was that a higher a proportion of TT carriers of the rs2295633 SNP with a positive history of cannabis use was treated with high potency antipsychotics. This finding could be related to the fact that, under cannabis use, this genotype was linked

to a greater presence of more striking or disruptive symptoms (positive symptoms, disorganization or disruptive behavior), leading clinicians to choose a more incisive treatment. In fact, in this TT genotype, there were differences of more than ten points in the PANSS mean scores according to cannabis history (Table 5, group 3: 69.3 vs. group 4: 79.8), suggesting that cannabis use affects the severity of symptoms in TT carriers, but not in the other two variants. On the other hand, this genotype was not significantly associated to any other clinical or neurocognitive specific feature.

Some limitations should be taken into account when analyzing these results. Firstly, the sample size limits the statistical power and did not allow us to perform a whole genome genotyping analysis. However, we achieved enough statistical power to identify certain significant GxE associations. Secondly, we used a candidate gene strategy limited by our current understanding of psychosis pathology and the genetic variability was limited to the information available in public databases. Thirdly, the majority of the participant sites are tertiary care centers, so patient samples and therapeutic strategies may differ from other areas. Fourthly, none of the participants corresponding to certain genotypes groups did not receive high potency antipsychotic (see Table 5), which could be limiting the capacity of the statistical test to find differences. Fifthly, we could not quantify the levels of AEA and 2AG due to technical difficulties for isolating and measuring their circulating levels, a methodology not easily available. Finally, in a FEP cohort as ours we cannot assure whether cannabis use preceded the onset of psychoses or if it was a response to early symptoms.

In conclusion, in this study we have identified a GxE interaction between a genetic polymorphism from the ECS and cannabis use involved in the risk of presenting a FEP. Although this preliminary data should be replicated with independent samples, our results highlight the importance of the pro-psychotic effects exogenous cannabis use over the ECS in certain subjects. These results also point the interest to use the ECS elements as biomarkers of the FEP and to explore its pharmacological modulation. Such findings warrant greater attention in future investigations and in the translational significance of these data.

Conflicts of interest

All authors report no competing interests for this study.

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Contributors

MBi conducted the literature review, collected data, assisted with the analysis, wrote the first draft of the manuscript and handled subsequent drafts after receiving coauthors feedback. SM conducted the main statistical analysis, wrote the first draft of the manuscript and handled subsequent drafts after receiving coauthors feedback. MBe coordinated the PEPs study. The rest of coauthors collected data and commented on drafts. PEPs Group collected the data and revised the manuscript. All of the authors contributed to the final version of the paper.

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.euroneuro.2019.04.005.

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