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Brain structural and clinical changes after first episode psychosis: Focus on cannabinoid receptor 1 polymorphisms

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ABSTRACT

Cannabinoid receptor 1 (*CNR1*) gene polymorphisms have been associated with central and peripheral effects of cannabis and schizophrenia pathophysiology. Here, we have tested whether three CNR1 variants (rs1049353, rs1535255 and rs2023239) are associated with changes in brain volumes, body mass index (BMI) or psychopathological scores in a 3-year longitudinal study of 65 first-episode psychosis patients. The rs1049353 at-risk allele was significantly associated with a greater reduction of caudate volume, and the rs2023239 T/C polymorphism showed a significant decrease in thalamic volume after the 3-year period. For those who were not cannabis users, the rs1535255 and rs2023239 polymorphisms had effects in lateral ventricle (LV), and LV and white matter, respectively. The rs2023239 variant also was associated with significant improvements in positive and negative symptoms of schizophrenia. There was no significant effect of any of the variants on changes in BMI over the 3-year study. Finally, an interaction between all three polymorphisms was found involving evolution of positive symptoms. These findings suggest that the cannabinoid pathway is associated with schizophrenia evolution over time. However, further studies using larger cohorts are needed to confirm these results. If confirmed, the present findings could lead in subsequent investigations for identification of novel drug targets for improved treatment of patients suffering from schizophrenia.

1. Introduction

Schizophrenia is a complex disorder with a heritable component. Common genetic variations of genes expressed both in brain and in peripheral tissues have been implicated in the etiology of the disorder (PGC-SCZ, 2014; Ripke et al., 2013). Growing evidence suggests that a combination of genetic (Cannon et al., 1998; Ross et al., 2006; Stefansson et al., 2009) and environmental factors, such as cannabis use (van Os et al., 2005; Torrey et al., 2012), may significantly increase the risk of illness.

The endocannabinoid system has been implicated in brain functions associated with neurogenesis, synaptic plasticity and neuroprotection (Skaper and Di Marzo, 2012), as well as in peripheral actions, such as immune and metabolic functions (Pertwee, 2006). Cannabinoid receptors and their ligands are part of the endocannabinoid system. Two well-characterized CB1 and CB2 cannabinoid receptors are G_i/G_o-protein-coupled receptors. Other cannabinoid receptor candidates include the putative abnormal cannabinoid receptor 18 (GPR18), G-protein coupled receptor 55 (GPR55), peroxisome proliferator-activated receptors (PPARS) and the vanilloid transient receptor potential V1 (TRPV1) (Onaivi et al., 2012). CB1 receptors are localized mainly in the central nervous system (CNS), but they are also present in the periphery. Conversely, CB2 receptors are predominant in peripheral tissues and have also been found in the brain (Galiegue et al., 1995; Onaivi, 2009).

The CB1 receptor is coded by the central cannabinoid receptor-1 (*CNR1*) gene located on chromosome 6 (6q14-15), a region of replicated linkage for schizophrenia (Lewis et al., 2003). Some single nucleotide polymorphisms (SNPs) of this gene have been associated with CNS effects (Dinu et al., 2009; Okahisa et al., 2011) and with certain neuropsychiatric features. For example, the rs2023239

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polymorphism has been linked to substance dependence (Hirvonen et al., 2013), the rs806378 polymorphism with tardive dyskinesia (Tiwari et al., 2012) and the rs1535255, rs2023239 and rs1049353 polymorphisms have been also related to impulsive behaviors (Ehlers et al., 2007). Also *CNR1* polymorphisms have been associated with psychosis-related disorders (Eggan et al., 2008, 2012; Brown et al., 2014). However, some studies have failed to reveal a significant association between schizophrenia and *CNR1* genetic mutations through studies of the rs1049353, rs77660229, rs86366 and rs6454674 polymorphisms (Seifert et al., 2007; Costa et al., 2013), whereas others have suggested that differences in the gene are related to schizophrenia vulnerability, independent of substance abuse (Martinez-Gras et al., 2006). Moreover, the rs1049353 polymorphism has been associated with predisposition to the hebephrenic schizophrenia subtype (Ujike et al., 2002). In addition, the rs2501431 and rs1049353 polymorphisms have been linked to depression (Monteleone et al., 2010; Mitjans et al., 2013) and the latter has also been associated with antidepressant response (Mitjans et al., 2013).

CNR1 is also a candidate gene for metabolic disorders such as obesity, hypercholesterolemia and insulin resistance (Benzinou et al., 2008; Feng et al., 2010). For example, the minor alleles of rs1535255 and rs2023239 were associated with lower risk of metabolic syndrome in schizophrenia (Yu et al., 2013). However, other authors found no significant association between CNR1 genes and obesity (Muller et al., 2007; Zhuang et al., 2012).

Some investigations have shown that some SNPs of the *CNR1* gene are associated with morphological brain abnormalities, consistent with the link to some psychiatric disorders. Studies of cannabis use in schizophrenia patients (Onwuameze et al., 2013) and healthy volunteers (Schacht et al., 2012) have identified specific variants of *CNR1* (rs12199654 and rs2023239) related to reductions in brain white matter (WM) volume. Furthermore, neuroanatomical studies have found a selective increase of CB1 receptors in schizophrenia patients, especially in the pons (Wong et al., 2010), nucleus accumbens (Ceccarini et al., 2010) and prefrontal cortex, independent of cannabis use (Dean et al., 2001). Under inflammatory conditions, *CNR1* mRNA was shown to be over-expressed in multiple regions of the brain (Lou et al., 2012). This effect may be related to the inflammatory theory of schizophrenia, which proposes that inflammation of the brain during prenatal, neonatal and pubertal periods may be involved in the subsequent development of psychosis-related disorders (Busse et al., 2012; Zavitsanou et al., 2013). Consistent with this speculation, some studies have suggested that exposure to cannabis during a vulnerable stage is associated with a two-fold increased risk of developing psychotic symptoms or a psychotic illness in later years (Arseneault et al., 2002).

Because of the hypothesized role of cannabis and the endocannabinoid system in the pathogenesis of schizophrenia (Ujike and Morita, 2004) and the potential linkages with effects on central and peripheral functions outlined above, we have tested whether there are any interactions of three *CNR1* polymorphisms (rs1049353, rs1535255 and rs2023239) with changes in regional brain volumes, body mass index (BMI) or clinical psychopathology scores after a 3-year follow-up of patients with a first episode of psychosis.

2. Methods

2.1. Subjects and clinical assessments

The subjects formed part of a naturalistic longitudinal cohort of patients with a first episode non-affective psychosis (PAFIP), treated at the University Hospital Marques de Valdecilla, Santander, Spain. This program included patients who met Diagnostic and Statistical Manual of Mental Disorders (DSM)–IV criteria for schizophrenia, schizophreniform disorder, brief psychotic disorder, or psychosis not otherwise specified, and excluded those with psychotic disorders caused directly by a general medical condition or the use of substances including prescription drugs. Cases were diagnosed using the Structured Clinical Interview for DSM Disorders (SCID-I) (Spitzer et al., 1990).

Only those patients who underwent *CNR1* genotyping and two magnetic resonance imaging (MRI) scans at baseline and 3 years after illness onset were included in the analyses ($n=65$). The mean time from the first assessment to the MRI scan was 31.9 ± 26.9 days. The gender distribution was 44 males (67.7%) and 21 females (32.3%), and the mean age of onset was 29.9 ± 8.3 years. The mean duration of untreated psychosis (DUP) at initial assessment was 13.8 ± 20.7 months (median=5). Sociodemographic and clinical assessments were performed by psychiatrists during medical examinations, using the Scale for the Assessment of Negative Symptoms (SANS) (Andreasen, 1984a), the Scale for the Assessment of Positive Symptoms (SAPS) (Andreasen, 1984b), the Calgary Depression Scale (CDG) (Addington et al., 1993) and the Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962). Height and weight measurements were made regularly by an experienced nurse. BMI was calculated at baseline and after 3 years by dividing body weight in kilograms by height in meters squared (kg/m^2). The amount and type of medication prescribed during the 3-year follow-up period were recorded. After the first clinical assessment, patients were randomly assigned to haloperidol ($n=14$), olanzapine ($n=16$), risperidone ($n=13$), quetiapine ($n=6$), ziprasidone ($n=10$), and aripiprazole ($n=6$). To derive total antipsychotic dose, each antipsychotic was converted to chlorpromazine (CPZ) milligram equivalent units (Woods, 2003; Andreasen et al., 2010). The cumulative CPZ equivalents were 234641.52 ± 157361.57 mg over the 3-year follow-up.

Patients included in the analyses did not significantly differ from the rest of the patients in the program with regards to age, gender, BMI, symptom severity, duration of untreated psychosis, and frequency of tobacco, alcohol or cannabis consumption. To describe genotype distribution and inheritance models, 12 healthy individuals (5 males and 7 females) were recruited from the same geographic area and used as a comparison group with the patients. Subjects volunteered to provide blood samples for *CNR1* genotyping and to undergo two MRI scans at baseline and after 3 years. None of the healthy volunteers had a current or past history of a psychiatric disorder, a neurological or general medical disorder, or a first-degree relative with a psychotic illness. The study was approved by the local institutional review board and conformed to international standards for research ethics. Patients, their families and healthy volunteers provided written informed consent for inclusion in the study.

2.2. MRI analysis

MRI scans of the whole brain were obtained using a 1.5 T General Electric SIGNA System (GE Medical Systems; Milwaukee, WI, USA). Three-dimensional (3D) T1-weighted images and 2D proton density (PD) and T2 sequences were acquired as described previously (Mata et al., 2010). Images were processed using the BRAINS2 software (Andreasen et al., 1992). In order to classify volumes as gray matter (GM), WM or cerebrospinal fluid (CSF), the data were segmented using multispectral discriminant analysis, based on automated training class selection (Harris et al., 1999). The resulting 3D isosurface approximated the spatial center of the cortex and was used to provide estimates for direct quantitative measurements of volume. In the present study, we examined total brain volume, surface area, cortical thickness, GM and WM volumes, lateral ventricle (LV) sizes, CSF and subcortical (thalamus and caudate nucleus) volumes.

2.3. Genotyping

Three *CNR1* SNPs were genotyped: rs1049353 (guanine (G)/adenine (A)), located in the coding region, and rs1535255 (thymine (T)/cytosine (C)) and rs2023239 (T/C), located in the distal region of intron 2. Genomic DNA was extracted from whole venous blood samples, and polymorphisms were assayed using the SNPlex technology (Applied Biosystems, Foster City, CA, USA) (Tobler et al., 2005).

2.4. Statistical analysis

Deviation from Hardy–Weinberg equilibrium (HWE) and differences in allele and genotype frequencies between patients and healthy volunteers were evaluated using Pearson's Chi-square test. Associations of the different genetic models were tested using logistic regression on the minor allele of each SNP. The Akaike information (AIC) criteria were used to compare the likelihood of inheritance models (Ziegler et al., 2010) and the selected model was used in the final analyses. AIC compares the quality of several statistical models and it allows selection of the "best" of them. AIC is defined as $-2\ln(L)+$twice the number of estimated parameters, the model with the lower AIC value corresponding to minimize the expected entropy (Akaike, 1974, 1992). Therefore, a model that minimizes the AIC is preferable.

In patients, potential differences in distribution of clinical and sociodemographic variables between the genotype groups were assessed using Pearson Chi-square tests and one-way analysis of variance (ANOVA) for categorical and continuous variables. When variances were not equal (as determined using Levene's test), unequal variance (Welch's) *t*-tests were used. If any variable was found to be significantly different, it was included as a covariate in subsequent analyses.

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