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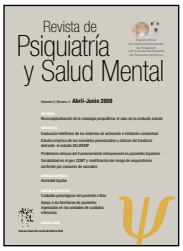

ORIGINAL

Variability of the *COMT* gene and modification of the risk of schizophrenia conferred by cannabis consumption

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KEYWORDS
Cannabis;
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Abstract
Introduction: The risk of schizophrenia conferred by cannabis has recently been proponed to be modulated by the Val158Met polymorphism (rs4680) at the *COMT* gene. To date, these findings have not been replicated in independent samples.
Material and methods: We tested the potential gene-by-environment interaction between Val158Met genotype at the *COMT* gene and previous use of cannabis in schizophrenia in 192 healthy controls and 91 inpatients with DSM-IV schizophrenia. The functional *COMT* Val158Met polymorphism was analyzed using TaqMan technology. Cannabis use was measured by taking into account the frequency of intake during the previous month. Logistic regression models were used to test the interaction between genetic and environment factors.
Results: Cannabis use was strongly associated with the case condition ($p < 0.0001$). The Val158Met polymorphism at the *COMT* gene was not associated with schizophrenia, although Val/Val homozygosity tended to be more frequent in the case group than in the control group (34% vs 27%; OR = 1.39; 95% CI, 0.78-2.47). Finally, in women we found a non-significant trend toward the association when we tested for the interaction between cannabis use, the number of Val alleles and susceptibility to schizophrenia ($p = 0.152$).

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Conclusions: Our results tend to support recent findings suggesting that the Val-158Met polymorphism at the *COMT* gene modifies the risk of schizophrenia conferred by cannabis use. In our study, this possible effect was only detected in women.

PALABRAS CLAVE
Cannabis;
COMT;
Esquizofrenia;
Interacción genético-ambiental

Variabilidad en el gen COMT y modificación del riesgo de esquizofrenia conferido por consumo de cannabis

Resumen
Introducción: Recientemente se ha propuesto que el incremento de riesgo de esquizofrenia en relación con el consumo de cannabis podría modificarse por el polimorfismo Val-158Met (rs4680) del gen *COMT*. Estos hallazgos no han sido replicados hasta el momento en muestras independientes.
Material y métodos: En una muestra de 192 controles sanos y 91 pacientes con diagnóstico DSM-IV de esquizofrenia se exploró la posible interacción genético-ambiental de *COMT* y consumo de cannabis y su efecto en el riesgo de esquizofrenia. El polimorfismo funcional Val158Met del gen *COMT* se analizó mediante tecnología TaqMan. El consumo de cannabis se determinó teniendo en cuenta la frecuencia de su uso durante el último mes. Se aplicaron modelos de regresión logística para probar perfiles de riesgo genético y/o ambiental concretos definidos a priori para la esquizofrenia.
Resultados: El consumo de cannabis tiene firme relación con la condición de enfermo ($p < 0{,}0001$). El polimorfismo Val158Met del gen *COMT* no tenía relación con la esquizofrenia, si bien los individuos homocigotos Val/Val tendían a ser más frecuentes en el grupo de enfermos que en el de controles (el 34 frente al 27%; odds ratio = 1,39; intervalo de confianza del 95%, 0,78-2,47). Por último, se detectó un incremento no significativo ($p = 0{,}152$) de mujeres consumidoras de cannabis y portadoras del alelo Val en el grupo de enfermas al compararlo con el grupo control.
Conclusiones: Nuestros resultados apoyarían hallazgos recientes que describen un efecto modulador del gen *COMT* en el riesgo que confiere para la esquizofrenia el consumo y/o el abuso de cannabis. En nuestro estudio, este efecto sólo se observa en el grupo de mujeres.

Introduction

Cannabis is one of the most widely consumed drugs in the world. Traditionally, its consumption has been linked to an increased risk of psychotic symptoms and schizophrenia, an assumption corroborated by prospective studies conducted on large population samples.[1] Two recent reviews specifically indicate that cannabis might actually act as a causal factor in the onset of psychoses, as it increases the risk of these diseases from 1.4 to 1.9 times.[2,3] This risk could be increased in the case of young teenage consumers, in whom the deleterious effects of cannabis could be greater, owing to the fact that in these particularly vulnerable individuals brain development has not yet been completed.[4,5]

The effect of cannabis on the total risk for psychosis, and particularly for schizophrenia, may also be modulated by genetic factors.[6,7] In a study carried out in Dunedin (New Zealand), on a cohort of individuals monitored from birth until the age of 26, it was found that homozygotes for the Val (Valine) allele of the Val158Met polymorphism of the catechol-O-methyltransferase (*COMT*) gene, who had also consumed cannabis during their adolescence, were at least 5 times more at risk of exhibiting psychotic symptoms and developing a schizophreniform disorder than individuals with the same genotype who had not previously consumed cannabis.[8] Catechol-O-methyltranspherase is the principal metabolizer of dopamine. The gene containing the information for its synthesis is located on the 22q11 chromosome, a region that has consistently been linked to schizophrenia[9] and in which a specific microdeletion has been associated with the velocardiofacial syndrome, in which psychotic symptoms are very common.[10] In the *COMT* gene the Val158Met polymorphism, also known as rs4680, is characterized by the fact that it has two possible alleles or genetic variants (Val or Met) with different functional repercussions on the protein they code for, the Val allele determining greater activity of the catechol-O-methytranspherase enzyme than the Met allele.[11,12]

In addition, the Val allele appears to determine greater concentrations of dopamine in mid-brain neurons[13] and lower densities of grey matter in the anterior cingulate cortex.[14] These functional differences between one allele and another could translate as different degrees of vulnerability to schizophrenia, in which there definitely seems to be a general and non-specific dysfunction of the dopaminergic neurotransmission pathway.

Studies on families have described a differential transmission of the high-activity Val allele in individuals with schizophrenia; however, case-control studies have generally provided inconclusive results about the effect in isolation of this gene in the etiology of the disorder.[15,16] In this respect, the study by Caspi[8] et al contributes revealing findings because they demonstrate for the first time the genetic-environmental interaction (*COMT* gene and cannabis consumption) in the causality of psychosis. The effect of the gene alone is not very great, but when combined with a stressful environmental factor it increases significantly.

Although there have not yet been any replications of these findings in population samples, there are 3 studies whose results support those of Caspi et al,[8] and at least one which does not support their results. The first of these studies supporting the research done by Caspi et al is an entirely case-based study, which included 223 first psychotic episodes. In this study Di Forti et al[17] concluded that the effect of cannabis on the appearance of psychosis was moderated by the Val158Met polymorphism of the *COMT* gene and that Val/Val homozygotes, who had also consumed cannabis, were at greater risk of developing a psychotic disorder in the future. The second study corresponds to an experimental design in which THC (the main psychoactive component of cannabis) or placebo was administered to volunteers.[18] In this study it was noted that Val/Val homozygous individuals were more sensitive to the effect of THC and had a higher probability of manifesting psychotic symptoms after its administration than individuals with other genotypes. In relation to these results, an independent study executed by the same research team describes an increased risk of hallucinations after consuming cannabis in psychotic patients carrying the Val allele of the Val158Met polymorphism.[19] However, all these findings, which, although they are from heterogenous design studies, appear to support the interaction between the *COMT* gene and cannabis consumption in the etiology of schizophrenia, contrast with those described by Zammit et al,[20] who failed to find any evidence of such an interaction in a recent study entirely based on cases of schizophrenia.

Given the potential transcendence of the findings described and the current lack of data, in the present study our aim was to explore the possible interaction between cannabis consumption, *COMT* gene variability and the risk of schizophrenia in a Spanish sample of schizophrenic patients and controls.

Sample and methods

Sample

The sample included in this study consisted of 283 individuals, 91 (66 men and 25 women) of whom were patients with a DSM-IV schizophrenia diagnosis and 192 (96 men and 96 women) of whom were healthy individuals, who were not related to one another and originated from the same population as the schizophrenic patients. The patients were recruited from acute units in 2 Spanish hospitals (Sant Joan de Déu-Serveis de Salut Mental in Barcelona and the San Cecilio University Hospital in Granada). The controls were from primary care centres in Andalusia who attended consecutive appointments with their GP and agreed to give a genetic sample. All the participants signed an informed consent in order to join the study; previously the ethics committees of the respective hospitals had approved the protocols.

State of patients or controls

The patients had been clinically diagnosed with schizophrenia. This was confirmed, following DSM-IV criteria for schizophrenia, by independent and experienced clinical psychiatrists. Subsequently, the symptomatology was evaluated using the Spanish version of the Positive and Negative Symptoms of Schizophrenia (PANSS) scale.[21] For their part, the controls, who came from primary care centres, were evaluated using the Spanish version of the 28-item General Health Questionnaire (GHQ-28)[22] and their disease-free status was confirmed when their score on this mental disorder screening scale was lower than the most stringent cut-off point (2/3).

Environmental factors

Socio-demographic data, including sex, age, marital status and educational level, was collected. In addition, both patients and controls were asked about their current habits with regard to cannabis consumption to clarify whether they were regular consumers of this substance. Consumption was regarded as habitual when cannabis had been used at least once a week during a minimum of 2 weeks in the preceding month. Questions related to the age at which cannabis consumption was initiated were included in the study protocol once sampling had begun but this information is only available for a small part of the sample. This is why the age when consumption began is not a variable that we can take into account in our analyses.

Genetic analyses

After giving their informed consent, both patients and controls donated a biological sample of blood or saliva (in cases in which these subjects preferred the sample to be obtained in this way). The Val158Met polymorphism of the *COMT* gene was analyzed in the entire sample using TaqMan technology. The oligonucleotide mould and the probe employed in this analysis were designed using Primer Express 2.0 software (ABI, Foster City, California). The DNA sequences were obtained through GenBank. Allele discrimination was performed using a TaqMan 7900 machine. 4 types of signal were identified as clusters by means of SDS 1.7 *software* (ABI, Foster City, California). These clusters corresponded to Val/Val homozygotes, Met/Met homozygotes, Val/Met heterozygotes and negative

controls. The validity of each genotype group or *cluster* was confirmed by sequencing 3 randomly selected samples from each group.

Statistical analysis

The Hardy Weinberg equilibrium was analyzed for the genotype frequencies in patients and controls using the χ^2 test (EPI Info VI statistical package and SPSS v.12.0). The same statistical test was employed to compare the distribution of consumers and non-consumers of cannabis and the allele and genotype frequencies of groups of schizophrenics and controls. Logistic regression models were applied to control specific genetic and/or environmental risk profiles, designed a priori, for schizophrenia. These logistic regression models were used to adjust associations and interactions due to possible confounding factors, such as sex and age. The associations (odds ratio [OR] with confidence intervals [CI] of 95%) between the disease, cannabis consumption and the genetic variants which were analyzed were also estimated.

Results

Consumption of cannabis and schizophrenia

It was found that the consumption of cannabis was firmly linked to the condition. Thus, the use of this substance was significantly more common in cases of schizophrenia than in controls (χ^2 = 62.812; p < 0.0001) (Table 1). This link was maintained when cannabis consumption was analyzed according to sex. Although in our study the women smoked less cannabis than the men, in the female group greater consumption was also observed amongst the women who had the disease than amongst women who did not (χ^2 = 7.36; p = 0.006; OR = 3.831; CI 95%, 1.392-10.542). In the case of males this association between cannabis consumption and schizophrenia increased (χ^2 = 49.7; p = 0.0001; OR = 14; CI 95%, 6.29-31.162).

COMT gene and schizophrenia

The genotype frequencies for the Val158Met polymorphism of the *COMT* gene were in Hardy Weinberg equilibrium in both patients and controls.

The analysis of the genetic association between this gene and schizophrenia revealed that there were no statistically significant differences in the distribution of allele and genotype frequencies when patients and controls were compared (table 2), although the proportion of homozygous individuals carrying the Val allele tended to be greater in the patient group (34 compared to 27%; χ^2 = 1.45; p = 0.228; OR = 1.39; CI 95%, 0.78-2.47). Neither were differences found when the analyses were sex-stratified. In both patients and controls the allele and genotype distribution was similar in males and females. Once again, although statistical significance was not reached, in both cases a higher proportion of Val/Val genotypes was observed in patients than in controls (data not shown).

Table 1 Cannabis consumption in patients with schizophrenia and controls

	Cannabis consumption	
	Yes	No
Patients	51 (58.6%)	36 (41.4%)
Controls	25 (13%)	167 (87%)

χ^2 = 62.812; gl = 1; p < 0.001.
Odds ratio = 9.463 (confidence interval 95%, 5.199-17.225)

Table 2 Distribution of the allele and genotype frequencies for the val158met polymorphism of the *COMT* gene in patients and controls

	Controls	Patients
Allele frequencies		
Met	182 (47%)	75 (41%)
Val	202 (53%)	107 (59%)
	χ^2 = 1.907; gl = 1; p = 0.167	
Genotype frequencies		
Met/Met	42 (22%)	15 (16%)
Met/Val	98 (51%)	45 (50%)
Val/Val	52 (27%)	31 (34%)
	χ^2 = 1.948; gl = 2; p = 0.378	

Schizophrenia, the *COMT* gene and cannabis consumption

we found that cannabis consumption was independent of genotype for the Val158Met polymorphism of the *COMT* gene both in patients (χ^2 = 1.146; gl = 2; p = 0.564) and in controls (χ^2 = 1.676; gl = 2; p = 0.433). When the possible interaction of *COMT* gene variability with cannabis consumption and the risk of schizophrenia that these two factors jointly confer was analyzed, no significant general effects were detected on comparing patients and controls. However, when the data was analyzed according to sex, we found that the maximum risk of schizophrenia corresponded to women who regularly consumed cannabis and who were also homozygous for the Val allele. In the case of Val/Met heterozygous females who consumed cannabis there was an intermediate level of risk for schizophrenia and, finally, Met/Met homozygous females who consumed cannabis had the least risk of developing the disease (Figure 1). However, this dose-dependent pattern between Val alleles, cannabis consumption and risk of schizophrenia in women did not attain statistical significance (p = 0.152).

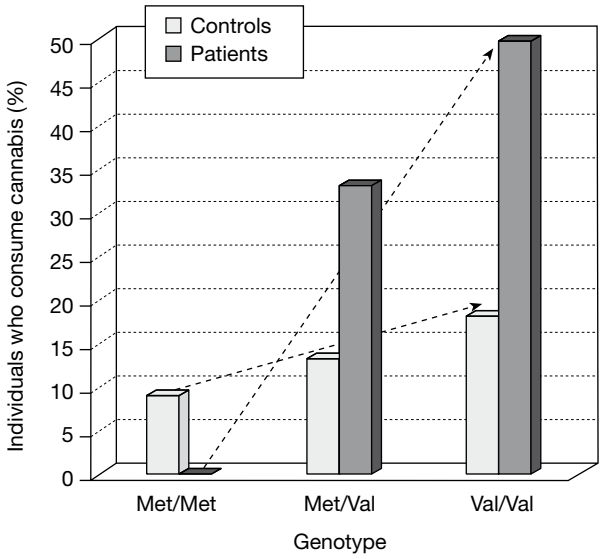

Figure 1 Female cannabis consumers and the val158met genotype of the *COMT* gene

Discussion

The continued consumption and/or abuse of cannabis has consistently been associated with an increased risk of developing psychotic symptoms and/or schizophrenia.[1,23] This risk could be greater in genetically vulnerable individuals. To be specific, recent findings by the Caspi et al[8] group indicate that cannabis consumption during adolescence could increase the risk of schizophrenia by 5 in Val/Val homozygotes for the Val158Met polymorphism of the *COMT* gene. This increase in risk was not observed in people with the same genotype who had not previously consumed cannabis.[8]

The *COMT* gene codes for catechol-O-methyltransferase, a key enzyme in dopaminergic neurotransmission and the Val158Met polymorphism is, specifically, a functional polymorphism which affects the transcriptional activity of the gene and, consequently, the efficiency with which dopamine is metabolized in the prefrontal cortex.[12] Traditionally, the *COMT* gene has been regarded as a candidate for schizophrenia and within this gene the Val158Met functional polymorphism has been widely studied in relation to the origin of the disease. However, in general the results of genetic association studies have proved inconclusive. Two recent meta-analyses found minimal or no evidence of an association between the Val158Met polymorphism of the *COMT* gene and schizophrenia.[24,25] Taking into account the evidence above, it seems plausible that, rather than having a major effect, the *COMT* gene could have a modulatory role on the effect of specific environmental risk factors for schizophrenia, for example, cannabis consumption. So far this hypothesis, supported by Caspi et al's study,[8] has not been replicated in an independent sample, although 3 studies have indirectly shown favourable results.[17-19]

In this study, as our main objective, we proposed to explore the possible interaction of these two factors (cannabis consumption and *COMT* gene variability) and their role in the risk of schizophrenia in a Spanish sample. Firstly, as other authors have described,[3,7] our results show a firm association between cannabis consumption and schizophrenia. This association was observed in both the male and female group, although in the former the magnitude of the effect was significantly greater than in the latter. It is true that our study is based on a transversal design, in which it is not possible to establish cause-effect relationships, and, therefore, we can only affirm that in our sample cannabis consumption is significantly more common in people who have the disease than in people who do not, without being able to determine whether consumption preceded its manifestation or whether it became established afterwards.

With respect to the possible role of the *COMT* gene in causing schizophrenia, our results do not appear to support the contention that the variability analyzed in this gene is related to a differential risk for this disease, although in both the general analysis and in the sex-stratified analysis we found a non-significant tendency towards an increase in homozygotes of the Val (Valine) allele amongst patients in comparison to controls. It is likely that, if these tendencies were maintained in a larger sample, the results could attain statistical significance. However, taking into account the data currently at our disposal, the frequency of risk genotypes in the non-schizophrenic population and the size of our sample, we estimate that we have a probability of 80% (CI 95%) of detecting a minimal risk of 2.2, if we consider the Val/Val homozygote a risk genotype, or 3.2, if we consider the Val/Met and Val/Val combinations to be risk genotypes. In both cases these are risks of certain magnitude, probably higher than those expected for the *COMT* gene. To detect smaller effects we would need a considerably larger sample and our current sampling capacity is limited.

Finally, and in relation to the increased risk of schizophrenia which the interaction between cannabis consumption and *COMT* gene variability might confer, in our study we only found evidence that could support this interaction in the female group and not in the male group. These tendencies did not reach statistical significance, possibly due to a lack of sampling capacity. However, they point in the same direction as the findings of Caspi et al[8] and should be explored in greater depth in a larger sample. New studies along these lines should be developed, ideally in the context of longitudinal designs, in order to clarify, on the one hand, the modulatory role of the *COMT* gene on the risk cannabis poses in the development of schizophrenia and, on the other, on the magnitude of this effect.

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