ABSTRACT

Evidence suggests that childhood trauma and cannabis use sinergistically impact on psychosis risk, although a non-replication of this environment-environment interaction was recently published. Gene-environment interaction mechanisms may partially account for this discrepancy. The aim of the current study was to test whether the association between childhood abuse, cannabis use and psychotic experiences (PEs) was moderated by the *COMT* gene. PEs, childhood abuse, cannabis use and *COMT* Val158Met genotypes were assessed in 533 individuals from the general population. Childhood abuse was shown to have a significant main effect on PEs (B=.08; SE=.04; p=.047). Furthermore, a significant three-way interaction among childhood abuse, cannabis use and the *COMT* gene was found (B=-.23; SE=.11; p=.006). This indicates that *COMT* genotypes and cannabis use only influenced PE scores among individuals exposed to childhood abuse. Exposure to childhood abuse and cannabis use increased PE scores in Val carriers. However, in individuals exposed to childhood abuse but who do not use cannabis, PEs increased as a function of the Met allele copies of the *COMT* gene. Our findings suggest that the psychosis-inducing effects of childhood abuse and cannabis use are moderated by the Val158Met polymorphism of the *COMT* gene, which supports a gene-environment-environment interaction. Cannabis use after exposure to childhood abuse may have opposite effects on the risk of PEs, depending on the *COMT* genotypes. Val carriers are vulnerable to the psychosis-inducing effects of cannabis.

Key words: childhood abuse, cannabis, the *COMT* gene, psychosis, gene-environment interaction, general population

INTRODUCTION

There is renewed interest in the complex role of environmental risk factors in the aetiology of psychosis [1]. Exposure to environmental variation during developmentally sensitive periods is essential for the development of neuronal connectivity, which underlies the functional abilities of the adult human brain including adequate interpretation of perceptual and social stimuli. Stresses, such as social adversity and drug use, in childhood, adolescence or early adult life may trigger the expression of psychosis, which can progress from subclinical to full-blown psychotic states [2]. Individual differences underlying the organism's response to environmental risk factors, which may be at least partially accounted for by genetic factors, would be decisive in shaping the final expression of psychotic experiences (PEs) or symptoms.

Thus, it is well-established that attenuated PEs occur in some individuals from the general population [3-5]. In the absence of illness or the need for treatment, these milder forms of psychotic symptoms are referred to as PEs [6]. Furthermore, it has been suggested that clinical and subclinical expression of psychosis share genetic and/or environmental factors in their aetiology [6]. Therefore, the study of the risk factors for PEs would ultimately contribute to the understanding of the aetiology of psychotic disorders.

In this context, childhood adversity constitutes an environmental risk factor that has been related to the expression of clinical and subclinical psychotic symptoms as well as PEs [7-8]. More recently, cannabis use has been associated consistently with an increased risk of developing psychosis in clinical and non-clinical populations [9-10]. Furthermore, an interaction effect between childhood adversity and cannabis use has been reported. Studies indicate that joint exposure to these two environmental factors may increase the likelihood of developing psychotic symptoms and/or experiences to a greater extent than the risk expected for each factor working independently [11-14]. These results are neurobiologically plausible, since both stressful experiences and delta-9-tetrahydrocannabinol (THC, the main psycho-active constituent of cannabis) have been found to increase dopaminergic signalling in the mesolimbic system [15], which is hypothesized to result in an increased risk of delusions and hallucinations [16].

However, a recent study of a large sample drawn from the general population reports non-replication of the interaction effect between cannabis and childhood trauma on the risk of developing psychotic symptoms [17]. Individual differences in neurobiological susceptibility to the impact of childhood abuse and cannabis use might help to explain this failure to replicate. In addition, gene-environment interaction studies have shown that the Val158Met polymorphism of the catechol-*O*-methyltransferase (*COMT*) gene moderates i) the association between cannabis use and psychosis [18-19] and ii) the association between childhood trauma and schizotypal traits [20]. However, to our knowledge, no study to date has investigated whether the impact of the joint effect of exposure to childhood adversity and cannabis use on the subsequent development of PEs partially depends on the COMT-Val158Met polymorphism.

The *COMT* gene is located in chromosome 22q11 and contains a functional polymorphism (COMT-Val158Met) that results in two common variants of the enzyme (Val and Met) [21]. The *COMT* gene encodes the enzyme catechol-*O*-methyltransferase, which plays an important role in the degradation of dopamine in the brain. The Val variant is associated with increased COMT activity, which results in a combination of reduced dopamine neurotransmission in the prefrontal cortex, associated with impairments in working memory, attention and executive functioning [22], and increased levels of dopamine in mesolimbic areas. Individuals carrying the Met/Met genotype have the lowest COMT activity and heterozygotes are considered to be of intermediate activity, as the two alleles are codominant [23]. Therefore, exposure to childhood abuse, cannabis use and the genotypes of the COMT-Val158Met polymorphism can influence dopaminergic neurotransmission in the brain and ultimately lead to the development of PEs. For these reasons, in the present study we aimed to explore whether the impact of the joint effect of childhood adversity and cannabis use on the development of PEs varies according to COMT-Val158Met polymorphism genotypes.

METHODS

Sample

The sample consisted of 533 individuals who were recruited from the campus of the Jaume I University in Castelló (Spain), as well as from university offices and community technical schools in the metropolitan area of Barcelona (Spain). All the participants were adults (mean age: 22.9 years; SD=5.4) and 45.4% were males. At assessment, 77% of the participants were students.

Exclusion criteria were the presence of any major medical illness affecting brain function, neurological conditions, and a history of head injury. All participants were of Spanish (Caucasian) ancestry, thereby reducing the possibility of confounding genetic differences by population stratification.

Ethical approval was obtained from local research ethics committees. All participants provided written informed consent before inclusion in the study. All procedures were carried out according to the Helsinki Declaration.

Measures

The Community Assessment of Psychic Experiences (CAPE; [24]) was used to assess positive and negative PEs in the sample. This validated self-report questionnaire measures the lifetime prevalence of PEs on a frequency scale ranging from 'never' to 'nearly always'. Examples of the items that assess the positive and negative dimension are, respectively, 'do you ever feel as if things in magazines or TV were written especially for you?' and 'do you ever feel that you experience few or no emotions at important events?' The CAPE provides a total score *per* dimension by adding up the scores on the frequency items.

Childhood abuse was assessed by the shortened version of the Childhood Trauma Questionnaire (CTQ; [25]). This questionnaire consists of 28 items that measure five types of childhood trauma: emotional abuse, physical abuse, sexual abuse, emotional neglect and physical neglect. In the current study, the subscales that assess abuse were combined to yield a total score of childhood abuse. Neglectful events were discarded, since only abusive events were shown to be associated

with PEs in a previous study conducted in this sample [3]. An example of an item on childhood abuse is 'people in my family hit me so hard that it left me with bruises or marks'. The score for each item ranges from 1 to 5 ('never true' – 'very often true'), depending on the extent to which individuals agree with the statement. The reliability and validity of the CTQ have been demonstrated [26].

Cannabis use was assessed with one question regarding the frequency of consumption: 'never', 'once', 'monthly', 'weekly' or 'daily' (this variable was then dichotomized into two categories: 'not exposed to cannabis': never, once; and 'exposed to cannabis': monthly, weekly, daily).

All analyses were corrected by sex, age, schizotypal personality and anxiety levels as in a previous study conducted in this sample [3]. Schizotypal personality was measured with the Schizotypy Personality Questionnaire-Brief (SPQ-B; [27]). Anxiety as a behavioural trait was assessed using the State-Trait Anxiety Inventory (STAI-T; [28]).

Laboratory methods

Genomic DNA was extracted from saliva samples using the Collection Kit BuccalAmp DNA extraction kit (Epicentre, ECOGEN, Barcelona, Spain). The SNP rs4680 (Val158Met) of the *COMT* gene was genotyped using Applied Byosystems (AB) TaqMan technology. The AB assay-on-demand service was used to order the probes. Genotype determinations were performed blind to the clinical condition. Randomized individuals were re-tested for their genotypes in order to confirm the pattern reproducibility.

Statistical Analysis

Multiple linear regressions were conducted using STATA 10.0 for Windows. Separate models were tested for positive and negative PEs (continuous variables) as dependent variables. The independent variables for main and interaction effects were childhood abuse, cannabis use and the Val158Met polymorphism of the *COMT* gene (continuous childhood abuse, dichotomous cannabis use and three categories in the *COMT* gene: Val/Val, Val/Met and Met/Met). Data were analysed hierarchically. In the first step, the main effects of childhood abuse, cannabis use and the Val158Met polymorphism of the *COMT* gene on positive PEs were tested in the same

model on positive and negative PEs separately. Two-way interaction terms (childhood abuse*cannabis use; childhood abuse**COMT* gene and *COMT* gene*cannabis use) were added in a second step. In the third step, a three-way interaction term (childhood abuse*cannabis use**COMT* gene) was entered.

Age, sex, schizotypy and trait anxiety were included as covariates in all analyses.

Additional analyses were carried out using logistic regression analysis to investigate whether childhood abuse increased the risk of cannabis use and whether the COMT-Val158Met polymorphism was associated with cannabis use.

The log-likelihood ratio test was used to assess the difference between nested models. In our case, if a significant interaction effect was detected, the log-likelihood ratio test was used to examine whether the addition of the interaction term (either two-way or three-way) significantly improved the model fit compared to the main effects model.

A power analysis was performed using the QUANTO V.1.2 program [29]. The sample of 419 individuals had 0.72 power to detect a gene-environment interaction effect, accounting for at least 1.5% of the variance of the studied outcome at an α level of 0.05. If a gene-environment interaction was detected, the effect size was calculated using eta squared (η^2). This parameter can be used to estimate the proportion of variance in the outcome that is accounted for by the predictor. In addition, $P<0.05$ was considered to indicate statistical significance, but we used a more stringent P-value, based on the Bonferroni correction, for the interactions tested. We conducted three tests (main effects, two-way interaction effects and three-way interaction effects) for two outcomes (positive and negative PEs). Therefore, for a Bonferroni correction on the P-values for interactions, we used $P=0.05/6=0.0083$ as a threshold for significance.

RESULTS

To obtain the prevalence of PEs, CAPE scores were recoded as 0 (never, sometimes) and 1 (often, almost always). The resulting prevalence rate indicated that 40.7% of the sample often or almost always experienced at least one positive PE. Childhood abuse was also recoded as 0 (never true) and 1 (rarely true, sometimes true, often true and very often true) to calculate the

prevalence. In the current sample, 25.5% of the individuals were exposed to at least one abusive event during childhood. In regard to cannabis use, 29.1% of the sample used cannabis monthly, weekly or daily.

All the variables included in the model were available for 419 individuals from the total sample. In this final sample, the genotype frequencies for the Val158Met polymorphism of the *COMT* gene were: Val/Val: 30.3% (n=127); Val/Met: 48.0% (n=201); and Met/Met: 21.7% (n=91). These frequencies did not differ from others described in Caucasian individuals [18]. The Hardy-Weinberg equilibrium was verified for the present population (χ^2=.47; df=2; p=.49).

A main effect of childhood abuse was found in both positive (B=.08; SE=.04; p=.047) and negative PEs (B=.09; SE=.05; p=.038). Cannabis use showed a main effect on negative PEs (B=.08; SE=.44; p=.047) but not on positive PEs. No main effect was found for the Val158Met polymorphism of the *COMT* gene on either dimension of PEs. None of the two-way interactions tested (childhood abuse*cannabis use; childhood abuse**COMT* gene or cannabis use**COMT* gene) were significant. However, a significant three-way interaction among childhood abuse, cannabis use and the *COMT* gene was found in positive PEs (B=-.23; SE=.11; p=.006) (Table 1; Fig 1). This result was significant even after correction for multiple testing. It accounted for 2% of the variance of positive PEs (η^2=0.2).

In individuals exposed to childhood abuse who used cannabis, positive PEs score increased as a function of the Val allele dose of the *COMT* gene. However, among individuals exposed to childhood abuse who did not use cannabis, the positive PEs score increased as a function of the Met allele copies of the *COMT* gene. When individuals were exposed to low rates of childhood abuse, cannabis use and the Val158Met polymorphism of the *COMT* gene had a negligible effect on the presence of positive PEs scores.

The log-likelihood ratio test indicated that addition of the three-way interaction term in the third step resulted in a statistically significant improvement in model fit compared to the main effects (χ^2=12.7; df=2; p=.013).

Additional logistic regression analyses revealed that neither childhood abuse (OR=1.01; 95% CI .96-1.07; p=.671) nor the COMT-Val158Met polymorphism (OR=1.19; 95% CI .71-1.98; p=.513) were associated with cannabis use.

DISCUSSION

Our findings suggest that the psychosis-inducing effects of childhood abuse and cannabis use are moderated by the Val158Met polymorphism of the *COMT* gene, which supports a gene-environment-environment interaction effect. Cannabis use after exposure to childhood abuse may have opposite effects on the risk of PEs development, depending on the *COMT* genotypes. Specifically, Val carriers are vulnerable to the psychosis-inducing effects of cannabis.

Rates for PEs and childhood trauma in the current sample were in line with previous reports in European and North American samples [6, 25] (further details can be found elsewhere [3]). In addition, the rate of individuals using cannabis (monthly, weekly or daily) was 29.1%, which is similar to the rates reported in other European countries [30].

As previously shown in this sample, childhood abuse was associated with both positive and negative PEs [3]. These findings support the role of childhood abuse in the development of PEs in the general population, as reported in previous research [7-8]. Furthermore, the fact that cannabis use did not show a main effect on positive PEs in the current study may be related to the inclusion of childhood abuse in the model. As previous studies have suggested, explorations of the association between cannabis and psychosis need to consider the effects of childhood trauma as an important potential effect modifier [11, 13]. Nevertheless, both childhood abuse and cannabis use were associated independently with negative PEs. This association has also been reported previously [10, 31].

Additional analyses enabled us to rule out the possibility that childhood abuse increased the likelihood of using cannabis (environment-environment correlation). A gene-environment correlation can also be discarded, since *COMT* genotypes were not associated with cannabis use. In accordance with recent evidence, we did not find an interaction between the effect of childhood abuse and cannabis use on PEs [17]. However, we believe that this may be related to the

inclusion of *COMT* genotypes in the analyses, since a significant gene-environment-environment interaction effect was detected. This finding is in line with previous studies indicating that environmental exposures, in interaction with genetic factors, may induce psychological or physiological alterations that can be traced to a final common pathway of altered dopamine neurotransmission. This pathway facilitates the onset and persistence of psychotic symptoms [32].

Therefore, our main findings suggest that the psychosis-inducing effects of childhood abuse and cannabis use are moderated by the Val158Met polymorphism of the *COMT* gene, which supports a gene-environment-environment interaction effect.

This three-way interaction effect indicated that positive PEs showed almost no variation for individuals exposed to low rates of childhood abuse, regardless of their cannabis use frequency or their genotype for the Val158Met polymorphism of the *COMT* gene. However, among individuals exposed to childhood abuse, cannabis use only increased the likelihood of reporting positive PEs if individuals were carriers of the Val allele of the *COMT* gene. Furthermore, Met carriers exposed to childhood abuse were more likely to report positive PEs without cannabis use. Thus, our findings suggest that use of cannabis after exposure to childhood abuse may have opposite effects on the development of positive PEs depending on the *COMT* genotypes. These results may partially account for previous discrepancies found when examining the role of cannabis use, childhood trauma and the *COMT* gene in the risk of developing psychotic symptoms [17, 33].

The fact that exposure to both childhood abuse and cannabis was associated with higher scores of positive PEs in Val carriers may be explained by sensitization involving dopaminergic signalling. Evidence from animal studies suggests a possible interaction (exposure to one factor increases sensibility to the effects of the other factor) between stress and THC. Rats living under normal conditions (i.e. access to water and food), that were exposed to THC, showed only minor behavioural changes and no change in dopaminergic transmission [34]. In contrast, under stressful conditions (i.e. isolation and food deprivation), THC administration had marked behavioural consequences and was associated with a significant increase in dopamine uptake [34].

Similarly, it has been shown in humans that the psychosis-inducing effect of cannabis may be stronger in subjects exposed to early stress [14]. Based on previous findings, our results indicate that variability in the *COMT* gene confers different neurobiological vulnerability to cannabis use in the risk of developing PEs. In accordance with previous studies, Val carriers are more vulnerable to the psychosis-inducing effects of cannabis than Met/Met individuals [18-19], but only when exposed to childhood abuse. In line with previous studies indicating that Met carriers were more vulnerable to stress than carriers of the Val/Val genotype [35], Met carriers were vulnerable to the psychosis-inducing effects of childhood abuse, but only when they did not use cannabis. Previous evidence indicates that the risk of psychosis did not increase in Met carriers of the COMT gene who used cannabis [18]. However, in the current study, individuals who are homozygous for the Met allele appeared to be able to use cannabis without any increase in risk of developing PEs, but only when they were exposed to childhood abuse. Indeed, cannabis use by Met/Met carriers seems to benefit these individuals. The proposed role of the endocannabinoid system in extinguishing fear-related memories may help to explain these results [36]. In this regard, the use of cannabis for some psychiatric patients has been proposed, to alleviate the stress associated with childhood traumatic experiences [37]. Nevertheless, this result must be interpreted with caution and needs further research and replication.

The results of the present study should be interpreted in the context of its limitations. Firstly, we used a relatively small sample size to detect a three-way interaction. Indeed, power analysis indicated that the present study was slightly underpowered to detect gene-environment interaction effects accounting for at least 1.5% of the outcome. Nevertheless, to prevent false positive results, all analyses were hypothesis-driven, the interaction effects remained significant after correcting for multiple testing and the effect size of the interaction that was detected accounted for 2% of the variance of positive PEs. Post-hoc power analyses revealed that the current sample had 0.85 power to detect the interaction effect. Secondly, the cross-sectional nature of the design does not allow causal inference. Thirdly, childhood abuse was measured retrospectively, which may constitute an inherent source of bias. That said, the Childhood Trauma Questionnaire has been validated and is considered a reliable measure of childhood

adversity [26]. Finally, frequency of cannabis use was dichotomously defined in the present study, and other parameters that have been related to the expression of psychotic symptoms such as onset, duration or potency of cannabis consumed [19, 38], were not specified.

Although the findings of gene-environment interaction studies have been exciting, there is increasing concern about the reliability and contribution of such results to the understanding of complex traits such as PEs. Dismissal of gene-environment interaction studies arises mainly as a result of the failure to replicate [39]. As there are powerful reasons to expect that gene-environment interaction effects are involved in the aetiology of complex traits and psychiatric disorders [40], the debate is more focused on the reliability of such findings. To prevent false positive results or statistically significant results that may not represent true insights, the current study was developed with an a priori hypothesis that guided the choice of the gene, the polymorphism and the environmental risk factors that were explored. Moreover, as abovementioned, power analyses are specified and correction for multiple testing was applied. To our knowledge, this is the first study reporting a gene-environment- environment interaction effect among childhood abuse, cannabis use and the COMT-Val158Met polymorphism underlying the development of positive PEs. Although these findings need further research and replication, they offer a new vision of the role of cannabis use and the COMT-Val158Met polymorphism in the expression of PEs. Furthermore, our findings may help to explain previous discrepancies in the association between cannabis, the COMT gene and psychosis [18, 33]. Of note, public health messages about the potential risk of cannabis use should not be tempered by results indicating that its use may not be harmful for a subgroup of the population.

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Figure Legends

Table 1. Main effects (1), two-way interaction effects (2) and three-way interaction effects (3) of childhood abuse, cannabis use and the COMT Val158Met polymorphism are presented for positive psychotic experiences (PEs) and negative PEs. All the models were corrected by age, sex, schizotypal personality and trait anxiety. Adjusted R^2 (Adj-R^2) are presented for each step for positive and negative PEs. Significant results are indicated in bold.

Figure 1. Graphic representation of the interaction effect among childhood abuse, cannabis use and the Val158Met polymorphism of the COMT gene on positive psychotic experiences (PEs) corrected for age, sex, schizotypal personality and trait anxiety. Cannabis use and the Val158Met polymorphism of the COMT gene have a negligible effect on positive PEs when individuals are not exposed to childhood abuse or exposed to low rates of such events. The use of cannabis in individuals exposed to childhood abuse has opposite effects depending on their genotype. Positive PEs score increases as a function of the number of copies of the Met allele of the COMT gene in individuals exposed to childhood abuse who do not use cannabis. Met carriers seem to be especially vulnerable to the effect of childhood abuse for their later development of PEs and cannabis use may have a protective effect. However, in individuals exposed to childhood abuse who use cannabis, the positive PEs score increases as a function of the Val allele copies of the COMT gene.

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	Positive PEs			Negative PEs		
	B	**SE**	**p**	**B**	**SE**	**p**
1) Main Effects						
Childhood abuse	**.088**	**.044**	**.047***	**.089**	**.051**	**.038***
Cannabis use	.041	.384	.325	**.081**	**.443**	**.047***
COMT	.025	.241	.541	-.022	.278	.573
2) Two-way interaction effects						
Childhood abuse * Cannabis use	.034	.089	.516	-.031	.103	.539
Childhood abuse * *COMT*	.114	.053	.065	.066	.061	.269
Cannabis use * *COMT*	-.063	.519	.384	-.027	.602	.702
3) Three-way interaction effects						
Childhood abuse * Cannabis use * *COMT*	**-.230**	**.110**	**.006***	-.099	.129	.228

B, standardized coefficient; **SE,** standard error; *p<.05.
Positive PEs: 1) Adj-R^2=.29; 2) Adj-R^2=.29; 3) Adj-R^2=.30
Negative PEs: 1) Adj-R^2=.33; 2) Adj-R^2=.33; 3) Adj-R^2=.33

Table 1. *Main effects (1), two-way interaction effects (2) and three-way interaction effects (3) of childhood abuse, cannabis use and the COMT Val158Met polymorphism are presented for positive psychotic experiences (PEs) and negative PEs. All the models were corrected by age, sex, schizotypal personality and trait anxiety. Adjusted R^2 values (Adj-R^2) are presented for each step for positive and negative PEs. Significant results are indicated in bold.*

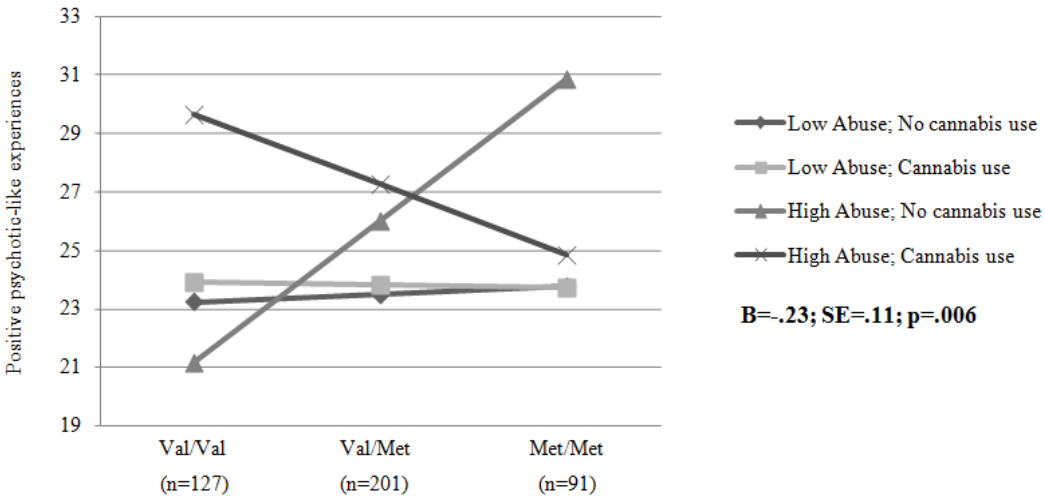

Figure 1. *Graphic representation of the interaction effect among childhood abuse, cannabis use and the Val158Met polymorphism of the COMT gene on positive psychotic experiences (PEs) corrected for age, sex, schizotypal personality and trait anxiety. Cannabis use and the Val158Met polymorphism of the COMT gene have a negligible effect on positive PEs when individuals are not exposed to childhood abuse or exposed to low rates of such events. The use of cannabis in individuals exposed to childhood abuse has opposite effects depending on their genotype. A positive PEs score increases as a function of the number of copies of the Met allele of the COMT gene in those individuals exposed to childhood abuse who do not use cannabis. Met carriers seem to be especially vulnerable to the effect of childhood abuse on their later development of PEs and cannabis use may have a protective effect. However, in individuals exposed to childhood abuse who use cannabis, a positive PEs score increases as a function of the Val allele copies of the COMT gene.*