

ORIGINAL ARTICLE

Lifetime cannabis use and childhood trauma increase risk of psychosis in carriers of *CNR1* genetic variants: findings from the STREAM study

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Objectives: Gene-environment interactions increase the risk of psychosis. The objective of this study was to investigate gene-gene and gene-environment interactions in psychosis, including single nucleotide variants (SNVs) of dopamine-2 receptor (D2R), N-methyl-d-aspartate receptor (NMDAR), and cannabinoid receptor type 1 (CB1R), lifetime cannabis use, and childhood trauma.

Methods: Twenty-three SNVs of genes encoding D2R (*DRD2*: rs1799978, rs7131056, rs6275), NMDAR (*GRIN1*: rs4880213, rs11146020; *GRIN2A*: rs1420040, rs11866328; *GRIN2B*: rs890, rs2098469, rs7298664), and CB1R (*CNR1*: rs806380, rs806379, rs1049353, rs6454674, rs1535255, rs2023239, rs12720071, rs6928499, rs806374, rs7766029, rs806378, rs10485170, rs9450898) were genotyped in 143 first-episode psychosis patients (FEP) and 286 community-based controls by Illumina HumanCoreExome-24 BeadChip. Gene-gene and gene-environment associations were assessed using nonparametric Multifactor Dimensionality Reduction software.

Results: Single-locus analyses among the 23 SNVs for psychosis and gene-gene interactions were not significant ($p > 0.05$ for all comparisons); however, both environmental risk factors showed an association with psychosis ($p < 0.001$). Moreover, gene-environment interactions were significant for an SNV in *CNR1* and cannabis use. The best-performing model was the combination of *CNR1* rs12720071 and lifetime cannabis use ($p < 0.001$), suggesting an increased risk of psychosis.

Conclusion: Our study supports the hypothesis of gene-environment interactions for psychosis involving T-allele carriers of *CNR1* SNVs, childhood trauma, and cannabis use.

Keywords: Cannabis use; childhood trauma; first-episode psychosis; single nucleotide variants

Introduction

Schizophrenia and other psychoses are complex psychiatric disorders characterized by gene-environment interactions.¹ Genome-wide association studies (GWAS) have implicated functional single nucleotide variants (SNVs) in schizophrenia pathogenesis.²⁻⁴ Specifically, the largest and most recent GWAS reported that rare and common variants of the *GRIN2A* gene, which encodes a subunit of the N-methyl-D-aspartate receptor (NMDAR), have a pathogenic role in schizophrenia.⁵

Environmental factors, including cannabis use and childhood trauma, are also associated with first-episode

psychosis and schizophrenia,^{6,7} and growing evidence supports gene-environment interactions in additive models including both cannabis use and childhood trauma.⁸ It has also been suggested that gene-environment interactions can be traced to a final common pathway involving the dopaminergic, glutamatergic, and endocannabinoid systems, which underlies psychosis pathogenesis.⁹ There is a wealth of evidence suggesting that disruptions in NMDAR may lead to dopaminergic impairments (excess in the mesolimbic and reduction in the mesocortical pathways), as already described in schizophrenia.¹⁰ Recent evidence has indicated a key role of the endocannabinoid system in cellular and molecular signaling mechanisms involving

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Submitted Sep 27 2022, accepted Feb 03 2023.

How to cite this article: Loureiro CM, Corsi-Zuelli F, Fachim HA, Shuhama R, de Oliveira AM, Menezes PR, et al. Lifetime cannabis use and childhood trauma increase risk of psychosis in carriers of *CNR1* genetic variants: findings from the STREAM study. *Braz J Psychiatry*. 2023;45:226-235. <http://doi.org/10.47626/1516-4446-2022-2882>

physiological neurotransmission pathways such as the dopamine¹¹ and glutamate systems.¹² However, whether interaction of variants in the dopamine-2 receptor (D2R), NMDAR, and cannabinoid type 1 receptor (CB1R) genes with both cannabis use and childhood trauma increase the risk of psychosis remains unclear.

It is well established that hyperregulation of the dopaminergic system is implicated in the etiology of psychosis.¹³ One of the longest-standing hypotheses in schizophrenia suggests dopaminergic hyperactivity at the D2R, encoded by the *DRD2* gene, which is considered a risk gene for schizophrenia.¹⁴

Abnormalities in the glutamatergic system, particularly hypofunction of NMDAR, have also been hypothesized to play a major role in psychosis pathogenesis, since glutamate is the major excitatory neurotransmitter and its dysfunction results in a wide range of impairments.¹⁵ NMDAR is composed of several subunits, three of which are NR1, NR2A, and NR2B, encoded by *GRIN1*, *GRIN2A*, and *GRIN2B*, respectively.¹⁶ One well-characterized model of glutamatergic hypofunction involves the administration of noncompetitive NMDAR antagonists. This has been shown to induce cognitive- and negative features reminiscent of schizophrenia, including reduction of spatial learning, social withdrawal, and anhedonia,¹⁷ in animal models.¹⁸ Additionally, exome-sequencing studies have revealed that rare damaging mutations in NMDAR genes are found in brain and blood samples from patients with schizophrenia.¹⁹

The glutamatergic system is highly interconnected with the endocannabinoid system.²⁰ The CB1R, encoded by the *CNR1* gene, is widely distributed in brain regions (cortex, olfactory bulb, hippocampus, basal ganglia, and cerebellum)²¹ and peripheral tissues, including skeletal muscle, hepatocytes, and pancreatic beta β -cells.²² Although earlier studies failed to find an association between genetic variants of *CNR1* and schizophrenia,^{23,24} and no GWAS to date has identified common genetic variants within *CNR1* associated with psychosis in large population studies,⁵ there may still be environmental risk factors contributing to psychosis that potentially act through interactions with variants of this gene.

Given that the etiology of psychosis may lie in gene-environment interactions, genetic variants could help define biological subgroups resilient or vulnerable to environmental factors that potentially increase the risk of psychosis. Thus, we hypothesized that SNVs in genes related to dopaminergic, glutamatergic, and endocannabinoid receptors could be involved in the pathogenesis of psychosis through interactions with environmental risk factors. Specifically, we tested for gene-gene interactions and interactions between 23 SNVs of D2R (*DRD2*), NMDAR (*GRIN1*, *GRIN2A*, and *GRIN2B*), and CB1R (*CNR1*) and two well-known environmental risk factors for psychosis: childhood trauma and lifetime cannabis use.

Methods

Sample

This study is part of Schizophrenia and Other Psychosis – Translational Research: Environment and Molecular

Biology (henceforth, STREAM).²⁵ STREAM is part of the international consortium European Network of National Schizophrenia Networks Studying Gene-Environment Interactions (EU-GEI WP2 Group) (<http://www.eu-gei.eu/>).^{26,27} The catchment area selected was the health administrative region of Ribeirão Preto, state of São Paulo, Brazil. The region comprises 26 municipalities corresponding to an area of 9,300 km² and a population of approximately 1.3 million.

In the initial STREAM dataset, DNA blood samples were collected from 202 first-episode psychosis patients (FEPp) and 293 controls. After quality control (QC) for genotyping, 159 FEPp and 289 community-based controls remained in the STREAM dataset. Of these, 429 subjects (143 FEPp and 286 community-based controls) had complete clinical, biological, and environmental assessments and were included in this study.

The sample was composed of incident cases of affective and non-affective psychosis identified in the mental health services of the Ribeirão Preto catchment area from 1 April 2012 to 31 March 2015. We included patients aged 16 to 64 years, with no gender restrictions, who lived in the catchment area and had sought medical care for psychotic symptoms for the first time.²⁵ We excluded patients with previous contact with mental health services for psychosis, psychotic symptoms due to other medical conditions, or presenting during substance intoxication/withdrawal.

We also included controls aged 16 to 64 years, both sexes, with no history of psychotic symptoms, from the same catchment area. For the recruitment of controls, we considered the total population defined by the latest (2010) census (<http://www.ibge.gov.br/>), according to place of residence, stratified by sex, in three age groups (16 to 24, 25 to 34, and > 35 years old).

Sociodemographic and clinical assessments

Face-to-face interviews were held to gather sociodemographic and clinical data, as described elsewhere.²⁸

All included patients met diagnostic criteria for FEPp based on a structured clinical interview for DSM-IV-CV axis I disorders (SCID-I).^{29,30} Moreover, we investigated the history of treatment and the duration of untreated psychosis (DUP) using the Nottingham Onset Schedule (NOS).³¹ Symptom severity at the time of peripheral blood collection was determined by the Brief Psychiatric Rating Scale (BPRS),^{32,33} and the use of cannabis and nine other types of psychoactive substances (inhalants, crack, cocaine, stimulants, sedatives, opioids, hallucinogens, ketamine, and other drugs – the latter covering new psychoactive substances, e.g., mephedrone) was assessed by applying the Cannabis Experiences Questionnaire (CEQ).³⁴ This self-report assessment gives information about lifetime cannabis use, age at first use, frequency, duration, and type of cannabis used. We considered the variable lifetime cannabis use as a measure of an individual's susceptibility, as well as its strong association with genetic variants, consistent with previous studies.^{35,36} Childhood trauma was assessed using Childhood Trauma Questionnaire (CTQ), which

yields a CTQ total score and allows classification of participants into two groups (maltreated or non-maltreated).^{37,38}

Blood samples and genetic analysis

Genotyping and quality control (QC)

Peripheral blood samples were genotyped using a custom Illumina HumanCoreExome-24 BeadChip genotyping array (Cardiff chip) that contains probes for 570,038 genetic variants (Illumina, San Diego, USA). Experiments were performed at the Institute of Psychological Medicine and Clinical Neurology of Cardiff University, as part of a multicenter study for GWAS analysis by DNA microarray techniques. Genotype data were obtained using the GenomeStudio package and transferred into PLINK format for further analysis.

The QC was performed using PLINK v1.07³⁹ or with Perl scripts customized by EU-GEI. Variants and samples with a call rate < 98% and minor allele frequency (MAF) \leq 0.01 were excluded from the dataset. Variants with Hardy-Weinberg equilibrium (HWE) $p < 1e-6$ were excluded. We used the sample QC steps for the genotyping as per EU-GEI WP2.²⁷ After QC, 559,505 variants remained, and we extracted from the array only those of interest.

Population stratification

We used the first 10 principal components (PC) from genotyping (using PLINK³⁹ and RStudio⁴⁰) as covariates to adjust for genetic population stratification. Briefly, as a first step, we merged our sample with the 1000 Genomes Project sample Phase 3⁴¹ to build ancestry PCs of the overlapping SNVs and, subsequently, we applied k-mean clustering to determine individual populations in our sample based on 1000 Genomes sample information. The principal components analysis (PCA) plot shows the dimensions 1 and 2 that correspond the PC1 and PC2, respectively (explaining the maximum and second greatest variance in the data, respectively) (Figure 1).

SNVs selection

We selected D2R and NMDAR genes that were previously significantly associated with schizophrenia and other psychoses,^{5,14} and the *CB1R* gene for its functional relevance to this disorder.⁴² We used three strategies to select SNVs from the five candidate gene regions (covering target sites, upstream, and downstream): i) tagging (Haploview 4.2) at an r^2 threshold of 0.6 to capture 98% of the variants most common in HapMap phase II and a MAF > 0.01; ii) SNV functionality according to data published on Ensembl (<http://www.ensembl.org>); and iii) previous associations with psychosis reported in the literature for *DRD2*,⁴³⁻⁴⁵ *GRIN1*, *GRIN2A*, *GRIN2B*,^{44,46-48} and *CNR1*.⁴⁹

Twenty-three SNVs of the D2R (*DRD2*: rs1799978, rs7131056, and rs6275), NMDAR (*GRIN1*: rs4880213, rs11146020; *GRIN2A*: rs1420040, rs11866328; *GRIN2B*: rs890, rs2098469, and rs7298664), and *CB1R* genes

(*CNR1*: rs806380, rs806379, rs1049353, rs6454674, rs1535255, rs2023239, rs12720071, rs6928499, rs806374, rs7766029, rs806378, rs10485170, and rs9450898) were analyzed.

We calculated the power to detect an odds ratio (OR) of 2 in a case-control study with 429 subjects, including 143 FEPp and 286 controls (case rate of 30%), over a range of MAF from 0.09 to 0.50 in order to cover the frequencies of the candidate genes. For all possible combinations of true and test models (additive, dominant, and recessive), assuming an alpha of 0.05, a binary outcome with prevalence of 0.01, and gene-environment interaction, the statistical power reached ~80% (RStudio Team 2020, CRAN.R-project.org/package=genpwr).

Statistical analysis

Statistical analyses were conducted in SPSS version 26 and RStudio.⁴⁰ Data were checked for normality using Shapiro-Wilk's test. We described the sociodemographic, clinical, and environmental (lifetime cannabis use and childhood trauma) variables between groups (community-based controls and FEPp) using Student's *t* test and Fisher's exact test. HWE was calculated for each selected SNV using an online calculator (Excel-based HWE Test by Michael H. Court [2005-2008], Court lab – HW calculator – important.xls), using a nominal $p < 0.05$ as the cutoff.

To investigate the effect of SNVs on psychosis, data were analyzed using binary logistic regression models (OR [β], 95%CI), including group as the binary outcome (community-based controls and FEPp) in both unadjusted and adjusted models (sex, age, years of education, tobacco smoking, and the first 10 PCs to correct the possible bias due to an association between SNVs and genetic ancestry in an admixed population), considering the frequencies of the genotypes under the dominant (homozygous ancestral \times heterozygous + homozygous variant), additive (homozygous ancestral \times heterozygous \times homozygous variant), and recessive models (homozygous variant \times homozygous ancestral + heterozygous). To determine the significance level for multiple test errors, we performed the Bonferroni's test and considered an adjusted $p < 0.002$ (0.05/23) as statistically significant, taking into account the 23 selected SNVs. Moreover, we used Fisher's exact test for comparisons between the environmental risk factors and psychosis.

Associations between and within gene-gene and between the 23 SNVs and environmental risk factors (lifetime cannabis use [yes or no] and childhood trauma [yes or no]) were tested using the nonparametric Multifactor Dimensionality Reduction (MDR) software (version 3.0.2) (www.epistasis.org), considering 10 data divisions as the same best model for cross-validation consistency (CVC), testing accuracy and an empirical $p < 0.05$.⁵⁰ We included a binary outcome (community-based controls and FEPp) to analyze associations between *GRIN1*, *GRIN2A*, *GRIN2B*, *DRD2*, and *CNR1* genotypes in three groups (ancestral homozygous vs. heterozygous vs. minor allele homozygous) in relation to the environmental

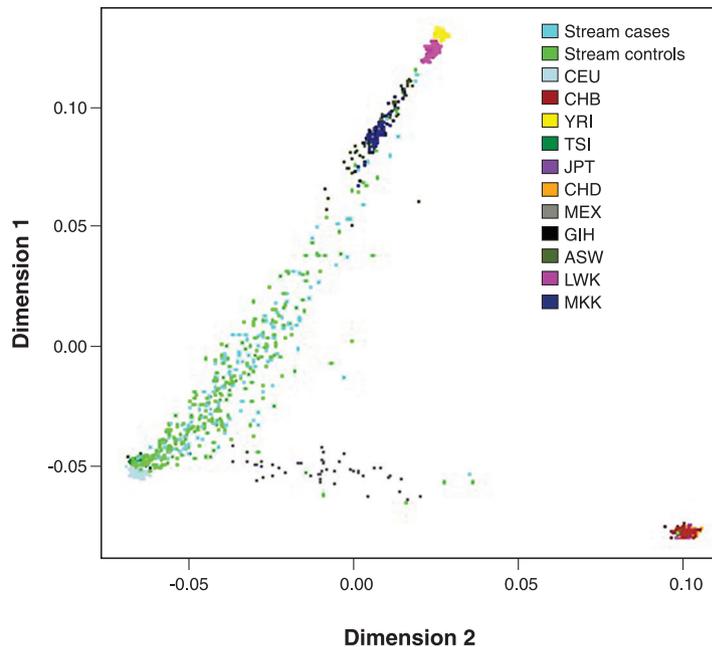


Figure 1 Principal components (PC) analysis plot of the studied Ribeirão Preto catchment area individuals in comparison to 1000 Genomes Project populations. The first two PCs (PC1 and PC2, corresponding to dimensions 1 and 2) are shown, and the colors illustrate the stream sample (143 cases, light blue; 286 controls, neon green) with the 1000 Genome Project sample Phase 3.⁴¹ ASW = Americans of African ancestry from Southwest United States; CEU = Utah residents with Northern and Western European ancestry from Utah, United States; CHB = Han Chinese from Beijing, China; CHD = Chinese from metropolitan Denver, Colorado, United States; GIH = Gujarati Indians from Houston, Texas, United States; JPT = Japanese from Tokyo, Japan; LWK = Luhya in Webuye, Kenya; MEX = Americans of Mexican ancestry from Los Angeles, California, United States; MKK = Maasai in Kinyawa, Kenya; TSI = Italians from Tuscany, Italy; YRI = Yoruba in Ibadan, Nigeria.

factors. For models deemed statistically significant in MDR analysis, we subsequently performed the Hosmer-Lemeshow test in the logistic regression to assess goodness of fit. The reference allele was based on population genetics data from African, European, and Native Americans (bank of allele frequency, <https://www.ensembl.org/index.html/>).

Considering the multifactorial etiology of psychosis (multiple environmental risk factors, polygenicity, and the interactions thereof), MDR has been proposed as a complementary method to linear and logistic regressions to improve the identification of gene-gene and gene-environment interactions given that no genetic mode of inheritance is assumed, and this approach avoids increased type II errors and decreasing the power by reducing the dimensionality of multilocus genotype combinations.^{51,52} The possible multifactor classes from n combinations between genetic and/or environmental variables are labeled as high-risk or low-risk to disease, and the ratio of the number of patients to the number of controls is calculated within these multifactor classes. MDR analysis calculated the empirical p -value derived from permutation testing (p -values based on 1,000 permutations) at a 0.05 significance level. In our results, we present the mean p -value from the p -value derived from permutation testing. The criteria for the best final interaction models included: i) the minimal prediction error; ii) the CVC 9 or 10-fold cross validation; iii) significance of the p -value (< 0.05); and iv) close values

of the training and testing balance accuracy (TBA = 0.55-0.69), as described by Moore.⁵³

We constructed three models (dominant, additive, and recessive) to estimate the average effects of the risk allele for the associations between genes and psychosis. However, an additive genetic model was employed to estimate the effects across each genotype for the gene-gene and gene-environment interactions to show the genetic variant contribution of each gene better.

Ethics statement

This study was approved by the ethics committee of Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto (opinion number 12.606/2012). Informed consent was obtained from each participant prior to study enrollment.

Results

Sociodemographic, clinical, and environmental profiles of the participants

FEPp had a relatively higher proportion of males and non-whites, lower education, higher frequency of tobacco smoking, and, as expected, higher BPRS scores than controls ($p < 0.001$ for all comparisons). Age ($p = 0.648$) and body mass index (BMI) ($p = 0.062$) did not differ significantly between groups (Table 1).

Table 1 Sociodemographic and clinical variables of participants (n=429)

Characteristics	Community-based controls (n=286)	FEPp (n=143)	p-value
Sociodemographic variables			
Sex [†]			< 0.001
Female	148 (51.7) ^a	57 (39.9) ^a	
Male	138 (48.3) ^a	86 (60.1) ^a	
Education in years [‡]			< 0.001
≤ 9	64 (22.4) ^a	58 (40.6) ^b	
> 9	222 (77.6) ^a	85 (59.4) ^b	
Skin color [†]			0.004
White	196 (68.5) ^a	76 (53.1) ^b	
Non-white	90 (31.5) ^a	67 (46.9) ^b	
Clinical variables			
Age, [‡] years: mean (SD)	33.7 (12.6)	32.8 (12.1)	0.648
BMI, [‡] kg/m ² : mean (SD)	26.4 (6.4)	25.1 (6.5)	0.062
BPRS, [‡] total score: mean (SD)	0.8 (1.9) ^a	9.0 (7.1) ^b	< 0.001
Age of onset, [‡] years: mean (SD)		31.3 (11.9)	
DUP, weeks: median (Min-Max)		10.0 (0.0-1.056.0)	
Duration of disease, weeks: median (Min-Max)		39.6 (1.6-1.094.4)	
Duration of treatment, weeks: median (Min-Max)		21.0 (0.0-155.4)	
Tobacco smoking [†]			< 0.001
Yes	44 (15.4) ^a	46 (32.2) ^b	
Lifetime cannabis use [†]			< 0.001
Yes	55 (19.2) ^a	71 (49.7) ^b	
Childhood trauma [†]			< 0.001
Yes	68 (23.8) ^a	74 (51.7) ^b	

Data presented as n (%), unless otherwise specified.

Significant results are highlighted in bold.

BMI = body mass index; BPRS = Brief Psychiatric Rating Scale; DUP = duration of untreated psychosis; FEPp = first-episode psychosis patients.

[†] Fisher's exact test.

[‡] Student's *t* test.

^{a,b} Different superscript letters denote statistically significant differences between means.

The environmental risk factors of lifetime cannabis use and childhood trauma were each significantly associated with FEPp compared to controls (Table 1) ($p < 0.001$ for both comparisons). However, these two factors were themselves significantly associated in the control group, but not in the FEPp group. The lifetime frequency of either cannabis use or childhood trauma among controls was 7.3% ($\chi^2 = 7.80$; $p = 0.008$), while in the FEPp group it was 25.2% ($\chi^2 = 0.06$; $p = 0.868$) (Table S1, available as online-only supplementary material).

Associations between SNVs and psychosis

Group differences in genotype frequencies and their association with psychosis using the dominant, additive, and recessive models are described in Tables S2, S3, and S4 (available as online-only supplementary material), respectively. Single-locus analysis showed no significant association between the 23 SNVs and psychosis ($p > 0.05$ for all comparisons; adjusted models for sex, age, 10 PCs, years of schooling, and tobacco).

Gene-gene and gene-environment interactions in psychosis

Regarding gene-gene interactions, no significant associations within or between genes were found ($p > 0.05$ for all comparisons, data not shown). However, MDR analyses

demonstrated approximately 60-70% of the ability to classify the individuals included in the analysis and showed significant gene-environment interactions for the polymorphic loci rs12720071 and rs7766029 of the *CNR1* gene (Table S5, available as online-only supplementary material). The best association model was represented by the combination of *CNR1* rs12720071 with lifetime cannabis use (testing accuracy 66.4%, CVC 10/10, $p < 0.001$) (Figure 2A). The results suggested a higher risk for psychosis when combining the TT genotype of rs12720071 with lifetime cannabis use (OR = 7.1; 95%CI 2.5-19.8; $p < 0.001$). The Hosmer-Lemeshow statistic predicts 69.2% of the model.

Moreover, *CNR1* rs12720071 (TT genotype) also showed a significant association with childhood trauma as a risk for psychosis (testing accuracy of 62.1%, CVC of 6/10 and $p = 0.005$) (Figure 2B). Likewise, as happened in the model including cannabis use, the TT genotype was detrimental when childhood trauma was combined (OR = 3.3; 95%CI 1.4-7.9; $p = 0.006$). Finally, an interaction between *CNR1* rs7766029 (T-allele) with both lifetime cannabis use and childhood trauma presented a testing accuracy of 69.3%, CVC of 8/10, and $p < 0.001$ for association with psychosis (Figure 2C). This model shows that the combination of rs7766029-T homozygosity and the presence of both environmental risk factors increases the risk for psychosis (OR = 3.2; 95%CI 1.5-6.5; $p = 0.002$). Although models 2(B-C) are significant

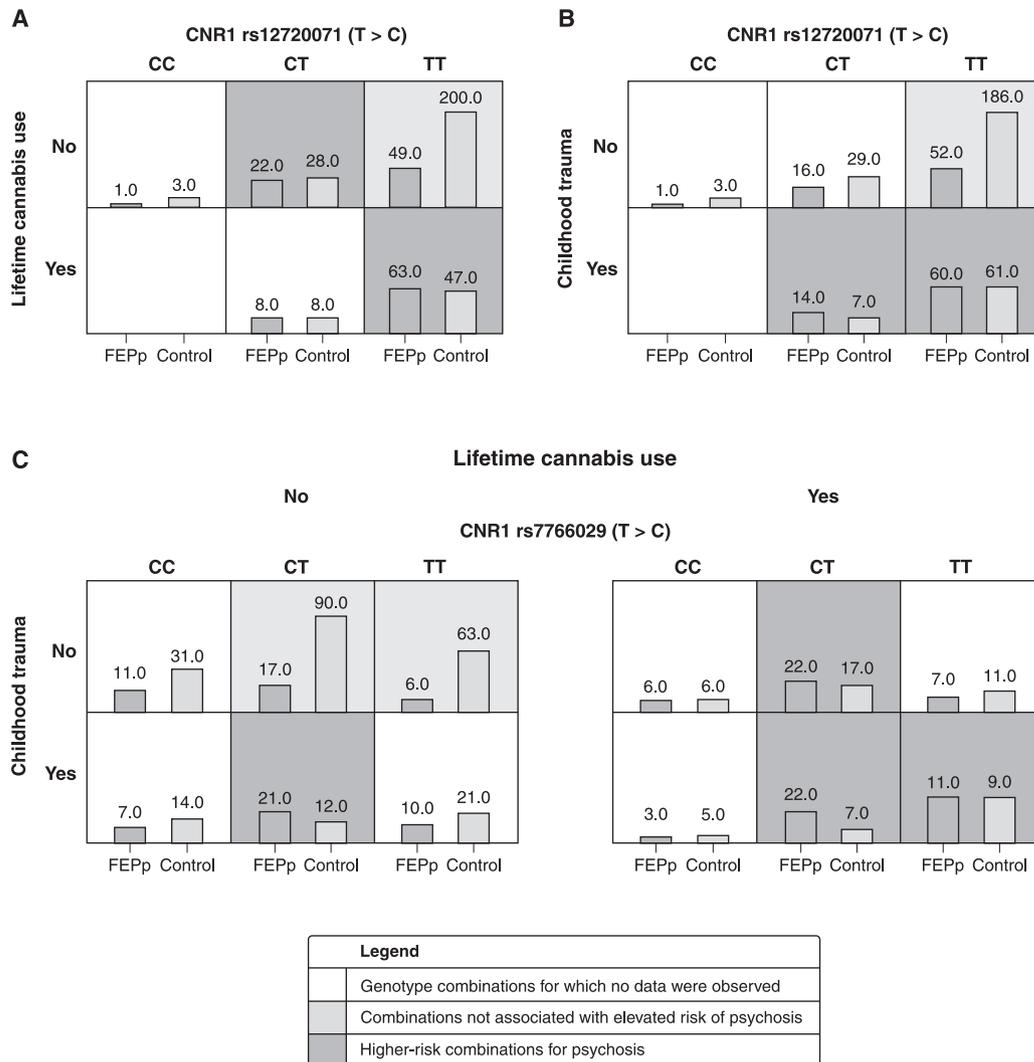


Figure 2 Models assessing gene-environment and gene-gene associations considering: A) Lifetime cannabis use: individuals with lifetime cannabis use who were *CNR1* rs12720071 T-allele carriers had a greater risk of psychosis (cross-validation consistency [CVC] = 10/10, testing accuracy = 66.4%, $p < 0.001$). The bars show the frequency distribution of genotypes for the first-episode psychosis patients (FEPp) and controls (left and right columns, respectively) in relation to lifetime cannabis use; B) Childhood trauma: risk association between individuals with childhood trauma and *CNR1* rs12720071-T-allele carriers (CVC = 6/10, testing accuracy = 62.1%, $p = 0.005$). The bars show the frequency distribution of genotypes for the FEPp and controls (left and right columns, respectively) in relation to childhood trauma; and C) Lifetime cannabis use and childhood trauma: increased risk for developing psychosis in individuals with lifetime cannabis use, childhood trauma, and *CNR1* rs7766029 T-allele carrier status (CVC = 8/10, testing accuracy = 69.3%, $p < 0.001$). The bars show the frequency distribution of genotypes for the FEPp and controls (left and right columns, respectively) in relation to both environmental risk factors.

and clinically relevant, neither reached the considerations described by Moore⁵³ to be classified as a good model. No significant associations between the environmental factors and other SNVs were found.

Discussion

In this case-control study of selected genetic and environmental risk factors for psychoses, we have demonstrated the interaction of SNVs of the *CNR1* gene (rs12720071 and rs7766029) with both lifetime cannabis use and childhood exposure to trauma as increasing risk for psychosis based on analyses with the MDR approach. This

approach has been applied to many psychiatric diseases, including non-affective^{54,55} and affective disorders.^{56,57}

Various lines of evidence point to associations of cannabis use and childhood trauma with psychosis.⁵⁸ We have confirmed both these associations in our sample. A relationship between childhood trauma and cannabis use in the control group indicates that early life stress might be a risk factor for subsequent cannabis use; the loss of this relationship in the FEPp suggests that other factors underlie the greater incidence of cannabis use in this latter group.

A fast-growing field of schizophrenia research has recently suggested the interactive effects of environmental

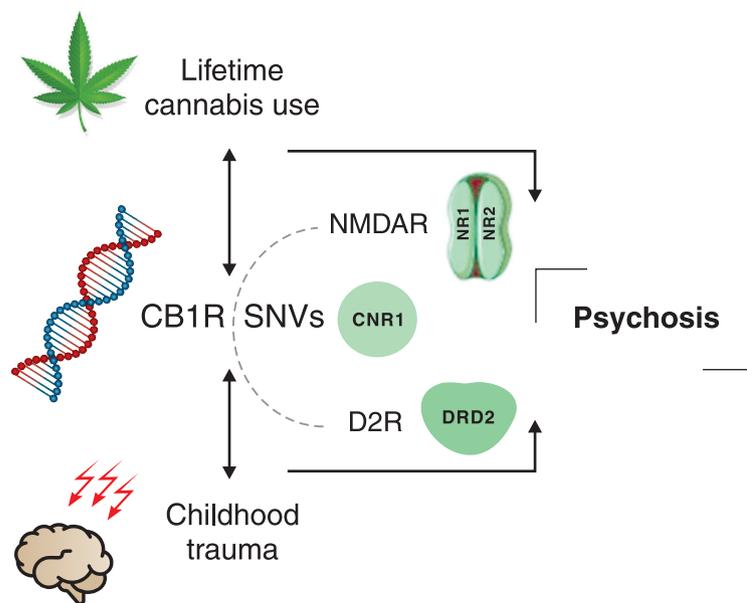


Figure 3 Schematic representation of our main findings. In the presence of *CNR1* gene variants, lifetime cannabis use and childhood trauma may modulate the N-methyl-D-aspartate receptor (NMDAR) and dopamine-2 receptor (D2R) genes, increasing the risk of psychosis. CB1R = cannabinoid receptor type 1; SNVs = single nucleotide variants.

and genetic factors moderating the risk of psychosis.⁹ We found a significant interaction between *CNR1* genotype rs12720071 and cannabis use – an important environmental risk factor – in its association with psychosis. T-allele carriers of *CNR1* presented a lower risk of psychosis when cannabis use was absent, while the same allele increased the risk of psychosis when the environmental risk factor was present. Moreover, T-allele carriers of *CNR1* (rs12720071) presented an increased risk of psychosis when childhood trauma was present, while the model that included both environmental factors also showed that cannabis use abolished the protective association of rs7766029-T-allele with psychosis in the absence of childhood trauma. However, although we found statistical significance in MDR analysis, neither model met established criteria to be considered “good” in terms of representing the predictive ability of the gene-environment interactions in the case-control study. Thus, our findings may suggest that lifetime cannabis use is moderated by the *CNR1* genetic variant for psychosis. Our results demonstrate that *CNR1* rs12720071-T homozygosity interacts with lifetime cannabis use, suggesting combined gene-environment influences in mediating phenotypic features of psychosis.

The CB1 receptor, encoded by the *CNR1* gene, is the primary brain receptor stimulated by endocannabinoids and exogenously by Δ^9 -THC.⁵⁹ Despite conflicting evidence, SNVs of *CNR1* gene polymorphic loci (rs12720071 and rs7766029), localized to chromosome 6q14-15, have been associated with cannabis use and psychosis risk.^{49,60} For instance, cannabis use was reported as a moderating factor between *CNR1* rs12720071 genotypes and changes in cognitive performance in schizophrenia.^{61,62}

The two *CNR1* SNVs (rs12720071 and rs7766029) are localized in the 3'-untranslated region (3'-UTR) of exon 4, which plays a major role in gene expression regulation.⁶³ Evidence shows that the presence of the variant on rs12720071 could be a binding site of a transcription factor for CCAAT/enhancer-binding protein beta (C/EBP-beta),⁶⁴ with effects on neurogenesis.^{65,66} Consequently, the nucleotide switch could potentially change the C/EBPbeta transcription factor binding site, repressing CB1R expression and resulting in cognitive deficits in rs12720071-T-allele carriers who engage in cannabis use.⁶¹

Studies have also suggested a functional and direct relationship between NMDA and CB1 receptors that may be dysregulated by cannabinoid agonists, such as Δ^9 -THC, in precipitating psychosis susceptibility and inducing NMDAR hypofunction,¹² which may compromise glutamate signaling and, consequently, essential processes including synaptic plasticity, memory formation, and learning.²⁰

Additionally, although evidence has shown alterations in dopaminergic signaling and NMDAR genes in psychoses,¹⁰ inconsistencies remain concerning their associations. Considering our criteria for the MDR analysis, our findings did not show associations among D2R or NMDAR variants with environmental factors as a risk for psychosis, as we did not also reproduce the associations between these variants and psychosis reported previously,^{5,44,46-48} potentially attributable to the sample size and genetic diversity in different populations.

To the best of our knowledge, this is the first study to investigate the potential interactions of D2R, NMDAR, and CB1R SNVs in psychosis using the MDR statistical tool. We also speculate that the interaction between lifetime cannabis use or childhood trauma and *CNR1*

genotypes may induce neuromodulatory dysfunctions in dopaminergic and glutamatergic pathways, especially in the presence of another environmental risk factor, contributing to the multigene interaction involved in the pathophysiology of psychosis (Figure 3).

Despite our small sample size and exploratory nature of the study, our findings demonstrate the risk-increasing effects driven by gene-environment interactions of previously established environmental factors,⁸ supporting the multifactorial etiology and polygenicity of psychotic disorders. Recent findings have demonstrated that many common variants of risk genes with minor effects have been associated with schizophrenia and other psychosis,⁵ reinforcing the strong evidence of polygenic inheritance associated with multifactorial risk factors for psychosis. However, our gene-environment interaction findings may be relevant to genetic studies in non-European countries due to the genetic and cultural heterogeneity of the Brazilian population. The results of this study have some limitations. Due to the cross-sectional nature of the analysis presented, we are unable to make comparisons over time. Thus, larger and longitudinal cohorts are required to validate these findings and expand the interpretations about the causal effects of gene-environment interactions. In addition, the higher prevalence of lifetime cannabis use in FEP patients may be skewing the association of gene-environment interaction; however, we do not believe this occurred in our study, since the frequency of the rs12720071 genotype was not significantly different between the FEP group and the controls. Further research should attempt to control the discrepancies in genotype and environmental factor prevalences to understand better their association with psychosis. Finally, we did not include linkage disequilibrium analysis or haplotype-based approaches. Future studies should include haplotypes to identify the *cis* interaction between selected SNVs in addition to the main effects in relation to psychosis susceptibility.

We stress that the power of our results should be interpreted with caution, due to the small sample size after group comparisons. Our findings merit further investigation in a larger sample to elucidate the minor contributions of each SNV and their relationships with environmental factors.

In conclusion, our study supports the hypothesis of gene-environment interactions for psychosis and uncovers a genetic liability dependent on such interactions, even when the SNVs involved showed no independent effect. Specifically, we suggest a gene-environment interaction involving the SNVs rs12720071 and rs7766029-T-allele of the *CNR1* gene, childhood trauma, and lifetime cannabis use in psychosis.

Acknowledgements

This study was supported by the research grant from the clinical research fund of Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; grants 2012/05178-0 and 2013/08216-2) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; Finance Code 001). CML, FC-Z, HAF, and RS are

recipients of fellowships from FAPESP (grants 2020/15752-1; 2021/07448-3, and 2019/13229-2; 2017/00624-5 and 2015/02948-7; 2013/11167-3, respectively). AMO receives grant from CAPES – Finance Code 001. PRM, PL-J, SIB, RL, and CMD-B are recipients of fellowships from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Disclosure

GPR has received honoraria for lectures and/or advisory panel membership from Janssen, Lundbeck, Otsuka, and Sunovion, and a research grant from Sunovion. The other authors report no conflicts of interest.

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