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

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Abstract

Background: Gene-environment interactions increase the risk of psychosis. Aim: 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 related to D2R (DRD2: rs1799978, rs7131056, rs6275), NMDAR (GRIN1: rs4880213, rs11146020; GRIN2A: rs1420040, rs11866328; GRIN2B: rs890, rs2098469, rs7298664), and CB1R genes (CNR1: rs806380, rs806379, rs1049353, rs6454674, rs1535255, rs2023239, rs12720071, rs6928499, rs806374, rs7766029, rs806378, rs10485170, rs9450898) were genotyped in 143 first-episode psychosis patients (FEPp) and 286 community-based controls by Illumina HumanCoreExome-24 BeadChip. Associations between gene-gene and gene-environment were performed using nonparametric multifactor dimensionality reduction software.

Results: Single locus analysis among the 23 SNVs with 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 SNV in CNR1 and cannabis use. The best performing model was the combination between 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 the T allele carriers of CNR1 SNVs, childhood trauma and cannabis use in psychosis.

Keywords: Cannabis use; Childhood trauma; First-episode psychosis; Single Nucleotide Variants

## Introduction

Schizophrenia and other psychoses are complex psychiatric disorders characterised by gene-environment interactions [1]. Genome-wide association studies (GWAS) have revealed functional single nucleotide variants (SNVs) in schizophrenia pathogenesis [2–4]. Specifically, the largest and most recent GWAS reported the rare and common variants of the *GRIN2A* gene of N-methyl-d-aspartate receptor (NMDAR) to 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 plenty of evidence suggesting that disturbances in NMDAR may lead to dopaminergic impairments (an excess in the mesolimbic and reduction in the mesocortical pathways) as already described in schizophrenia [10]. Recently, the endocannabinoid system has been indicated to have a key role in cellular and molecular signalling mechanisms involving the physiological neurotransmission pathways such as dopamine [11] and glutamate systems [12]. However, whether interaction of the variants in the dopamine-2 receptor (D2R), NMDAR and cannabinoid type 1 receptor (CB1R) genes with both cannabis use and childhood trauma increases the risk of psychosis remains unclear.

It is well established that hyperregulation in the dopaminergic system is implicated in the aetiology of psychosis [13]. This is one of the longest-standing hypotheses in schizophrenia that suggest 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 hypothesised 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 non-competitive 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 schizophrenia patients [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 may act through interactions with genetic variants in this gene.

Given that the aetiology of psychosis may lie in gene-environment interactions, genetic variants could help to define biological subgroups resilient or vulnerable to environmental factors that potentially increase the risk of psychosis. Thus, we hypothesised that SNVs in genes related to dopaminergic, glutamatergic and endocannabinoid receptors could be involved in the pathogenesis of psychosis through their interaction with environmental risk factors. Specifically, we tested for gene-gene interactions and interactions between the 23 SNVs related to 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.

## 1. Methods

### 2.1. Sample

This study is part of *Schizophrenia and Other Psychosis - Translational Research: Environment and Molecular Biology*, from now on referred as 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, São Paulo state, Brazil. The region comprises 26 municipalities corresponding to an area of 9300 $km^2$ and a population of approximately 1.3 million inhabitants.

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 1st April 2012 to 31st March 2015. We included patients aged 16 to 64 years old, both sexes, living in the catchment area and searching for 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 only during substance intoxication/withdrawal.

We also included controls aged 16 to 64 years old, both sexes, with no history of psychotic symptoms and from the same catchment area. For the recruitment of controls, we considered the total number of inhabitants defined by the 2010 last 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 more than 35 years old).

This study was approved by the Clinics Hospital of the Ribeirão Preto Medical School ethics committee (Process number 12.606/2012) and informed consent was obtained from each participant prior to study enrolment.

2.2. Sociodemographic and clinical assessments

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

All included patients meet diagnostic criteria for FEPp based on 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]. The symptom severity at the moment of peripheral blood collection was determined by the Brief Psychiatric Rating Scale (BPRS) [32,33] and the use of cannabis and other nine types of psychoactive substances (inhalants, crack, cocaine, stimulants, sedatives, opioids, hallucinogens, ketamine and other drugs that covers new psychoactive substances; e.g. mephedrone) was assessed by applying the Cannabis Experiences Questionnaire [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 and its strong association with genetic variants, consistent with previous studies [35,36]. Childhood trauma was assessed using Childhood Trauma Questionnaire (CTQ), which allows a CTQ total score and a classification of participants in two groups (maltreated and non-maltreated) [37,38].

2.3. Blood samples and genetic analysis

*2.3.1. Genotyping and quality control*

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, CA). Experiments were performed at the Institute of Psychological Medicine and Clinical Neurology of Cardiff University, as part of the multicentre 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 quality control was performed using PLINK v1.07 [39] or with Perl scripts customised by EU-GEI. Variants and samples with a call rate <98% and minor allele frequency ≤0.01, were excluded from the dataset. Variants with Hardy-Weinberg Equilibrium (HWE) p-value <1e-6 were excluded. We used the sample quality control steps for the genotyping as EU-GEI WP2 [27]. After quality control, 559,505 variants remained and we extracted from the array only the variants of interest.

*2.3.2. Population stratification*

We used the first 10 principal components (PC) from genotyping (using PLINK [39] and RStudio [40]) as covariants to adjust for genetic population stratification. Briefly, as a first step, we merged our sample with the 1000 Genome project sample phase 3 [41] to build ancestry PCs of the overlapping SNVs, and subsequently, we applied k-mean clustering to determine individual populations in our sample based on Thousand Genomes sample information. The plot of principal component analysis (PCA) 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**).

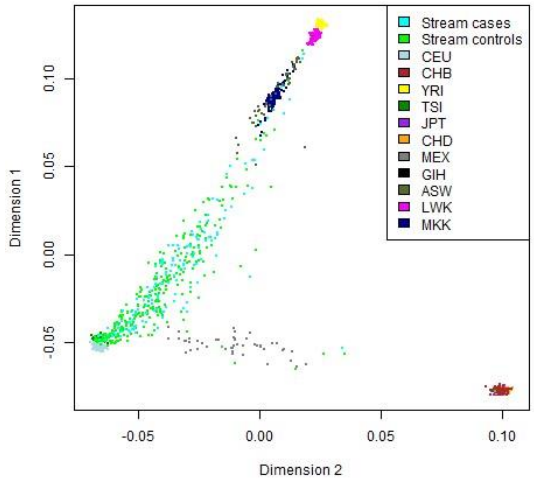


**Figure 1** Principal Component Analysis plot of the studied Ribeirão Preto catchment area individuals in comparison to 1000 Genomes Project populations. The first two PCs (PC1 and PC2, corresponding to dimensions 1 and 2) are shown, and the colours illustrate the Stream sample (143 cases, blue light and 286 controls, neon green) with the 1000 Genome Project sample phase 3 [41] (CEU: Utah residents with Northern and Western European ancestry from UT; CHB: Han Chinese from Beijing, China; YRI: Yoruba in Ibadan, Nigeria; TSI: Italians from Tuscany, Italy; JPT: Japanese from Tokyo, Japan, CHD: Chinese from metropolitan Denver, Colorado; MEX: Americans of Mexican ancestry from Los Angeles, CA; GIH: Gujarati Indians from Houston, TX; ASW: Americans of African ancestry from Southwest United States; LWK: Luhya in Webuye, Kenya; MKK: Maasai in Kinyawa, Kenya).

*2.3.3. SNVs selection*

We selected D2R and NMDAR genes that were previously significantly associated with schizophrenia and other psychoses [5,14], and the CB1R gene from their 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): (a) tagging (Haploview 4.2) at an $r^2$ threshold of 0.6 to capture 98% of the variants most common HapMap phase II and a minor allele frequency (MAF) > 0.01; (b) SNV functionality according to data published on Ensembl (http://www.ensembl.org); and (c) previous associations with psychosis reported in the literature of *DRD2* [43–45], *GRIN1*, *GRIN2A*, *GRIN2B* [44,46–48] and *CNR1* [49].

Twenty-three SNVs of 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 analysed.

We calculated the power to detect an odds ratio of 2 in a case-control study with 429 subjects, including 143 FEP and 286 controls (case rate of 30%) over a range of minor allele frequencies from 0.09 to 0.50 in order to cover the frequencies of the candidate genes. We calculated the power 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 power reached ~80% (RStudio Team 2020**,** CRAN.R-project.org/package=genpwr/).

## 2.4. Statistical analysis

Statistics were conducted in SPSS version 26.0 (SPSS Inc; IBM Corp: Armonk, NY, USA) 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. The 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 value<0.05 as the cutoff.

To investigate the effect of SNVs on psychosis, data were analysed 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 x heterozygous + homozygous variant), additive (homozygous ancestral x heterozygous x homozygous variant) and recessive models (homozygous variant x homozygous ancestral + heterozygous). To determine the significance level for multiple test errors, we performed the Bonferroni's test and considered an adjusted p-value < 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 performed 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-value < 0.05 [50]. We included a binary outcome (community-based controls and FEPp) to analyse associations between *GRIN1, GRIN2A*, *GRIN2B, DRD2* and *CNR1* genotypes in three groups (ancestral homozygous versus heterozygous versus minor allele homozygous) in relation to the environmental factors. For statistically significant models in MDR analysis, subsequently, we performed the Hosmer–Lemeshow test in the logistic regression to assess the goodness of fit the model. 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 aetiology of psychosis (multiple environmental risk factors and polygenicity as their interactions), 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 set of multifactor classes from n combinations between genetic and/or environmental variables are labelled 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 were based on 1000 permutations) at a 0.05 significance level. In this study, we presented the mean p-value from the p-value derived from permutation testing. The criteria for the best final interaction models included: (a) the minimal prediction error; (b) the CVC 9 or 10-fold cross validation; (c) the significance of p-value (less than 0.05); and (d) close values of the training and testing balance accuracy (TBA=0.55-0.69) as described by Moore (2015) to be considered a good model [53].

We performed 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.

## 3. Results

### 3.1. Sociodemographic, clinical and environmental variables of the participants

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

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

| Characteristics | Community-based controls (n=286) | FEPp (n=143) | p |
|---|---|---|---|
| **Sociodemographic variables** | | | |
| **Sex**[1]: n (%) | | | **<0.001** |
| Female | 148 (51.7)[a] | 57 (39.9)[a] | |
| Male | 138 (48.3)[a] | 86 (60.1)[a] | |
| **Education in years**[1]: n (%) | | | **<0.001** |
| ≤ 9 | 64 (22.4)[a] | 58 (40.6)[b] | |
| > 9 | 222 (77.6)[a] | 85 (59.4)[b] | |
| **Skin colour**[1]: n (%) | | | **0.004** |
| White | 196 (68.5)[a] | 76 (53.1)[b] | |
| Non-white | 90 (31.5)[a] | 67 (46.9)[b] | |
| **Clinical variables** | | | |
| **Age**[2], years: mean (SD) | 33.7 (12.6) | 32.8 (12.1) | 0.648 |
| **BMI**[2], Kg/m$^2$: mean (SD) | 26.4 (6.4) | 25.1 (6.5) | 0.062 |
| **BPRS total score**[2]: mean (SD) | 0.8 (1.9)[a] | 9.0 (7.1)[b] | **<0.001** |
| **Age of onset**[2], years: mean (SD) | | 31.3 (11.9) | |
| **DUP**, weeks: median (Min-Max) | | 10.0 (0.0–1056.0) | |
| **Duration of disease**, weeks: median (Min-Max) | | 39.6 (1.6–1094.4) | |
| **Duration of treatment**, weeks: median (Min-Max) | | 21.0 (0.0–155.4) | |
| **Tobacco smoking**[1]: n (%) | | | **<0.001** |
| Yes | 44 (15.4)[a] | 46 (32.2)[b] | |
| **Lifetime cannabis use**[1]: n (%) | | | **<0.001** |
| Yes | 55 (19.2)[a] | 71 (49.7)[b] | |
| **Childhood trauma**[1]: n (%) | | | **<0.001** |
| Yes | 68 (23.8)[a] | 74 (51.7)[b] | |

[1] *Fisher's Exact* Test.; [2] Student's t-test.
[a,b] Superscript letters were used for the mean values in the columns, in which the means followed by different letters differ statistically from each other.
FEPp: First-episode Psychosis Patients; SD: Standard Deviation; BMI: Body Mass Index; BPRS: Brief Psychiatric Rating Scale, DUP: Duration of Untreated Psychosis.
Significant results are highlighted in bold.



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 or childhood trauma among controls was 7.3% ($\chi^2$=7.80; p=0.008), while in the FEPp group was 25.2% ($\chi^2$=0.06; p=0.868) (**Supplementary Material, Table S1**).

3.2. 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 **Supplementary Material, Tables S2, S3 and S4**, 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).

3.3. 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 60.0-70.0% 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 (**Supplementary Material, Table S5**). The best association model was represented by the combination of *CNR1* rs12720071 with lifetime cannabis use (testing accuracy of 66.4%, CVC of 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.

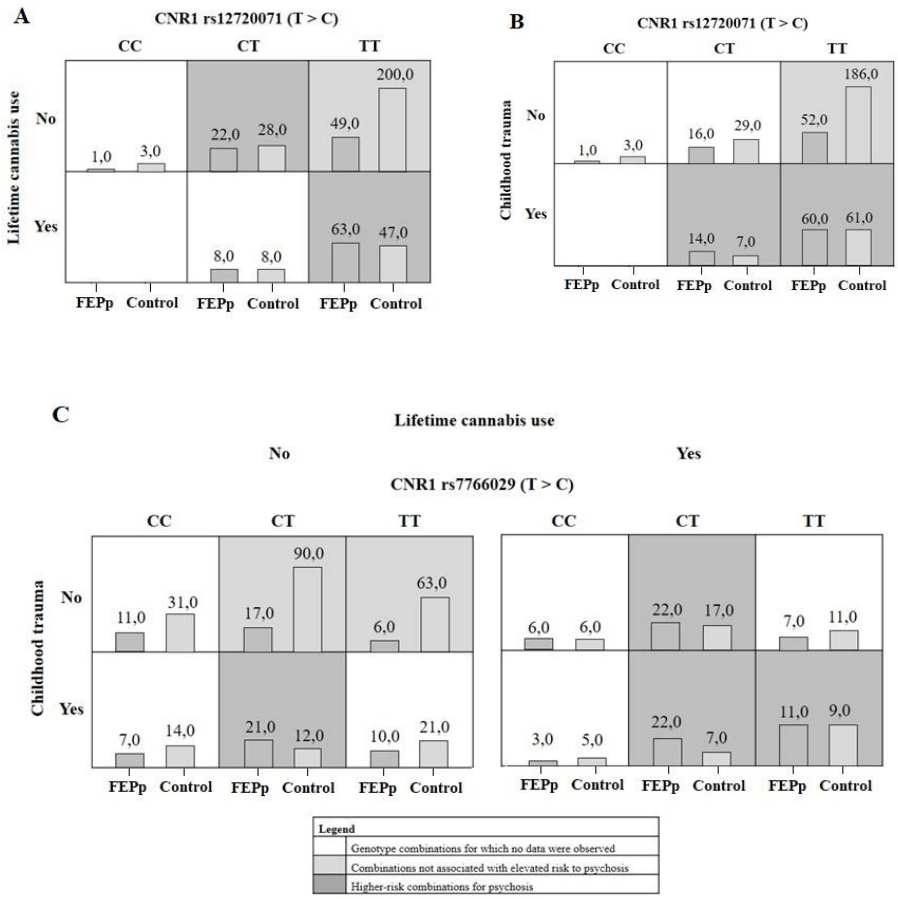


**Figure 2** Models assessing genetic-environment and genetic-genetic associations considering (A) Lifetime cannabis use: associations between individuals with lifetime cannabis use and *CNR1* rs12720071-T-allele carriers had a greater risk of psychosis (CVC=10/10, testing accuracy=66.4%, p<0.001). The bars show the frequency distribution of genotypes for the 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:** The carriers of *CNR1* rs7766029 (T-allele) related to

individuals with lifetime cannabis use and childhood trauma increased the risk for developing psychosis (CVC8/10, 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.

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 homozygotes and the presence of both environmental risk factors increase the risk for psychosis (OR=3.2; 95%CI=1.5-6.5; p=0.002). Although models 2(B-C) are significant and clinically relevant, neither reached the considerations described by Moore (2015) to be classified as a good model [53]. No significant associations between the environmental factors and other SNVs were found.

## 4. 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 increased risk for psychosis based on the analyses with the MDR approach. Similar to our study, MDR analysis 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 and genetic factors moderating the risk of psychosis [9]. We found 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 of MDR analysis, both models did not meet the specifications to consider an interesting model to represent the predictive ability of the gene-environment

interactions in the case-control study. Thus, our study may suggest that lifetime cannabis use is moderated by the *CNR1* genetic variant for psychosis. Our results demonstrate that *CNR1* rs12720071-T homozygotes interact 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 Δ9-THC [59]. Despite conflicting evidence, SNVs of *CNR1* gene polymorphic loci (rs12720071 and rs7766029), localised in chromosome 6q14-15, have been associated with cannabis use and psychosis risk [49,60]. For instance, cannabis use was reported as a moderator 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/EBPbeta) [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 may result in cognitive deficits between individuals with rs12720071-T-allele and 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 $\Delta^9$-THC, in precipitating psychosis-susceptibility and inducing NMDAR hypofunction [12], which may compromise the glutamate

signalling, and essential processes including synaptic plasticity, memory formation and learning [20].

Additionally, although evidence has shown alterations in dopaminergic together with NMDAR genes in psychoses [10], there are still inconsistencies about their associations. Considering our criteria in 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, as 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 that investigates these interactions associated to D2R, NMDAR and CB1R in psychosis using MDR statistical tool. We also speculate that the interaction between lifetime cannabis use or childhood trauma with 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**).

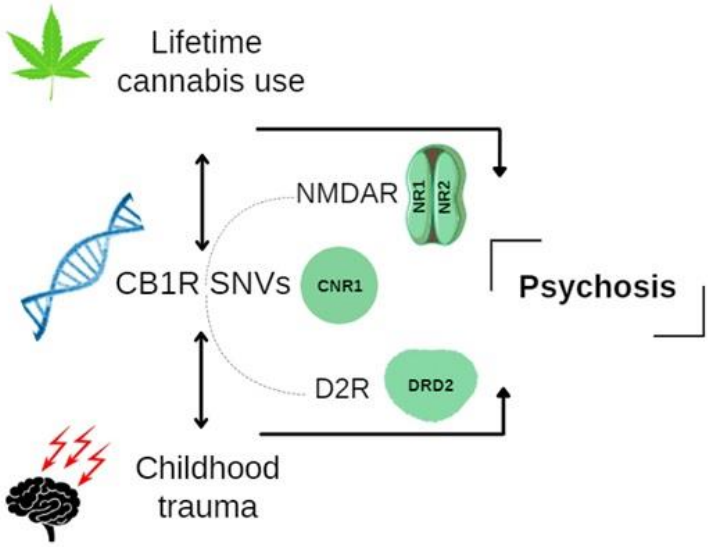


**Figure 3** Schematic representation of the main results. Lifetime cannabis use and childhood trauma associated with *CNR1* genetic variants may modulate the NMDAR and DR2 genes increasing the risk of psychosis.

The results of this study have some limitations. Despite our small sample size and explorative 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 aetiology and polygenicity of psychotic disorders. Second, 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. Third, 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. Fourth, the higher prevalence of lifetime cannabis use in FEP patients may be skewing the association of gene-environment interaction; however, we do not believe it happened 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 the linkage disequilibrium analysis and 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 highlight that the power of our results should be interpreted with caution due to the small sample size after group comparisons. The finding merits further investigation in a larger sample to elucidate the minor contributions of each SNV and its relationship with environmental factors.

## 5. Conclusion

Our study supports the hypothesis of gene-environment interactions for psychosis and uncovers a genetic liability dependent on gene-environment interaction, 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.

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**Disclosures**

The authors report no biomedical financial interests or potential conflicts of interest.

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## Supplementary Material

### Results

**Supplementary Table 1.** Associations between the environmental risk factors in community-based controls and psychosis

| | | Lifetime cannabis_lifetime, n (%) | | $\chi^2$; p-value[1] |
|---|---|---|---|---|
| | | No | Yes | |
| | | Community-based controls (n=286) | | |
| **Childhood trauma, n (%)** | No | 184 (64.3)[a] | 34 (12.0)[b] | **7.80; 0.008** |
| | Yes | 47 (16.4)[a] | 21 (7.3)[b] | |
| | Total | 231 (80.7) | 55 (19.3) | |
| | | FEPp (n=143) | | |
| | No | 34 (23.8) | 35 (24.5) | 0.06; 0.868 |
| | Yes | 38 (26.5) | 36 (25.2) | |
| | Total | 72 (50.3) | 71 (49.7) | |

[1] *Fisher's Exact* Test.
[a,b] Superscript letters were used for the mean values in the columns, in which the means followed by different letters differ statistically from each other.
FEPp: First-episode Psychosis Patients
Significant results are highlighted in bold.

**Supplementary Table 2.** Genotype frequencies between FEPp and controls and the risk effects of the NMDAR, D2R and CB1R SNVs in psychosis using a dominant model

| SNVs: Dominant Model | Variant | Controls (n=286) | FEPp (n=143) | Association with psychosis | |
|---|---|---|---|---|---|
| | | | | Unadjusted[a] | Adjusted[b] |
| | | n (%)[1] | | β (95% CI); p | |
| ***GRIN1**** | | | | | |
| rs4880213 (C>T) | CC | 83 (29.0) | 35 (24.5) | 1.00 | 1.00 |
| | CT or TT | 203 (71.0) | 108 (75.5) | 1.3 (0.8-2.0); 0.321 | 1.2 (0.7-2.2); 0.455 |
| rs11146020 (G>C) | GG | 239 (83.6) | 117 (81.8) | 1.00 | 1.00 |
| | GC or CC | 47 (16.4) | 26 (18.2) | 1.1 (0.7-1.9); 0.650 | 1.1 (0.6-2.2); 0.755 |
| ***GRIN2A**** | | | | | |
| rs1420040 (A>G) | AA | 109 (38.1) | 49 (34.3) | 1.00 | 1.00 |
| | AG or GG | 177 (61.9) | 94 (65.7) | 1.2 (0.8-1.8); 0.436 | 1.4 (0.8-2.3); 0.220 |
| rs11866328 (G>T) | GG | 103 (36.0) | 55 (38.5) | 1.00 | 1.00 |
| | TG or TT | 183 (64.0) | 88 (61.5) | 0.9 (0.6-1.4); 0.620 | 0.9 (0.6-1.6); 0.772 |
| ***GRIN2B**** | | | | | |
| rs890 (A>C) | AA | 82 (28.7) | 55 (38.5) | 1.00 | 1.00 |
| | AC or CC | 204 (71.3) | 88 (61.5) | 0.6 (0.4-1.0); 0.041 | 0.6 (0.4-1.0); 0.073 |
| rs7298664 (T>C) | TT | 212 (74.1) | 100 (69.9) | 1.00 | 1.00 |
| | TC or CC | 74 (25.9) | 43 (30.1) | 1.2 (0.8-1.9); 0.358 | 1.2 (0.7-2.0); 0.564 |
| rs2098469 (T>G) | TT | 235 (82.2) | 111 (77.6) | 1.00 | 1.00 |
| | TG or GG | 51 (17.8) | 32 (22.4) | 1.3 (0.8-2.2); 0.262 | 1.4 (0.8-2.7); 0.265 |
| ***DRD2**** | | | | | |
| rs1799978 (T>C) | TT | 244 (85.3) | 115 (80.4) | 1.00 | 1.00 |
| | TG or CC | 42 (14.7) | 28 (19.6) | 1.4 (0.8-2.4); 0.197 | 1.9 (1.0-3.6); 0.061 |
| rs7131056 (C>A) | CC | 107 (37.4) | 55 (38.5) | 1.00 | 1.00 |
| | AC or AA | 179 (62.6) | 88 (61.5) | 1.0 (0.6-1.4); 0.833 | 0.8 (0.5-1.3); 0.355 |
| rs6275 (G>A) | GG | 117 (40.9) | 48 (33.6) | 1.00 | 1.00 |

| | | | | | |
|---|---|---|---|---|---|
| | AG or AA | 169 (59.1) | 95 (66.4) | 1.4 (0.9-2.1); 0.141 | 1.5 (0.9-2.4); 0.147 |
| ***CNR1**** | | | | | |
| rs806380 (A>G) | AA | 154 (53.8) | 85 (59.4) | 1.00 | 1.00 |
| | AG or GG | 132 (46.2) | 58 (40.6) | 0.8 (0.5-1.2); 0.272 | 0.7 (0.4-1.2); 0.169 |
| rs806379 (A>T) | AA | 92 (32.2) | 44 (30.8) | 1.00 | 1.00 |
| | AT or TT | 194 (67.8) | 99 (69.2) | 1.1 (0.7-1.6); 0.769 | 1.2 (0.7-2.0); 0.592 |
| rs1049353 (C>T) | CC | 186 (65.0) | 95 (66.4) | 1.00 | 1.00 |
| | CT or TT | 100 (35.0) | 48 (33.6) | 0.9 (0.6-1.4); 0.774 | 1.1 (0.6-1.8); 0.815 |
| rs6454674 (T>G) | TT | 144 (50.3) | 72 (50.3) | 1.00 | 1.00 |
| | GT or GG | 142 (49.7) | 71 (49.7) | 1.0 (0.7-1.5); 1.000 | 0.8 (0.5-1.2); 0.268 |
| rs1535255 (T>G) | TT | 194 (67.8) | 85 (59.4) | 1.00 | 1.00 |
| | GT or GG | 92 (32.2) | 58 (40.6) | 1.4 (0.9-2.2); 0.086 | 2.0 (1.2-3.4); 0.008 |
| rs2023239 (T>C) | TT | 194 (67.8) | 86 (60.1) | 1.00 | 1.00 |
| | CT or CC | 92 (32.2) | 57 (39.9) | 1.4 (0.9-2.1); 0.155 | 2.0 (1.2-3.4); 0.009 |
| rs12720071 (T>C) | TT | 242 (84.6) | 117 (81.8) | 1.00 | 1.00 |
| | TC or CC | 44 (15.4) | 26 (18.2) | 0.8 (0.5-1.4); 0.460 | 1.5 (0.8-3.0); 0.248 |
| rs6928499 (G>C) | GG | 194 (67.8) | 86 (60.1) | 1.00 | 1.00 |
| | GC or CC | 92 (32.2) | 57 (39.9) | 1.4 (0.9-2.1); 0.115 | 2.0 (1.2-3.4); 0.009 |
| rs806374 (T>C) | TT | 113 (39.5) | 45 (31.5) | 1.00 | 1.00 |
| | CT or CC | 173 (60.5) | 98 (68.5) | 1.4 (0.9-2.2); 0.104 | 1.7 (1.0-3.0); 0.042 |
| rs7766029 (T>C) | TT | 89 (31.1) | 49 (34.3) | 1.00 | 1.00 |
| | TC or CC | 197 (68.9) | 94 (65.7) | 0.9 (0.6-1.3); 0.511 | 0.8 (0.5-1.4); 0.533 |
| rs806378 (C>T) | CC | 173 (60.5) | 94 (65.7) | 1.00 | 1.00 |
| | CT or TT | 113 (39.5) | 49 (34.3) | 0.8 (0.5-1.2); 0.291 | 0.7 (0.4-1.2); 0.180 |

| | | | | | |
|---|---|---|---|---|---|
| rs10485170 (T>C) | TT | 221 (77.3) | 102 (71.3) | 1.00 | 1.00 |
| | CT or CC | 65 (22.7) | 41 (28.7) | 1.4 (0.9-2.2); 0.179 | 2.1 (1.2-3.7); 0.012 |
| rs9450898 (T>C) | TT | 195 (68.2) | 88 (61.5) | 1.00 | 1.00 |
| | CT or CC | 91 (31.8) | 55 (38.5) | 1.3 (0.9-2.0); 0.172 | 1.8 (1.1-3.1); 0.024 |

SNVs: Single Nucleotide Variants; FEPp: First-episode Psychosis Patients; 1.00: Reference Category; β: Beta Coefficient; 95% CI: 95% Confidence Interval.

*Genotype frequencies analysed under dominant model (ancestral homozygous versus heterozygote + minor allele homozygous).

[1] Genotype frequency difference assessment by chi-square test.

[a] Unadjusted: binary logistic regression model including a binary outcome (community-based controls and FEPp). Alpha criterion ≤ 0.20 to univariate model as significant.

[b] Adjusted: binary logistic regression model including a binary outcome (community-based controls and FEPp). β adjusted by covariates sex, age, first 10 PCs, years of education and tobacco smoking. For multiple test errors, the Bonferroni's test was used and considered an adjusted p-value < 0.002 (0.05/23 SNVs) statistically significant.

**Supplementary Table 3.** Genotype frequencies between FEPp and controls and the risk effects of the NMDAR, D2R and CB1R SNVs in psychosis using an additive model

| SNVs: Additive Model | Variant | Controls (n=286) | FEPp (n=143) | Association with psychosis | |
|---|---|---|---|---|---|
| | | | | Unadjusted[a] | Adjusted[b] |
| | | n (%)[1] | | β (95% CI); p | |
| ***GRIN1**** | | | | | |
| rs4880213 (C>T) | CC | 83 (29.0) | 35 (24.5) | 1.00 | 1.00 |
| | CT | 147 (51.4) | 79 (55.2) | 1.3 (0.8-2.1); 0.322 | 1.2 (0.7-2.2); 0.463 |
| | TT | 56 (19.6) | 29 (20.3) | 1.2 (0.7-2.2); 0.500 | 1.2 (0.6-2.5); 0.600 |
| rs11146020 (G>C) | GG | 239 (83.6) | 117 (81.8) | 1.00 | 1.00 |
| | GC | 45 (15.7) | 25 (17.5) | 1.1 (0.7-1.9); 0.644 | 1.1 (0.6-2.2); 0.749 |
| | CC | 2 (0.7) | 1 (0.7) | 1.0 (1.0-11.4); 0.986 | 1.0 (0.1-14.5); 0.992 |
| ***GRIN2A**** | | | | | |
| rs1420040 (A>G) | AA | 109 (38.1) | 49 (34.3) | 1.00 | 1.00 |
| | AG | 125 (43.7) | 70 (49.0) | 1.2 (0.8-2.0); 0.335 | 1.5 (0.8-2.5); 0.169 |
| | GG | 52 (18.2) | 24 (16.8) | 1.0 (0.6-1.9); 0.930 | 1.2 (0.6-2.4); 0.678 |
| rs11866328 (G>T) | GG | 103 (36.0) | 55 (38.5) | 1.00 | 1.00 |
| | TG | 147 (51.4) | 66 (46.2) | 0.8 (0.5-1.3); 0.437 | 0.8 (0.5-1.4); 0.419 |
| | TT | 36 (8.4) | 22 (15.4) | 1.1 (0.6-2.1); 0.671 | 1.7 (0.8-3.7); 0.205 |
| ***GRIN2B**** | | | | | |
| rs890 (A>C) | AA | 82 (28.7) | 55 (38.5) | 1.00 | 1.00 |
| | AC | 146 (51.0) | 62 (43.4) | 0.6 (0.4-1.0); 0.048 | 0.6 (0.3-1.0); 0.049 |
| | CC | 58 (20.3) | 26 (18.2) | 0.7 (0.4-1.2); 0.170 | 0.8 (0.4-1.5); 0.446 |
| rs7298664 (T>C) | TT | 212 (74.1) | 100 (69.9) | 1.00 | 1.00 |
| | TC | 68 (23.8) | 37 (8.6) | 1.2 (0.7-1.8); 0.548 | 1.1 (0.6-1.9); 0.851 |
| | CC | 6 (2.1) | 6 (4.2) | 2.1 (0.7-6.7); 0.203 | 4.8 (0.8-30.9); 0.095 |
| rs2098469 (T>G) | TT | 235 (82.2) | 111 (77.6) | 1.00 | 1.00 |
| | TG | 48 (16.8) | 30 (21.0) | 1.3 (0.8-2.2); 0.281 | 1.5 (0.8-2.9); 0.197 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | GG | 3 (1.0) | 2 (1.4) | 1.4 (0.2-8.6); 0.708 | 0.5 (0.1-5.4); 0.530 |
| ***DRD2**** | | | | | | |
| rs1799978 (T>C) | | TT | 244 (85.3) | 115 (80.4) | 1.00 | 1.00 |
| | | TG | 41 (14.3) | 26 (18.2) | 1.3 (0.8-2.3); 0.281 | 1.8 (0.9-3.5); 0.083 |
| | | CC | 1 (0.3) | 2 (1.4) | 4.2 (0.4-47.3); 0.240 | 8.5 (0.1-0.1); 0.999 |
| rs7131056 (C>A) | | CC | 107 (37.4) | 55 (38.5) | 1.00 | 1.00 |
| | | AC | 138 (48.3) | 60 (42.0) | 0.8 (0.5-1.3); 0.460 | 0.7 (0.4-1.3); 0.264 |
| | | AA | 41 (14.3) | 28 (19.6) | 1.3 (0.7-2.4); 0.337 | 1.0 (0.5-2.0); 0.937 |
| rs6275 (G>A) | | GG | 117 (40.9) | 48 (33.6) | 1.00 | 1.00 |
| | | AG | 132 (46.2) | 66 (46.2) | 1.2 (0.8-1.9); 0.386 | 1.3 (0.7-2.2); 0.411 |
| | | AA | 37 (12.9) | 29 (20.3) | 1.9 (1.1-4.5); 0.032 | 2.2 (1.1-4.4); 0.036 |
| ***CNR1**** | | | | | | |
| rs806380 (A>G) | AA | 154 (53.8) | 85 (59.4) | 1.00 | 1.00 | |
| | AG | 117 (40.9) | 50 (35.0) | 0.8 (0.5-1.2); 0.237 | 0.7 (0.4-1.1); 0.121 | |
| | GG | 15 (5.2) | 8 (5.6) | 1.0 (0.4-2.4); 0.940 | 1.2 (0.4-4.0); 0.781 | |
| rs806379 (A>T) | AA | 92 (32.2) | 44 (30.8) | 1.00 | 1.00 | |
| | AT | 148 (51.7) | 69 (48.3) | 1.0 (0.6-1.5); 0.913 | 0.9 (0.5-1.6); 0.786 | |
| | TT | 46 (16.1) | 30 (21.0) | 1.4 (0.8-2.4); 0.298 | 2.3 (1.1-4.8); 0.029 | |
| rs1049353 (C>T) | CC | 186 (65.0) | 95 (66.4) | 1.00 | 1.00 | |
| | CT | 90 (31.5) | 45 (31.5) | 1.0 (0.6-1.5); 0.924 | 1.1 (0.6-1.8); 0.822 | |
| | TT | 10 (3.5) | 3 (2.1) | 0.6 (0.2-2.2); 0.427 | 1.1 (0.2-5.2); 0.927 | |
| rs6454674 (T>G) | TT | 144 (50.3) | 72 (50.3) | 1.00 | 1.00 | |
| | GT | 110 (38.5) | 57 (39.9) | 1.0 (0.7-1.6); 0.870 | 0.7 (0.4-1.3); 0.275 | |
| | GG | 32 (11.2) | 14 (9.8) | 0.9 (0.4-1.7); 0.704 | 0.8 (0.3-1.8); 0.585 | |
| rs1535255 (T>G) | TT | 194 (67.8) | 85 (59.4) | 1.00 | 1.00 | |
| | GT | 83 (29.0) | 49 (34.3) | 1.3 (0.9-2.1); 0.180 | 1.9 (1.1-3.2); 0.025 | |
| | GG | 9 (3.1) | 9 (6.3) | 2.3 (0.9-6.0); 0.092 | 4.8 (1.1-20.4); 0.034 | |

| | | | | | |
|---|---|---|---|---|---|
| rs2023239 (T>C) | TT | 194 (67.8) | 86 (60.1) | 1.00 | 1.00 |
| | CT | 83 (29.0) | 49 (34.3) | 1.3 (0.9-2.1); 0.197 | 1.8 (1.1-3.2); 0.027 |
| | CC | 9 (3.1) | 8 (5.6) | 2.0 (0.7-5.4); 0.167 | 4.6 (1.1-19.6); 0.042 |
| rs12720071 (T>C) | TT | 242 (84.6) | 117 (81.8) | 1.00 | 1.00 |
| | TC | 41 (14.3) | 25 (17.5) | 1.3 (0.7-2.2); 0.403 | 1.6 (0.8-3.3); 0.176 |
| | CC | 3 (1.0) | 1 (0.7) | 0.7 (0.1-6.7); 0.749 | 0.5 (0.1-6.2); 0.563 |
| rs6928499 (G>C) | GG | 194 (67.8) | 86 (60.1) | 1.00 | 1.00 |
| | GC | 84 (29.4) | 49 (34.3) | 1.3 (0.9-2.0); 0.215 | 1.8 (1.0-3.1); 0.037 |
| | CC | 8 (2.8) | 8 (5.6) | 2.3 (0.8-6.2); 0.115 | 7.9 (1.5-41.9); 0.016 |
| rs806374 (T>C) | TT | 113 (39.5) | 45 (31.5) | 1.00 | 1.00 |
| | CT | 126 (44.1) | 72 (50.3) | 1.4 (0.9-2.3); 0.116 | 1.7 (1.0-3.0); 0.049 |
| | CC | 47 (16.4) | 26 (18.2) | 1.4 (0.8-2.5); 0.275 | 1.7 (0.8-3.6); 0.561 |
| rs7766029 (T>C) | TT | 89 (31.1) | 49 (34.3) | 1.00 | 1.00 |
| | TC | 145 (50.7) | 63 (44.1) | 0.8 (0.5-1.2); 0.310 | 0.7 (0.4-1.2); 0.219 |
| | CC | 52 (18.2) | 31 (21.7) | 1.1 (0.6-1.9); 0.783 | 1.3 (0.7-2.7); 0.427 |
| rs806378 (C>T) | CC | 173 (60.5) | 94 (65.7) | 1.00 | 1.00 |
| | CT | 104 (36.4) | 42 (29.4) | 0.7 (0.5-1.2); 0.184 | 0.7 (0.4-1.1); 0.104 |
| | TT | 9 (3.1) | 7 (4.9) | 1.4 (0.5-4.0); 0.490 | 1.8 (0.5-7.0); 0.399 |
| rs10485170 (T>C) | TT | 221 (77.3) | 102 (71.3) | 1.00 | 1.00 |
| | CT | 60 (21.0) | 37 (25.9) | 1.3 (0.8-2.1); 0.229 | 1.9 (1.1-3.5); 0.027 |
| | CC | 5 (1.7) | 4 (2.8) | 1.7 (0.5-6.6); 0.420 | 5.7 (0.7-47.2); 0.107 |
| rs9450898 (T>C) | TT | 195 (68.2) | 88 (61.5) | 1.00 | 1.00 |
| | CT | 82 (28.7) | 49 (34.3) | 1.3 (0.9-2.0); 0.205 | 1.7 (1.0-2.9); 0.060 |
| | CC | 9 (3.1) | 6 (4.2) | 1.5 (0.5-4.3); 0.472 | 5.5 (1.0-28.9); 0.044 |

SNVs: Single Nucleotide Variants; FEPp: First-episode Psychosis Patients; 1.00: Reference Category; β: Beta Coefficient; 95% CI: 95% Confidence Interval.

*Genotype frequencies analysed under additive model (ancestral homozygous versus heterozygote versus minor allele homozygous).

[1] Genotype frequency difference assessment by chi-square test.

[a] Unadjusted: binary logistic regression model including a binary outcome (community-based controls and FEPp). Alpha criterion ≤ 0.20 to univariate model as significant.

[b] Adjusted: binary logistic regression model including a binary outcome (community-based controls and FEPp). β adjusted by covariates sex, age, first 10 PCs, years of education and tobacco smoking. For multiple test errors, the Bonferroni's test was used and considered an adjusted p-value < 0.002 (0.05/23 SNVs) statistically significant.

**Supplementary Table 4.** Genotype frequencies between FEPp and controls and the risk effects of the NMDAR, D2R and CB1R SNVs in psychosis using a recessive model

| SNVs: Recessive Model | Variant | Controls (n=286) | FEPp (n=143) | Association with psychosis | |
|---|---|---|---|---|---|
| | | | | Unadjusted[a] | Adjusted[b] |
| | | n (%)[1] | | β (95% CI); p | |
| ***GRIN1**** | | | | | |
| rs4880213 (C>T) | CC or CT | 230 (80.4) | 114 (79.7) | 1.00 | 1.00 |
| | TT | 56 (19.6) | 29 (20.3) | 1.0 (0.6-1.7); 0.864 | 1.0 (0.6-1.9); 0.878 |
| rs11146020 (G>C) | GG or GC | 284 (99.3) | 142 (99.3) | 1.00 | 1.00 |
| | CC | 2 (0.7) | 1 (0.7) | 1.0 (0.1-11.1); 1.000 | 1.0 (0.1-14.2); 0.996 |
| ***GRIN2A**** | | | | | |
| rs1420040 (A>G) | AA or AG | 233 (81.8) | 119 (83.2) | 1.00 | 1.00 |
| | GG | 52 (18.2) | 24 (16.8) | 0.9 (0.5-1.5); 0.709 | 1.2 (0.6-2.3); 0604 |
| rs11866328 (G>T) | GG or TG | 250 (87.4) | 121 (84.6) | 1.00 | 1.00 |
| | TT | 36 (12.6) | 22 (15.4) | 1.3 (0.7-2.2); 0.425 | 1.9 (0.9-4.0); 0.082 |
| ***GRIN2B**** | | | | | |
| rs890 (A>C) | AA or AC | 228 (79.7) | 117 (81.8) | 1.00 | 1.00 |
| | CC | 58 (20.3) | 26 (18.2) | 0.9 (0.5-1.5); 0.606 | 1.1 (0.6-2.0); 0.840 |
| rs7298664 (T>C) | TT or TC | 280 (97.9) | 137 (95.8) | 1.00 | 1.00 |
| | CC | 6 (2.1) | 6 (4.2) | 2.0 (0.6-6.5); 0.223 | 4.8 (0.8-30.2); 0.097 |
| rs2098469 (T>G) | TT or TG | 282 (99.0) | 141 (98.6) | 1.00 | 1.00 |
| | GG | 3 (1.0) | 2 (1.4) | 1.3 (0.2-8.1); 0.751 | 0.4 (0.1-4.8); 0.469 |
| ***DRD2**** | | | | | |
| rs1799978 (T>C) | TT or TG | 285 (99.7) | 141 (98.6) | 1.00 | 1.00 |
| | CC | 1 (0.3) | 2 (1.4) | 4.0 (0.4-45.0); 0.256 | 0.7 (0.1-0.1); 0.999 |
| rs7131056 (C>A) | CC or AC | 245 (85.7) | 115 (80.4) | 1.00 | 1.00 |

| | | | | | |
|---|---|---|---|---|---|
| | AA | 41 (14.3) | 28 (19.6) | 1.5 (0.9-2.5); 0.165 | 1.1 (0.6-2.3); 0.701 |
| rs6275 (G>A) | GG or AG | 249 (87.1) | 114 (79.7) | 1.00 | 1.00 |
| | AA | 37 (12.9) | 29 (20.3) | 1.7 (1.0-2.9); 0.049 | 1.9 (1.0-3.7); 0.053 |
| ***CNR1**** | | | | | |
| rs806380 (A>G) | AA or AG | 271 (94.8) | 135 (94.4) | 1.00 | 1.00 |
| | GG | 15 (5.2) | 8 (5.6) | 1.1 (0.4-2.6); 0.880 | 1.4 (0.4-4.6); 0.575 |
| rs806379 (A>T) | AA or AT | 240 (83.9) | 113 (79.0) | 1.00 | 1.00 |
| | TT | 46 (16.1) | 30 (21.0) | 1.4 (0.8-2.3); 0.212 | 2.4 (1.2-4.7); 0.009 |
| rs1049353 (C>T) | CC or CT | 276 (96.5) | 140 (98.6) | 1.00 | 1.00 |
| | TT | 10 (3.5) | 2 (1.4) | 0.4 (0.1-1.8); 0.394 | 0.7 (0.1-4.5); 0.732 |
| rs6454674 (T>G) | TT or GT | 254 (88.8) | 129 (90.2) | 1.00 | 1.00 |
| | GG | 32 (11.2) | 14 (9.8) | 1.0 (0.4-1.7); 0.659 | 0.9 (0.4-2.0); 0.820 |
| rs1535255 (T>G) | TT or GT | 277 (96.9) | 134 (93.7) | 1.00 | 1.00 |
| | GG | 9 (3.1) | 9 (6.3) | 2.1 (0.8-5.3); 0.133 | 3.9 (0.9-16.2); 0.064 |
| rs2023239 (T>C) | TT or CT | 277 (96.9) | 135 (94.4) | 1.00 | 1.00 |
| | CC | 9 (3.1) | 8 (5.6) | 1.8 (0.7-4.8); 0.227 | 3.7 (0.9-15.7); 0.077 |
| rs12720071 (T>C) | TT or TC | 283 (99.0) | 142 (99.3) | 1.00 | 1.00 |
| | CC | 3 (1.0) | 1 (0.7) | 0.7 (0.1-6.4); 0.724 | 0.4 (0.1-5.9); 0.539 |
| rs6928499 (G>C) | GG or GC | 278 (97.2) | 135 (94.4) | 1.00 | 1.00 |
| | CC | 8 (2.8) | 8 (5.6) | 2.1 (0.8-5.6); 0.157 | 6.4 (1.2-33.9); 0.028 |
| rs806374 (T>C) | TT or CT | 239 (83.6) | 117 (81.8) | 1.00 | 1.00 |
| | CC | 47 (16.4) | 26 (18.2) | 1.1 (0.7-2.0); 0.650 | 0.8 (0.4-1.6); 0.583 |
| rs7766029 (T>C) | TT or TC | 234 (81.8) | 112 (78.3) | 1.00 | 1.00 |

| | | | | | |
|---|---|---|---|---|---|
| | CC | 52 (18.2) | 31 (21.7) | 1.2 (0.8-2.1); 0.388 | 1.6 (0.9-3.0); 0.110 |
| rs806378 (C>T) | CC or CT | 277 (96.9) | 136 (95.1) | 1.00 | 1.00 |
| | TT | 9 (3.1) | 7 (4.9) | 1.6 (0.6-4.3); 0.371 | 2.1 (0.6-8.1); 0.278 |
| rs10485170 (T>C) | TT or CT | 281 (98.3) | 139 (97.2) | 1.00 | 1.00 |
| | CC | 5 (1.7) | 4 (2.8) | 1.6 (0.4-6.1); 0.479 | 4.9 (0.6-40.0); 0.141 |
| rs9450898 (T>C) | TT or CT | 277 (96.9) | 137 (95.8) | 1.00 | 1.00 |
| | CC | 9 (3.1) | 6 (4.2) | 1.3 (0.9-2.0); 0.172 | 4.6 (0.9-24.0); 0.069 |

SNVs: Single Nucleotide Variants; FEPp: First-episode Psychosis Patients; 1.00: Reference Category; β: Beta Coefficient; 95% CI: 95% Confidence Interval.

*Genotype frequencies analysed under recessive model (ancestral homozygous + heterozygote versus minor allele homozygous).

[1] Genotype frequency difference assessment by chi-square test.

[a] Unadjusted: binary logistic regression model including a binary outcome (community-based controls and FEPp). Alpha criterion ≤ 0.20 to univariate model as significant.

[b] Adjusted: binary logistic regression model including a binary outcome (community-based controls and FEPp). β adjusted by covariates sex, age, first 10 PCs, years of education and tobacco smoking. For multiple test errors, the Bonferroni's test was used and considered an adjusted p-value < 0.002 (0.05/23 SNVs) statistically significant.

**Supplementary Table 5.** Multifactor dimensionality reduction analysis of genetic-environment interactions

| Models of gene-environment interactions in psychosis | Adj. Bal. Acc CV Training | Adj. Bal. Acc CV Testing | CV Consistency | p-value For testing bal. Acc |
|---|---|---|---|---|
| **Lifetime cannabis use (Figure 2A)** | | | | |
| Lifetime cannabis use | 0.6521 | 0.6521 | 10/10 | 0.0010-0.0020 |
| ***CNR1* rs12720071, Lifetime cannabis use** | **0.6757** | **0.6641** | **10/10** | **0.0000-0.0010** |
| *CNR1* rs12720071, *GRIN1* rs4880213, Lifetime cannabis use | 0.692 | 0.64 | 5/10 | 0.0010-0.0020 |
| *CNR1* rs806379, *CNR1* rs7766029, *GRIN2B* rs890, Lifetime cannabis use | 0.7179 | 0.5818 | 5/10 | 0.0770-0.0780 |
| *DRD2* rs7131056*, DRD2* rs6275*, CNR1* rs806374, *CNR1* rs7766029, Lifetime cannabis use | 0.7306 | 0.5599 | 2/10 | 0.2250-0.2260 |
| **Childhood trauma (Figure 2B)** | | | | |
| Childhood trauma | 0.6399 | 0.6399 | 10/10 | 0.0010-0.0020 |
| ***CNR1* rs12720071, Childhood trauma** | **0.6549** | **0.6206** | **6/10** | **0.0040-0.0050** |
| *CNR1* rs7766029, *GRIN2A* rs11866328, Childhood trauma | 0.6956 | 0.6171 | 6/10 | 0.0060-0.0070 |
| *CNR1* rs7766029, *CNR1* rs806378, *GRIN2A* rs1420040 Childhood trauma | 0.7157 | 0.5819 | 2/10 | 0.0780-0.0790 |
| *CNR1* rs806374, *CNR1* rs7766029, *GRIN2A* rs11866328, *GRIN2A* rs1420040, Childhood trauma | 0.7275 | 0.5494 | 2/10 | 0.3110 |
| **Lifetime cannabis use and childhood trauma (Figure 2C)** | | | | |
| Lifetime cannabis use | 0.6544 | 0.6066 | 8/10 | 0.0170 |
| Childhood trauma, Lifetime cannabis use | 0.7028 | 0.7028 | 10/10 | 0.000-0.0010 |

| | | | | |
|---|---|---|---|---|
| ***CNR1* rs7766029, Childhood trauma, Lifetime cannabis use** | **0.7249** | **0.6929** | **8/10** | **0.0000-0.0010** |
| *DRD2* rs7131056*, CNR1* rs7766029, Childhood trauma, Lifetime cannabis use | 0.7432 | 0.6826 | 7/10 | 0.0000-0.0010 |
| *DRD2* rs7131056, *CNR1* rs6454674, *GRIN2B* rs890, Childhood trauma, Lifetime cannabis use | 0.7502 | 0.589 | 2/10 | 0.0490-0.0500 |

MDR analysis for each factor summarising the average CVC, adjusted testing balance accuracy and the empirical p-value derived from permutation testing (p-values were based on 1000 permutations) at a 0.05 significance level.
The best model of gene-environment interaction per analysis that shows a) the minimal prediction error; b) the CVC upper of 9 or 10-fold cross validation; c) the significance of p-value (less than 0.05); and d) close values of the training and testing balance accuracy (TBA=0.55-0.69) (in bold) as described by Moore (2006).
Adj. Bal. Acc CV Training: Adjusted Balance-Accuracy Cross-Validation Training; Adj. Bal. Acc CV Testing: Adjusted Balance-Accuracy Cross-Validation Testing; CV Consistency: Cross-Validation Consistency; P-value For Testing bal. Acc.: P-value For Testing Balance-Accuracy. Results were demonstrated in mean p-value.