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REVIEW ARTICLE

The role of the *CNR1* gene in schizophrenia: a systematic review including unpublished data

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Objective: Schizophrenia is a multifactorial disorder. It is known that a combination of extensive multiple common alleles may be involved in its etiology, each contributing with a small to moderate effect, and, possibly, some rare alleles with a much larger effect size. We aimed to perform a systematic review of association studies between schizophrenia (and its subphenotypes) and polymorphisms in the *CNR1* gene, which encodes cannabinoid receptors classically implicated in schizophrenia pathophysiology, as well as to present unpublished results of an association study in a Brazilian population.

Methods: Two reviewers independently searched for eligible studies and extracted outcome data using a structured form. Papers were retrieved from PubMed and ISI Web of Knowledge using the search term schizophrenia in combination with *CNR1* or CB1 or cannabinoid receptor. Twenty-four articles met our inclusion criteria. We additionally present data from a study of our own comparing 182 patients with schizophrenia and 244 healthy controls.

Results: No consistent evidence is demonstrated.

Conclusion: Some seemingly positive association studies stress the need for further investigations of the possible role of endocannabinoid genetics in schizophrenia.

Keywords: Cannabinoid receptors; cannabinoid receptors type-1; gene; association studies

Introduction

Schizophrenia is well known for its heterogeneous clinical symptom dimensions, interindividual variability in pharmacological treatment response, and the lack of an established understanding of its pathophysiology. It is a multifactorial disorder, as a complex interaction of genetic and environmental factors contribute to its etiology, and features high heritability (64-83%).^{1,2} Currently, it is known that a combination of extensive multiple common alleles - each one contributing with a small to moderate effect - and, possibly, some rare alleles with much larger effect size³ are implicated in schizophrenia. However, despite the progress achieved in recent years, particularly in genetics and neuroimaging, we still have more questions than answers regarding its causes.

Cannabis exposure, particularly during early adolescence, has long been considered a risk factor for psychosis and, more recently, for schizophrenia.^{4,5} However, the underlying neurobiological mechanisms responsible for this association are not well understood. Recent research has

pointed toward a complex gene-environment interaction that involves several variables, such as time of exposure (usually in early life) and genetic predisposition to psychosis.⁶ This interaction would lead to disrupted brain maturation and abnormalities in molecular functioning.

Although dopaminergic and glutamatergic transmissions are the classical neurobiological pathways implicated in the pathophysiology of schizophrenia, the endocannabinoid system has emerged as a possible link between cannabis and psychosis.⁷ This interaction may consider the effects of exogenous and endogenous cannabinoid compounds alike on the brain. It is well established that the psychogenic properties of cannabis are mainly mediated by delta-9-tetrahydrocannabinol (THC) stimulation of type-1 cannabinoid receptors (CB1).⁸ CB1 receptor activation may also modulate other pathways, such as glutamate, dopamine, and gamma-aminobutyric acid (GABA).⁹ In addition, some studies have verified abnormalities in endocannabinoid ligands such as anandamide in patients with schizophrenia,^{10,11} while others observed a potential antipsychotic property of CB1 receptor antagonists.^{7,12} Neuroimaging findings have also associated cannabis use with progressive cortical thickness in areas rich in CB1 receptors during the initial years of schizophrenia.¹³ This body of evidence suggests possible influences of the endocannabinoid system in the pathophysiology of the disorder.

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Therefore, considering the involvement of the endocannabinoid system in psychosis and the high heritability of schizophrenia, the *CNR1* gene, which encodes CB1 receptors, has been proposed as one candidate gene that may contribute to the etiology of schizophrenia and related phenotypes. *CNR1* is located on chromosome 6q14-15¹⁴ and is expressed widely in the central nervous system.¹⁵ Polymorphisms in this gene have been associated with schizophrenia¹⁶ and comorbid substance abuse,¹⁷ as well as with antipsychotic response¹⁸ and adverse effects¹⁹; however, other studies have reported negative findings,^{20,21} and these associations remain inconclusive.

Within this context, the aim of the present study was to conduct a systematic review of all association studies of the *CNR1* gene with schizophrenia and its related subphenotypes, including clinical response and side effects of antipsychotic drugs. In addition, we report unpublished results of a comparison of *CNR1* gene single nucleotide polymorphisms (SNPs) in a Brazilian sample of patients with schizophrenia and healthy controls.

Methods

Association study

This study was approved by the Ethics Committee of Universidade Federal de São Paulo (UNIFESP; protocol no. 1737/06). Written informed consent was obtained from all recruited participants or their caregivers, and all clinical and laboratory investigations were conducted in strict accordance with the principles expressed in the Declaration of Helsinki. All invited subjects agreed to enroll in this study. A total of 182 patients and 244 healthy controls were recruited through Programa de Esquizofrenia (PROESQ) and Laboratório Interdisciplinar de Neurociências Clínicas (LiNC), both at UNIFESP, São Paulo, Brazil. The diagnosis of schizophrenia was established according to the DSM-IV criteria, using the Structured Clinical Interview of the DSM-IV (SCID). Treatment-resistant (TR) status was defined in accordance with the International Psychopharmacological Criteria (IPAP; www.ipap.org) as failure to respond to 4- to 6-week trials of monotherapy with two different antipsychotics at adequate doses. Age- and gender-matched healthy controls with no family history of psychotic disease were recruited at UNIFESP. These subjects were also investigated using a modified version of SCID screening to exclude any with current or previous psychiatric diagnoses. The descriptive characteristics of the sample are presented in Table 1. Whole blood was collected from each subject and DNA was extracted using a Gentra Puregene kit (Qiagen, Germantown, United States). All four *CNR1* SNPs (rs806380, rs806379, rs1049353, and rs806368) were genotyped by TaqMan probe-based real-time polymerase chain reaction (PCR) assays (Life Technologies, Foster City, United States) performed under standard conditions. For each reaction, at least one positive control for each genotype was included. Statistical analysis was based on logistic regression, including group (control and patient or TR-schizophrenia [TRS] and non-TRS) as

the dependent variable and genotypes, age, gender, and ethnicity as independent variables.

Systematic review

The PubMed (<http://www.ncbi.nlm.nih.gov/pubmed>) and ISI Web of Knowledge (<http://apps.webofknowledge.com>) databases were searched from the first date available to June 2016, using the term schizophrenia in combination with CNR1 or CB1 or CB1. Two reviewers independently searched for eligible studies and extracted outcome data using a structured form designed for this purpose.

On PubMed, the final query resulted the following: ("schizophrenia" [MeSH Terms] OR "schizophrenia" [All Fields]) AND (CNR1 [All Fields] OR CB1 [All Fields] OR ("receptors, cannabinoid" [MeSH Terms] OR ("receptors" [All Fields] AND "cannabinoid" [All Fields]) OR "cannabinoid receptors" [All Fields] OR ("cannabinoid" [All Fields] AND "receptor" [All Fields]) OR "cannabinoid receptor" [All Fields])). On ISI Web of Knowledge the final query was: (schizophrenia AND (CNR1 or CB1 or cannabinoid receptor)). The results were downloaded and imported into EndNote X5 software (Thomson Reuters) to remove duplicates and select the papers that would be included in the systematic review.

Genetic analyses of *CNR1* polymorphisms with schizophrenia or its subphenotypes as outcomes were eligible for inclusion. Studies that did not genotype *CNR1* polymorphisms and did not have schizophrenia or its subphenotypes as outcomes were excluded.

Results

Association study (unpublished data)

The sample is described in Table 1. Our investigation did not show any significant association between schizophrenia and genotypes (Table 1), nor of TRS with rs806380 ($p = 0.351$), rs806379 ($p = 0.050$), rs1049353 ($p = 0.105$), or rs806368 ($p = 0.502$). After considering cannabis use (yes/no) as a dependent variable in the logistic regression, we did not find any positive association with TRS: rs806380 ($p = 0.375$), rs806379 ($p = 0.086$), rs1049353 ($p = 0.091$), and rs806368 ($p = 0.392$). When considering frequency of cannabis use (< 10 times; 10-50 times; > 50 times) as a dependent variable, no association was found: rs806380 ($p = 0.965$), rs806379 ($p = 0.266$), rs1049353 ($p = 0.985$), and rs806368 ($p = 0.684$). We did not evaluate adverse effects of antipsychotic pharmacological treatment in our study population. As cannabis use was an exclusion criterion for the control group, we could not investigate the effect of genotypes and cannabis use on schizophrenia risk, although we could assess the influence of these factors on TRS.

Systematic review

The final flow diagram is presented in Figure 1, and the selected studies listed in Table 2. A total of 24 studies involving *CNR1* polymorphisms and schizophrenia were

Table 1 Descriptive characteristics of the sample

Variable	Patients	Controls	Statistics
Gender			
Male	127	147	
Female	55	97	$\chi^2 = 4.129$; $df = 1$; $p = 0.042$
Age (years), mean (SD)	35.71 (10.56)	37.33 (12.40)	$t = 1.43$; $df = 402.67$; $p = 0.153$
Ethnicity			
European	101	151	
African	42	61	
Asian	22	9	
Not reported	17	23	$\chi^2 = 10.984$; $df = 2$; $p = 0.004$
Treatment-resistant status			
TR	73		
Non-TR	104		
Lifetime cannabis use			
Yes	56		
No	116		
Lifetime frequency of cannabis use			
< 10 times	17		
10-50 times	9		
> 50 times	30		
rs806380			
AA	93	133	
AG	78	92	
GG	11	19	$p = 0.330$
rs806379			
AA	50	75	
AT	89	122	
TT	43	47	$p = 0.157$
rs1049353			
CC	119	163	
CT	55	74	
TT	8	7	$p = 0.638$
rs806368			
CC	11	20	
CT	64	78	
TT	107	146	$p = 0.701$

Data presented as n unless noted otherwise.

χ^2 = chi-square; df = degrees of freedom; SD = standard deviation; TR = treatment-resistant.

selected; we divided the results into three sections according to primary outcome: schizophrenia, substance abuse comorbidity, and pharmacological treatment.

CNR1 gene polymorphisms and occurrence of schizophrenia and its symptoms

Studies of genetic variants affecting the *CNR1* gene in different populations showed conflicting results regarding a possible association with schizophrenia. One of the first such studies investigated the *CNR1* (AAT)_n repeat polymorphism in Caucasians and did not find evidence for linkage or association to schizophrenia using 23 multiplex schizophrenia pedigrees, 131 individuals with the disease, and 103 controls.²²

A few years later, Tsai et al.²³ compared the genotype and allele frequencies of the (AAT)_n repeat polymorphism in 127 patients with schizophrenia and 146 healthy controls from the Han Chinese population. The results indicated no significant association of this polymorphism with pathogenesis of schizophrenia in that population. Similarly, in a French Caucasian population, no significant association was found with the (AAT)_n repeat in a comparison of 102 patients with schizophrenia and 89 controls.²⁵

Ujike et al.¹⁶ conducted an association study with Japanese patients with schizophrenia (n=121) and age-matched healthy controls (n=148). Although the rs1049353 polymorphism was not associated with schizophrenia in this study, the allelic distributions of the (AAT)_n repeat were significantly different between controls and individuals with schizophrenia ($p = 0.046$), especially for hebephrenia

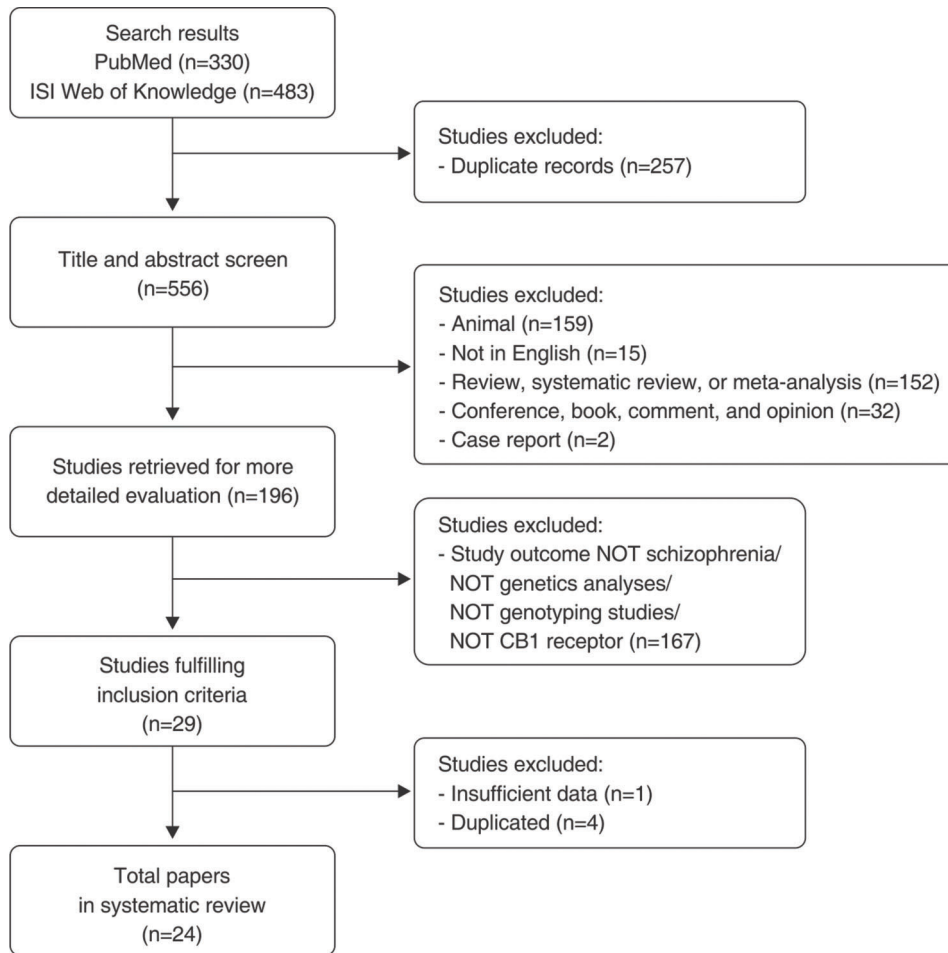


Figure 1 Flow diagram of the systematic review. CB1 = type 1 cannabinoid receptors.

Table 2 Studies included in the systematic review

Author	Year	Main outcome	Study sample	Population	SNPs	Association (for main outcome)
Dawson ²²	1995	SCZ	Multiplex SCZ pedigrees (23) SCZ patients (131) Controls (103)	Caucasian	(AAT)n	(-)
Tsai ²³	2000	SCZ	SCZ patients (127) Controls (146)	Han Chinese	(AAT)n	(-)
Leroy ²⁴	2001	SCZ; substance abuse; treatment response	SCZ patients (102) Controls (63)	French	rs1049353	(+) for substance abuse in patients
Krebs ²⁵	2002	SCZ; cannabis abuse	SCZ cannabis (102) Non-SCZ cannabis (85) Controls (89)	French	(AAT)n	(+) for cannabis-sensitive SCZ patients
Ujike ¹⁶	2002	SCZ; SCZ subcategories	SCZ patients (121) Controls (148)	Japanese	(AAT)n rs1049353	(+) (AAT)n for hebephrenic SCZ
Martinez-Gras ²⁶	2005	Substance abuse; PANSS scores; WCST (executive function)	SCZ patients (75 substance abusers/58 non-abusers) (133)	Spanish	(AAT)n	(+) for negative PANSS scale and WCST in substance abusers

Continued on next page

Table 2. (continued)

Author	Year	Main outcome	Study sample	Population	SNPs	Association (for main outcome)
Ballon ¹⁷	2006	SCZ; cocaine addiction	SCZ cocaine (45) Non-SCZ cocaine (97) Controls (88)	African-Caribbean	(AAT)n	(+) for cocaine addiction
Martinez-Gras ⁵	2006	SCZ; substance abuse	SCZ patients (113) Controls (111)	Spanish	(AAT)n	(+) for schizophrenia
Seifert ²⁷	2007	SCZ	SCZ patients (104) Controls (140)	German	(AAT)n rs1049353 rs6454674	(-)
Zammit ²⁸	2007	SCZ; cannabis use; other phenotype characteristics	SCZ patients (797) Controls (688)	UK	rs1049353	(-)
Chavarría-Siles ²⁹	2008	SCZ; SCZ subcategories	SCZ patients (244) SCZ relatives (481)	Costa Rica	(AAT)n	(+) for hebephrenic SCZ
Hamdani ¹⁸	2008	SCZ; cannabis use; treatment response	SCZ patients (133) Controls (141)	French	rs806468 rs1049353 rs806379 rs806380	(+) rs1049353 for treatment response
Monteleone ³⁰	2010	SCZ; weight gain	SCZ patients (83) Controls (80)	Italian	rs1049353	(-)
Tiwari ¹⁹	2010	Weight gain	SCZ patients (183)	German/U.S.	rs806368 rs1272071 rs1049353 rs806369 rs806370 rs806374 rs806375 rs806377 rs806378 rs2023239 rs806380 rs806381 rs7752758 rs12528858 rs12205430 rs6914429 rs2180619 rs754387 rs9450902 rs10485170	(+) rs806378 in patients of European ancestry treated with clozapine or olanzapine
Ho ³¹	2011	Cannabis abuse; MRI; neurocognitive assessment	SCZ patients (235)	U.S.	rs806365 rs7766029 rs806366 rs806368 rs12710071 rs1049353 rs806374 rs806375 rs806376 rs6454672 rs9450898 rs806380 rs806368 rs4707436 rs1049353	(+) rs7766029/ rs12720071/rs9450898 for MRI findings
Park ³²	2011	Weight gain (olanzapine)	SCZ patients (78)	Korean	rs806368 rs4707436 rs1049353	(-)
van Winkel ³³	2011	Psychosis; positive schizotypy; cannabis use	Patients with psychosis (801) Unaffected siblings (740)	Netherlands and Belgium	rs2023239 rs806379 rs1535255 rs6454674	(-)

Continued on next page

Table 2. (continued)

Author	Year	Main outcome	Study sample	Population	SNPs	Association (for main outcome)
					rs806308 rs806377 rs1049353 rs6928499	
Schennach ³⁴	2012	Treatment response	SCZ patients (274) Controls (427)	German	rs1049353	(-)
Tiwari ³⁵	2012	TD	SCZ patients (191)	U.S./ Canadian	rs806369 rs1272071 rs1049353 rs806369 rs806370 rs806374 rs806375 rs806377 rs806378 rs2023239 rs806380 rs806381 rs7752758 rs12528858 rs12205430 rs6914429 rs2180619 rs754387 rs9450902 rs10485170	(+) rs806374
Costa ³⁶	2013	SCZ	SCZ patients (170) Controls (350)	Italian	rs1049353 rs7766029 rs806366 rs12720071 rs806380 rs9450898 rs806375 rs806368 rs6454672 rs806374 rs806365 rs806376	(-)
Yu ³⁷	2013	MetS	SCZ patients (407)	Belgium	rs6454674 rs6928499 rs806379 rs1535355 rs806377 rs1049353 rs2023239	(+) rs6928499 rs1535355 rs2023239
Bae ²¹	2014	SCZ	SCZ patients (337) Controls (394)	Korean	rs806376 rs806368 rs806366 rs6689530 rs34570472	(-)
Copoglu ³⁸	2015	SCZ; symptoms	SCZ patients (66) Controls (65)	Turkish	rs6454674 rs806368 rs1049353	(+) rs6454674 for SCZ symptoms
Suárez-Pinilla ³⁹	2015	Cannabis use; MRI; BMI; clinical psychopathology	First-episode psychosis patients (65)	Spanish	rs1049353 rs1535255 rs2023239	(+) all SNPs for MRI and clinical psychopathology

BMI = body mass index; SCZ = schizophrenia; MetS = metabolic syndrome; MRI = magnetic resonance imaging; PANSS = Positive and Negative Syndrome Scale; SNP = single nucleotide polymorphism; TD = tardive dyskinesia; WCST = Wisconsin Card Sorting Test.

($p = 0.0028$). Hebephrenic-type schizophrenia - characterized by predominance of negative symptoms such as blunted affect, disorganized thought, and deterioration of personality - was associated with significantly increased frequency of the 9 repeat allele ($p = 0.032$; odds ratio [OR] = 2.30; 95% confidence interval [95%CI] 1.91-2.69) and decreased frequency of the 17 repeat allele ($p = 0.011$; OR = 0.208; 95%CI 0.098-0.439). This was the first study to verify a possible influence of *CNR1* gene variants on disorganized forms of schizophrenia.¹⁶

Later studies reported controversial findings regarding the association between this polymorphism and schizophrenia. Martinez-Gras et al.⁴⁰ studied possible influences of the *CNR1* gene in schizophrenia by analyzing the (AAT)_n repeat in 113 Spanish patients with schizophrenia and 111 healthy controls. Comparison of allele frequencies showed significant differences between the two groups. Allele 4 was overrepresented in controls (chi-square [χ^2] = 7.858; degrees of freedom [df] = 1; $p = 0.005$), suggesting that this allele could represent the protective variant of this gene for schizophrenia.⁴⁰

In an African Caribbean population, Ballon et al.¹⁷ did not find differences in allele or genotype distribution of the (AAT)_n polymorphism between cocaine dependents with schizophrenia ($n=45$) and those without schizophrenia ($n=97$), suggesting this polymorphism might not be associated with the disorder.

Chavarria-Siles et al.²⁹ replicated the findings of Ujike et al.¹⁶ in a population of 244 subjects with a DSM-IV diagnosis of schizophrenia and 481 family members recruited from psychiatric hospitals and clinics in the Central Valley of Costa Rica. The study found a positive correlation of prominent lifetime scores for disorganization and negative symptoms (hebephrenia dimension) with the (AAT)_n polymorphism (multiallelic $p = 0.0368$). These findings support the hypothesis that variation in the *CNR1* gene confers risk for a hebephrenic type of schizophrenia.²⁹ However, this study did not confirm the association between (AAT)_n polymorphism and schizophrenia as a broad phenotype.

Other studies in Caucasian populations did not show any association of *CNR1* gene polymorphisms with occurrence of schizophrenia. Seifert et al.²⁷ performed an association study in a sample of 104 German patients with schizophrenia and 140 healthy individuals, analyzing three known *CNR1* polymorphisms (rs6454674, rs1049353, and the AAT-repeat). No statistically significant differences were found between the two groups.²⁷

Zammit et al.²⁸ conducted the association study of the *CNR1* gene and schizophrenia with the largest sample to date. Individuals were recruited from outpatient and inpatient clinical settings and from volunteer support organizations in the United Kingdom. Overall, 797 participants with schizophrenia and 688 healthy controls were genotyped for the *CNR1* rs1049353 polymorphism. Allele frequencies were compared between the two groups, and there was no evidence for any association of this polymorphism with schizophrenia (OR = 0.97; 95%CI 0.82-1.13). This study also failed to demonstrate any association between rs1049353 genotypes and various phenotypes within schizophrenia, such as age at onset.²⁸

Two French studies also examined the allele frequencies of rs1049353 and other SNPs in patients with schizophrenia and controls. Both failed to demonstrate any association between the disorder and variations of *CNR1* gene, but found possible influences on treatment response and comorbidity with drug abuse, as we will discuss further below.^{18,24}

In an Italian population, two case-control association studies^{30,36} reported negative results. While Monteleone et al.³⁰ investigated only one *CNR1* SNP (rs1049353), Costa et al.³⁶ tested 12 SNPs in the *CNR1* gene. After running multiple comparisons analysis, none of the associations remained significant. Another study in 740 unaffected siblings of 801 patients with psychosis from the Netherlands and Belgium did not find a significant association between eight *CNR1* SNPs and schizotypal symptoms and signs, though a marginal effect was observed for rs1049353 ($p = 0.044$).³³

Bae et al.²¹ carried out a case-control association study in a Korean population and did not find any association between three SNPs in the *CNR1* gene (rs806376, rs806368, and rs806366) and schizophrenia.²¹ A more recent study conducted in Turkey also found no differences in allele frequencies of three SNPs in *CNR1* gene (rs6454674, rs806368, and rs1049353), though the authors reported a significant association between rs6454674 and schizophrenia symptoms assessed by the Positive and Negative Syndrome Scale (PANSS) and Clinical Global Impression Severity Scale (CGI-S).³⁸ Investigating three *CNR1* SNPs (rs1049353, rs1535255, and rs2023239) in a first-episode psychosis cohort of 65 patients, Suárez-Pinilla et al.³⁹ reported a significant association of the rs2023239 polymorphism with symptoms of schizophrenia at 3-year follow-up. Specifically, T/T patients showed a significant improvement in positive ($F_{1,61} = 8.76$; $p = 0.004$) and negative symptoms ($F_{1,61} = 4.51$; $p = 0.038$) compared with T/C patients. Moreover, a significant association was observed between the interaction of these three SNPs and positive symptoms.

In total, of the 16 studies that explored the association between *CNR1* polymorphisms and occurrence of schizophrenia, 15 failed to find a significant association with any SNP. However, some of the studies reported significant results for other categories or symptoms, such as an effect of the (AAT)_n polymorphism on hebephrenia^{16,29} and of rs6454674 on PANSS and CGI-S scores.³⁸ Moreover, a case-only study identified a significant association between *CNR1* SNPs and positive and negative symptoms.³⁹

CNR1 gene polymorphisms and comorbidity of schizophrenia with substance abuse

Substance abuse disorders, particularly cannabis abuse, are very common among patients with schizophrenia.⁴¹ Much research has been carried out in the search for possible neurobiological pathways and clinical implications underlying the high prevalence of this comorbidity.^{42,43} The endocannabinoid system appears as an interesting target due to its modulatory action and cross-

talk with the dopaminergic system⁴⁴; hence, this interaction could influence phenomena such as drug tolerance, craving, dependence, and relapse.⁴⁵

A meta-analysis conducted by Benyamina et al.⁴⁶ analyzed clinical studies regarding *CNR1* polymorphisms and addictive disorders in the general population. They found that only the (AAT)_n polymorphism was significantly associated with illicit substance dependence in Caucasian samples, using a risk allele of ≥ 16 repeats. The analysis also showed a small effect size (OR = 1.55; $p = 0.045$) with strong heterogeneity ($Q = 19.87$; $p < 0.01$ with $I^2 = 85\%$). This meta-analysis supports a minor implication of *CNR1* AAT polymorphism in illicit substance abuse vulnerability, taking a multifactorial model into account. The same analysis also explored studies of other *CNR1* polymorphisms, including rs1049353 and rs806379, and did not find a correlation with alcohol and drug dependence.⁴⁶

As described above, Ballon et al.¹⁷ evaluated the (AAT)_n polymorphism in 97 cocaine dependents without schizophrenia and 45 cocaine dependents with schizophrenia. They also compared both groups to 88 matched controls with no personal or family history of psychiatric disorders, including substance abuse. Although no significant difference was found between cocaine dependents with and those without schizophrenia, the frequency of the (AAT)₁₂ repeat allele was increased in both groups of cocaine dependents vs. controls (cocaine dependents without schizophrenia, 25.3%; cocaine dependents with schizophrenia, 26.7%; controls, 5.7%; $p < 0.001$). The author concluded that the (AAT)_n polymorphism near the *CNR1* gene could be associated with predisposition to cocaine dependency, with the same risk for individuals with and those without schizophrenia.¹⁷

Other three studies, all conducted in France, investigated the association between schizophrenia/substance abuse and *CNR1* polymorphisms. Leroy et al.²⁴ found, as a tendency, a non-significantly lower frequency of the rs1049353 SNP G-allele in non-substance-abusing patients with schizophrenia compared to substance-abusing patients with schizophrenia (69% vs. 81%, $p = 0.059$). The GG-genotype was significantly less frequent in non-substance-abusing than in substance-abusing patients (48% vs. 69%; $\chi^2 = 4.32$; $df = 1$; $p = 0.038$), the frequency in the latter being similar to that of controls (65%).²⁴ Later, the same group investigated another *CNR1* polymorphism and found a significant lack of allele 8 of the (AAT)_n repeat in "cannabis-sensitive" patients (i.e., patients with schizophrenia whose symptoms had been triggered or exacerbated in a context of cannabis consumption or misuse) when compared to the remaining patients ($p = 0.0028$) or to controls ($p = 0.014$).²⁵ There were no differences in allele or genotype distributions between the whole group of patients with schizophrenia and controls. A third group of addicted patients without schizophrenia were not different from controls ($p = 0.13$).⁴⁷ Finally, in an independent study, Hamdani et al.¹⁸ did not replicate this association between rs1049353 and substance abuse.

As mentioned, Martinez-Gras et al.⁴⁰ observed an association between the (AAT)_n polymorphism and schizophrenia; however, in the same study, they did not find significant differences with respect to substance abuse

when comparing 68 patients with schizophrenia and substance abuse and 45 without substance abuse. Zammit et al.,²⁸ in a larger sample, failed to find a significant effect of rs1049353 genotype on schizophrenia between those who did not use cannabis and those who did. However, van Winkel et al.,³³ evaluating 740 unaffected siblings of patients with schizophrenia, observed an interaction between *CNR1* SNPs rs806379 and rs806308 and earlier cannabis use, using positive schizotypy as the outcome, though the association was not significant after Bonferroni correction for multiple comparisons.

Evaluating other phenotypes, such as magnetic resonance imaging (MRI) findings and neurocognition, Martinez-Gras²⁶ compared substance abusers and non-substance abusers with schizophrenia and reported that patients with *CNR1* short alleles (from [AAT]_n polymorphism) who abused substances exhibited significantly higher scores on the negative PANSS scale ($p = 0.022$) and completed less categories on the Wisconsin Card Sorting Test (WCST) ($p = 0.039$) than patients who did not abuse substances.

Another study genotyped 235 patients with schizophrenia and investigated 12 SNPs that account for most of the genetic variability of the CB1 coding region. Genotypes were correlated with high-resolution anatomic brain MRI and cognitive assessment. Comorbidity with DSM-IV diagnosis of cannabis abuse and dependence, taken together, was found in % of the sample. There were no differences in allele frequencies of any of the analyzed SNPs, including rs1049353, between patients with or without cannabis abuse/dependence. However, there were significant rs12720071 genotype-by-marijuana use interaction effects on white matter (WM) volumes and neurocognitive impairment. Notably, carriers of the rs12720071 G-allele with cannabis abuse/dependence had the smallest mean parietal WM volumes ($p = 0.05$) and the worst cognitive performance on a Problem Solving Test. The authors concluded that heavy cannabis use in the context of specific *CNR1* genotypes might contribute to greater WM volume deficits and cognitive impairment, suggestive of gene-environment interactions conferring phenotypic abnormalities in schizophrenia.³¹

A more recent study investigated three *CNR1* SNPs (rs1049353, rs1535255, and rs2023239) to verify their association with brain volumes, body mass index (BMI), or psychopathological scores in 65 first-episode psychosis patients.³⁹ Data were obtained at the onset of first episode of psychosis and at 3-year follow-up. For those who were not cannabis users, the rs1535255 and rs2023239 polymorphisms had effects on lateral ventricle (LV) and LV and WM volume, respectively. Moreover, a significant interaction of the rs1049353 polymorphism with cannabis consumption on BMI was observed.

In total, 10 studies investigated *CNR1* polymorphisms and substance abuse in individuals with schizophrenia. Six of these reported significant results. One associated the (AAT)_n polymorphism with cocaine addiction¹⁷ and another with cannabis-sensitive schizophrenia.²⁵ A third study reported a significant effect of the rs1049353 SNP and substance abuse in patients with schizophrenia.²⁴ Moreover, three studies identified significant interaction effects on other phenotypes: Martinez-Gras²⁶ observed

an (AAT)n genotype-by-substance use interaction on psychopathology and neurocognition scores, Ho et al.³¹ reported a significant rs12720071 genotype-by-marijuana use interaction on MRI findings and neurocognition measures, and Suárez-Pinilla³⁹ identified a rs1049353 genotype-by-marijuana use interaction on BMI and rs1535255 and rs2023239 genotypes-by-marijuana use interaction on MRI findings.

CNR1 gene polymorphisms and pharmacological treatment of schizophrenia

In this section, we will discuss studies that investigated possible influences of *CNR1* gene polymorphisms on refractoriness to antipsychotic therapy and some adverse effects of these agents, including extrapyramidal symptoms and induced weight gain. This field clearly exemplifies the heterogeneity of schizophrenia in terms of treatment efficacy and tolerability. The future of psychopharmacology can be defined by the possibility of personalized treatment, in which clinicians could identify patients who might respond better to a certain drug or experience fewer adverse effects. This is crucial in a chronic disorder like schizophrenia, and could predict better prognosis and less functional impairment. In this sense, the field of pharmacogenomics seeks to identify biological markers for optimal treatment.⁴⁸ The dopaminergic and serotonergic systems have been the main targets of research into genetic determinants of schizophrenia refractoriness, but other systems (e.g., glutamatergic and endocannabinoid) are increasingly being studied. Authors have investigated possible influences of the *CNR1* gene on treatment response and found controversial results. The first study to investigate this hypothesis was conducted by Leroy et al.²⁴ in 102 patients with schizophrenia or schizoaffective disorder, 26 of whom were treatment-refractory. The authors analyzed the rs1049353 SNP and found that treatment-responding patients did not differ significantly from refractory patients with regard to allele frequency ($\chi^2 = 0.03$; $df = 1$; $p = 0.86$) or genotype distribution ($\chi^2 = 2.69$; $df = 2$; $p = 0.26$).²⁴

Another study, also conducted in a French population sample by Hamdani et al.,¹⁸ recruited 133 patients with schizophrenia from two hospitals in the Paris area. All were treated with atypical antipsychotics (including clozapine) for at least 4 weeks at appropriate dosages. Four *CNR1* SNPs were analyzed: rs806368, rs1049353, rs806379, and rs806380. The G-allele of rs1049353 was found in excess in patients with schizophrenia refractory to atypical antipsychotics compared with responders (85.6% vs. 71.6%; $\chi^2 = 7.420$; $p = 0.006$). This observation was independent of the geographic region of patient origin. The G-allele appeared to exert a dose-related influence on therapeutic response, increasing the risk of refractoriness in up to 76% of GG homozygous subjects. The association appeared to be specific of the genetic block where the rs1049353 polymorphism is located, because the three other SNPs encompassing the *CNR1* gene, but with no linkage disequilibrium (LD), showed no association with treatment refractoriness.¹⁸ In contrast to the previous study,²⁴ these findings suggest

that the G-allele of rs1049353 could be a pharmacogenetic factor for response to atypical antipsychotic drugs. In a recent letter to the editor, Schennach et al.³⁴ reported having found no significant association between rs1049353 and early improvement or clinical response.

Other authors also looked at influences of the *CNR1* gene on the development of adverse effects to antipsychotic treatment. Considering that *CNR1* has been implicated in the regulation of feeding behavior and body weight,⁴⁹ Tiwari et al.¹⁹ investigated the possible association of *CNR1* polymorphisms with antipsychotic-induced weight gain. This study analyzed 20 SNPs of the *CNR1* gene in a sample of 183 patients from three different sites in Germany and the U.S. Patients were treated with typical (haloperidol) and atypical (risperidone, olanzapine, and clozapine) agents and evaluated for antipsychotic-induced weight gain for up to 14 weeks. The results showed that the rs806378 polymorphism was associated with weight gain in patients of European ancestry treated with clozapine or olanzapine. T-allele carriers (CT + TT) had an additional weight gain of approximately 2.2 kg than patients homozygous for the CC genotype (4.33 ± 3.89 kg vs. 2.21 ± 4.51 kg; $p = 0.022$). The authors concluded that *CNR1* may be associated with antipsychotic-induced weight gain in patients with chronic schizophrenia, but these results still need to be replicated in larger sample sets.¹⁹

Another study, conducted in Korea with a small sample ($n=78$), found no significant association between *CNR1* polymorphisms (rs1049353, rs806368, and rs4707436) and olanzapine-induced weight gain in patients with chronic schizophrenia.³² Likewise, an Italian study found no association between *CNR1* polymorphism rs1049353 and antipsychotic-induced weight gain.³⁰

Besides weight gain, some studies have linked the *CNR1* gene to obesity and various metabolic parameters.^{50,51} Yu et al.³⁷ investigated whether genetic variation in *CNR1* was associated with the metabolic syndrome (MetS). A total of 407 patients with schizophrenia, schizoaffective disorder, and schizophreniform disorder were compared regarding occurrence of MetS ($n=143$ with MetS and $n=264$ without MetS). Seven *CNR1* SNPs were investigated (rs6454674, rs6928499, rs806379, rs1535355, rs806377, rs1049353, and rs3023239), and the minor alleles of rs6928499 ($p = 0.006$), rs1535255 ($p = 0.006$), and rs2023239 ($p = 0.001$) were significantly associated with lower risk of MetS. Further analysis revealed that this lower risk was attributable mainly to differences in HDL and fasting glucose.³⁷

Regarding extrapyramidal side effects, Tiwari et al.³⁵ evaluated the occurrence of tardive dyskinesia (TD) in a sample of 191 patients of European ancestry with schizophrenia (74 with TD). The authors analyzed 20 SNPs covering the *CNR1* gene and found significant genotype and allele ($p = 0.012$) associations with the rs806374 (T > C) polymorphism. CC genotype carriers were more likely to be TD-positive (CC vs. TT + TC, OR = 3.4 [1.5-7.8]; $p = 0.003$). These results point toward a possible role of the *CNR1* gene in the development of TD. However, the authors stress that these observations were marginal after correcting for multiple testing, and need to be replicated in a larger sample.³⁵

A total of eight studies included in this review explored the association between *CNR1* polymorphisms and pharmacological treatment of schizophrenia. Three of these investigated treatment response, and only one observed a significant effect.¹⁸ Three studies investigated antipsychotic-induced weight gain, and Tiwari et al.¹⁹ reported a positive association between rs806378 and weight gain in a subsample of patients of European ancestry treated with clozapine or olanzapine.¹⁹ Finally, one study investigated the association of *CNR1* SNPs with MetS, and another investigated its association with TD; both reported significant results.

Discussion

The (AAT)_n trinucleotide polymorphism was associated with schizophrenia, particularly the hebephrenic subtype, in several^{16,29,40} but not all studies included in this review. The same pattern was found for the rs1049353 SNP, which correlated positively with treatment refractoriness in a single study.¹⁸ Even in those studies, the authors did not establish how genetic variants could exert effects on disorder phenotype. The polymorphisms that were investigated are not capable of substituting amino acids and changing protein structure, and have not been found to alter mRNA expression or disrupt transcription at intronic promoter regions. Hence, the main possible explanation for these findings is the LD mechanism. Authors suggest that the tested variants could be in LD with another functional SNP along the *CNR1* gene, and that haplotype analyses should be well measured in further studies.

In previous research, *CNR1* variants have been associated with addictive disorders such as alcohol dependence and drug use.^{52,53} In a meta-analysis,⁴⁶ only the (AAT)_n polymorphism showed a significant association with illicit substance dependence in Caucasian population samples, but with a small effect size (OR = 1.55; $p = 0.045$). These data are in agreement with Ballon et al.,¹⁷ who concluded that the (AAT)_n polymorphism near the *CNR1* gene could be associated with predisposition to cocaine dependency, with the same risk for patients with and without schizophrenia. As pointed out by Martinez-Gras,²⁶ the presence of *CNR1* short alleles was associated with worse cognitive performance among substance abusers with schizophrenia. Based on these studies, we speculate that genetic variants involving the *CNR1* gene could be reflected by neurobiological dysfunctions in the endocannabinoid system. Given the neuromodulatory nature of this system, acting on dopaminergic and GABAergic molecular pathways, those variants could influence the pathophysiology of psychosis, brain reward processes, and vulnerability to addictive disorders.^{54,55}

Finally, gene-environment approaches integrate the evidence showing that *CNR1* gene polymorphisms and cannabis misuse could lead to brain abnormalities (WM volume reductions) and cognitive deficits in schizophrenia.³¹ This modern reasoning directs the future of research in this field, but is still far from a final conclusion. Several gaps, such as differences in genetic ancestry among study populations, phenotypic variations of schizophrenia, and the heterogeneity of drug compounds

(e.g., differential biological actions of THC and cannabidiol), should be better understood.⁵⁵

Although our search for the systematic review was very restrictive, we may have failed to include some eligible studies, such as genome-wide association studies (GWAS). These studies probably would present negative results regarding the *CNR1* gene, as they were not retrieved in our search. Searching for studies on schizophrenia in the GWAS catalog (<https://www.ebi.ac.uk/gwas/>) in June 2015, we found 45 studies with schizophrenia or response to treatment or substance dependence as outcome. Of these, none reported a significant association with *CNR1* polymorphisms.

Studies on this gene are very heterogeneous, reflecting the bias on investigating genotype associations using the candidate gene approach. The conflicting findings of this review, in part attributable to small sample sizes, do not allow us to reach a conclusion regarding the real influences of *CNR1* SNPs on the pathophysiology of schizophrenia. Moreover, some unpublished data (including ours reported herein) might not have been accepted for publication, reflecting publication bias. However, we included the grey literature, such as meeting abstracts and letters.

In conclusion, our systematic review on potential associations of *CNR1* polymorphisms with schizophrenia and its subphenotypes revealed conflicting data, with results tending towards a negative association. We also tested some *CNR1* SNPs on a sample of Brazilian subjects and did not find a significant association. Further studies should also go beyond genes that encode brain receptors and investigate the genetic basis of endocannabinoid system enzymes (e.g., fatty acid amide hydrolase), endogenous ligands (anandamide), and transporters. GWAS with large samples are revealing more *loci* associated to schizophrenia, which may finally allow us to fully understand the role of endocannabinoid system malfunctioning in the pathophysiology of schizophrenia and other psychiatric disorders.

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