


# Associations of **CB1** cannabinoid receptor (**CNR1**) gene polymorphisms with risk for alcohol dependence

## Evidence from meta-analyses of genetic and genome-wide association studies

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### Abstract

**Objectives:** Reported associations of the cannabinoid receptor 1 (*CNR1*) single nucleotide polymorphisms (SNPs) with alcohol dependence (AD) have been inconsistent, prompting a meta-analysis to obtain more precise estimates.

**Methods:** A Boolean search of 4 databases (PubMed, Scopus, Google Scholar, and Mednar) sought articles that evaluated the association between *CNR1* polymorphisms and risk of AD. We selected the articles with sufficient genotype frequency data to enable calculation of odds ratios (ORs) and 95% confidence intervals (CIs). Using the Population Intervention Comparators Outcome elements, AD patients (P) were compared by genotype data between AD-participants (I) and non-AD-participants (C) in order to determine the risk of AD (O) attributed to the *CNR1* SNPs. Analyzing 4 SNPs (rs1049353, rs1535255, rs2023239, and rs806379) using standard genetic models, we examined associations where multiple comparisons were Holm–Bonferroni corrected. The pooled ORs were assessed for aggregate statistical power and robustness (sensitivity analysis). Subgroups were Caucasians and African-Americans.

**Results:** From 32 comparisons, 14 were significant indicating increased risk, from which 5 outcomes (*P*-value for association [*P*<sup>a</sup>] = .003 to <.001) survived the Holm–Bonferroni-correction, which were deemed robust. In the rs1535255 outcomes, the codominant effect (OR = 1.43, 95% CIs = 1.24–1.65, *P*<sup>a</sup> < .001) had greater statistical power than the dominant effect (OR = 1.30, 95% CI = 1.08–1.57, *P*<sup>a</sup> = .006). In contrast, the rs2023239 codominant outcome was underpowered. Significance of both rs806379 Caucasian outcomes (ORs = 1.20–1.43, 95% CIs = 1.07–1.57, *P*<sup>a</sup> = .003) contrasted with the null effects in African-Americans (ORs = 0.98–1.08, 95% CIs = 0.70–1.53).

**Conclusions:** Three *CNR1* SNPs (rs1535255, rs2023239, and rs806379) were implicated in their associations with development of AD: based on aggregate statistical power, rs1535255 presented greater evidence for associations than rs2023239; rs806379 implicated the Caucasian subgroup. Multiple statistical and meta-analytical features (consistency, robustness, and high significance) underpinned the strengths of these outcomes. Our findings could render the *CNR1* polymorphisms useful in the clinical genetics of AD.

**Abbreviations:** AA = African-American, AD = alcohol dependence, ASP = aggregate statistical power, CB = Clark–Baudouin, *CB1* = cannabinoid receptor 1, CI = confidence interval, CIDI = Composite International Diagnostic Interview, *CNR1* = cannabinoid

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receptor 1 gene, *CNR1* = cannabinoid receptor 1 protein, *CNR2* = cannabinoid receptor 2 gene, DSM-III, IV, and V = Third, fourth, and fifth editions of the Diagnostic and Statistical Manual of Mental Disorders, ECS = endocannabinoid system, GAS = genetic association studies, GWAS = genome-wide association studies; HBC = Holm–Bonferroni-correction, HWE = Hardy Weinberg equilibrium,  $I^2$  = measure of variability,  $n$  = number of studies, OR = odds ratio,  $P^a$  =  $P$ -value for association,  $P_{\text{het}}$  =  $P$ -value for heterogeneity,  $P_{\text{HWE}}$  =  $P$ -value for HWE, SNP = single nucleotide polymorphism, *var* = variant, *wt* = wild-type.

**Keywords:** alcohol dependence, cannabinoid receptor 1 polymorphisms, meta-analysis

## 1. Introduction

The Diagnostic and Statistical Manual of Mental Disorders (DSM 5), alcohol use disorder including alcohol abuse and alcohol dependence (AD) defined as a psychiatric dysfunction, marked by compulsive drinking, leading to pathological alcohol seeking behavior.<sup>[1]</sup> Alcohol abuse corrodes the security of health, job, and family.<sup>[2,3]</sup> Moreover, the morbidity and mortality that result from AD, adversely impacts individuals and society, contributing to the global burden of disease.<sup>[4]</sup>

Persistence of AD in humans through the millennia may have come from the driving force of positive reinforcement mechanisms that stimulates reward pathways of the brain, which involve the endocannabinoid system (ECS).<sup>[5]</sup> Studies have shown that the ECS regulates dopamine reward circuits, which play an important role in the reward processes involved in substance dependence<sup>[6,7]</sup> and facilitate vulnerability to the progression of addiction.<sup>[8]</sup> The ECS is composed of cannabinoid receptors as well as enzymes that synthesize, degrade, and transport endocannabinoids (endogenous cannabinoids).<sup>[9]</sup> The cannabinoid receptors, CB1 and CB2 are encoded by cannabinoid receptor 1 (*CNR1*) and cannabinoid receptor 2 (*CNR2*) genes, respectively. *CNR2* is found at the brain periphery and appears to have an immune function.<sup>[10]</sup> *CNR1* is expressed at high levels in brain regions that act on drug reward and drug memories,<sup>[11]</sup> which include the hippocampus, striatum, and cerebral cortex.<sup>[12]</sup> The machinery of drug rewards and memories lead to risks of psychiatric disorders that include substance abuse which involves the ECS.<sup>[13]</sup> Activating the same reward pathways in the brain are cannabinoids, chemical substances that attach to the cannabinoid receptors of the brain and elicit pharmacological effects similar to marijuana (cannabis).<sup>[14]</sup> It has been shown that delta-9-tetrahydrocannabinol and alcohol share similar behavioral profiles where at low and high doses, both induce euphoria/motor incoordination and sedation, respectively.<sup>[15]</sup> delta-9-tetrahydrocannabinol is mediated by CB1, encoded by the *CNR1* gene, which maps to chromosome 6q14-q15. *CB1/CNR1* displays at least 4 exons that spans 25kb and produces several transcripts. Common single-nucleotide polymorphisms (SNPs) account for as much as 30% of the variance in AD.<sup>[16]</sup> Various locations of *CNR1* SNPs point to the silent rs1049353 found in the coding region involving a substitution of “G” to “A” at nucleotide position 1359 in codon 435 (threonine). The rs806379 SNP (A/T) is located in intron 2 of the *CNR1* gene (–385 to exon 3 alternate transcript initiation site). The rs2023239 and rs1535255 SNPs are non-coding intron variants in the 3 prime untranslated region.

These *CNR1* SNPs were found to be associated with polysubstance abuse.<sup>[17]</sup> A study showed that C allele carriers of rs2023239 had elevated cravings in response to alcohol-associated cues.<sup>[18]</sup> However, the association of rs1049353 with alcohol and drug dependence elicited variable results from

different studies.<sup>[10,19–22]</sup> A nicotine study found that the T allele on rs806379 reduced mRNA expression resulting in less mRNA activity,<sup>[23]</sup> which agreed with an earlier haplotype study involving rs2023239, rs806379 and rs1535255.<sup>[24]</sup> Yet, examinations of these SNPs for their associations with development of AD<sup>[25]</sup> have produced inconsistent outcomes, that ranged from reduced to increased risks. Hence, using the Population Intervention Comparators Outcome approach, we performed this meta-analysis to realize our objectives of obtaining less ambiguous and better estimates of associations. Here, we examined SNPs that might provide clues of the roles of their proteins in the neuro-metabolic pathways, fostering better understanding of risk biomarkers in AD.

## 2. Methods

### 2.1. Selection of studies

We searched MEDLINE using PubMed, Google Scholar, Scopus, and Mednar for association studies as of March 2, 2021. Using Boolean descriptors, search terms (Table S1, Supplemental Digital Content, <http://links.lww.com/MD2/A485>) were as follows: (CNR1 OR cannabinoid receptor 1 OR endocannabinoid OR CB1) and (polymorphisms, genetic OR gene OR single nucleotide polymorphism OR genome-wide association studies OR GWAS) and (alcoholism OR alcohol dependence OR alcohol abuse) medical subject heading and text, unrestricted by language. The Boolean search started the process of study selection and finalized with full-text examination of the included studies.

This provided the timeline of 2002 to 2010 indicating the years for including the studies. References in the retrieved articles were screened manually to identify additional eligible studies. In cases of duplicates, we selected the one with a later date of publication. The 4 Population Intervention Comparators Outcome elements were applied: Population: AD patients; Intervention: *CNR1* gene polymorphisms; Comparators: AD patients versus non-AD patients; and Outcome: AD risk. Inclusion criteria were: case-control design evaluating the association between *CNR1* polymorphisms and risk of AD; sufficient genotype frequency (provision of wild-type [*wt*], variant [*var*], and heterozygous [*wt-var*] numbers under a case-control design) data to enable calculation of odds ratios (ORs) and 95% confidence intervals (CIs). Exclusion criteria were: those not involving AD; reviews; functional articles; not about the *CNR1* SNPs; those without controls; studies whose genotype controls deviated from the Hardy-Weinberg equilibrium (HWE); and studies whose genotype or allele frequencies were unusable/absent.

### 2.2. Data extraction and HWE assessment

Two investigators (NP and PC) independently extracted data on March 3 and March 4, respectively. PT adjudicated disagree-

ments, which facilitated consensus. The following information was extracted from each article: first author's name, year of the study, country of origin, ethnicity, age of the subjects, *CNR1* SNPs (rs number), comparators, article features needed to tally the Clark–Baudouin (CB) scores, sample sizes, genotype data of AD, and controls and minor allele frequency. HWE was assessed using the application in <https://ihg.gsf.de/cgi-bin/hw/hwa1.pl> where a *P*-value for HWE [ $P_{HWE}$ ] > .05 indicated HW-compliance.

### 2.3. Statistical power and study quality

Using the G\*Power program,<sup>[26]</sup> we evaluated aggregate statistical power (ASP). Based on previous single-study results and meta-analysis outcomes,<sup>[27–29]</sup> we chose 3 OR levels (1.1, 1.2, and 1.5) at a genotypic risk level of  $\alpha=0.05$  (2-sided) where we considered power adequate at  $\geq 80\%$ . We used the CB scale to evaluate methodological quality of the included studies.<sup>[30]</sup> In this scale, low, moderate and high have scores of <5, 5 to 6, and  $\geq 7$ , respectively.

### 2.4. Meta-analysis protocol

Because the *CNR1* genotypes in the included studies were varyingly notated by rs number, we used the generic *var* and *wt* notations (Table S2, Supplemental Digital Content, <http://links.lww.com/MD2/A486>). Given the hypothesis of association between *CNR1* SNPs and risk of AD, we estimated the ORs with 95% CIs for each study by comparing cases with controls. Pooled ORs with 95% CIs were calculated for the following genetic models: homozygous: (*var-var* and *wt-wt*) genotypes compared with *wt-wt*; recessive: (*var-var* vs *wt-var* + *wt-wt*); dominant: (*var-var* + *wt-var* vs *wt-wt*); and codominant: (*var* vs *wt*). We used raw data for frequencies to estimate study specific ORs of AD development. Comparing the effects on the same baseline, we calculated pooled ORs using the Z-test, where we confined our analyses to  $\geq 3$  studies. Multiple comparisons were Holm–Bonferroni corrected (HBC).<sup>[31]</sup> Subgrouping was ethnicity-based (Caucasians and African-Americans [AA]). Heterogeneity<sup>[32]</sup> was estimated with the chi-squared-based *Q* test, the significance threshold of which was set at *P*-value for heterogeneity [ $P_{het}$ ]  $\leq .10$  and quantified with the measure of variability (*I*<sup>2</sup>) statistic, which measures variability between studies. Random-effects model<sup>[33]</sup> was used in the presence of heterogeneity, otherwise, the fixed-effects model was used.<sup>[34]</sup> Random-effects derived pooled ORs were subjected to outlier treatment, which dichotomized the comparisons into pre- and postoutlier. We used sensitivity analysis to assess robustness of the pooled ORs. Assessment of publication bias was considered for significant outcomes with  $\geq 10$  studies.<sup>[35]</sup> Except for heterogeneity estimation, 2-sided *P*-values of < .05 were considered significant. Data were analyzed using Review Manager 5.3 (Cochrane Collaboration, Oxford, England) and SIGMASTAT 2.03 (Systat Software, San Jose, CA).

## 3. Results

### 3.1. Search results and data extraction

Figure 1 outlines the study selection process following the guidelines in Preferred Reporting Items for Systematic Reviews and Meta-Analyses (Table S3, Supplemental Digital Content,

<http://links.lww.com/MD2/A487>). Initial search resulted in 16,152 citations, followed by a series of omissions that yielded 6 genetic association studies (GAS).<sup>[20–22,24,36,37]</sup> We found 7 GWAS that examined AD<sup>[38–44]</sup> in our search and 1<sup>[38]</sup> provided *CNR1* data (Table S1, Supplemental Digital Content, <http://links.lww.com/MD2/A485>) warranting inclusion in our analysis. Of the total 7 articles (6 GAS and 1 GWAS), 4<sup>[20,21,36,38]</sup> examined a single SNP (rs1049353) and the rest examined multiple SNPs (Table 1). Table S2 (Supplemental Digital Content, <http://links.lww.com/MD2/A486>) lists genotype data, sample sizes for each SNP, minor allele frequencies, and control  $P_{HWE}$ -values.

### 3.2. Study features

Table 1 shows that all 7 articles (2002–2010) had Caucasian subjects; with AA in 4 papers.<sup>[22,24,37,38]</sup> The Japanese samples in the Zhang et al<sup>[24]</sup> study were excluded in order to minimize ethnic heterogeneity. The mean and  $\pm$  standard deviation (42.3  $\pm$  4.5 years) of the age of the cases indicated a middle-aged demographic profile. Controls in most studies were screened for AXIS I or AXIS II psychiatric disorders, such as schizophrenia, with positive findings meriting exclusion from the studies. Phenotypic variation in the subjects was minimized with a battery of tests that involved third edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-III) and fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) guidelines<sup>[11]</sup> (Table 1). The sex ratio in GWAS<sup>[38]</sup> was 1:1 whereas in GAS, 3 articles ranged from 2.8 to 3.7 men for every women.<sup>[20,22,37]</sup> Table S2 (Supplemental Digital Content, <http://links.lww.com/MD2/A486>) lists the 4 *CNR1* SNPs used in the meta-analysis (rs1049353, rs1535255, rs2023239, and rs806379) which were Caucasians and AA. The number of studies (n) per SNP ranged from 4 to 6. Combined sample sizes in each of the 4 SNPs ranged from 90 to 1897 for cases and 46 to 1932 for controls. Table 1 shows that in 4 articles,<sup>[22,24,36,37]</sup> linkage disequilibrium and haplotype analyses were performed. The median (8.0) and interquartile range (8–9) of the CB scores indicated high methodological quality of the studies.

### 3.3. Meta-analysis outcomes

Table 2 shows 32 outcomes, 28 (88%) of which were fixed-effects and 4 (12%) were random-effects (heterogeneous). Of the 32, 14 were significant ( $P^a < .05$ ), found in all the genetic models. Of the 14, 6 robust outcomes survived the HBC, which implicated three *CNR1* SNPs (rs1535255, rs2023239, and rs806379) in risk for developing AD (Table 3). Publication bias was not performed as our results did not meet the 2 a priori criteria set in the Methods.

**3.3.1. rs1049353.** This SNP had the highest aggregate sample sizes (3862 cases and 2863 controls) (Table S2, Supplemental Digital Content, <http://links.lww.com/MD2/A486>). However, none of the 9 rs1049353 outcomes were significant (ORs = 1.13–1.49, 95% CIs = 0.85–2.60,  $P^a = .15–.38$ ), not even when confined to Caucasian (ORs = 1.13–1.31, 95% CIs = 0.90–1.82,  $P^a = .08–.18$ ) (Table 2).

**3.3.2. rs1535255.** Aggregate sample sizes for this SNP were 1135 cases and 1086 controls (Table S2, Supplemental Digital Content, <http://links.lww.com/MD2/A486>). This SNP generated 5 comparisons, 3 of which were significant. Of the 3, 2 survived the HBC. In the first, the dominant outcome was postoutlier

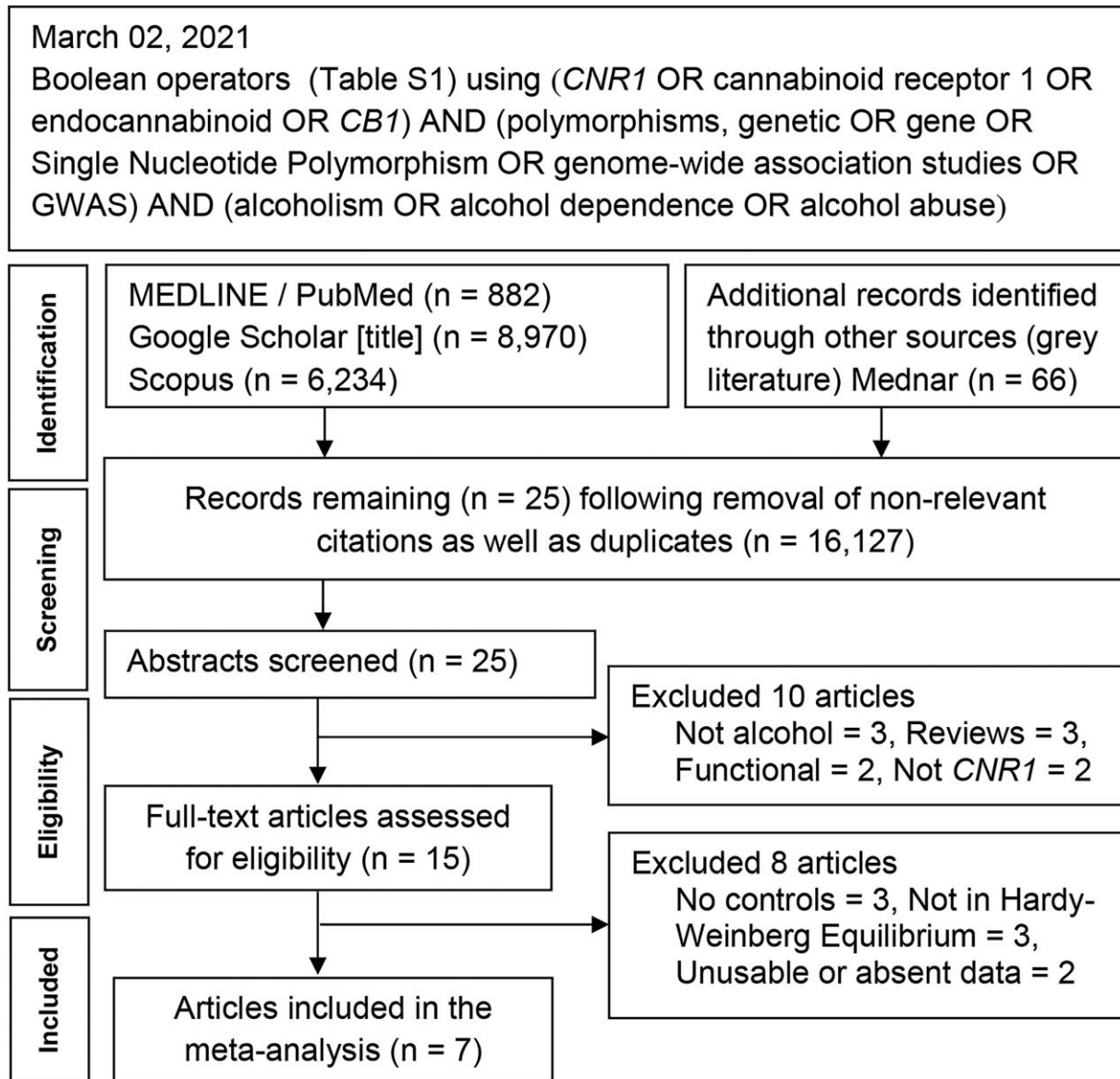


Figure 1. Summary flowchart of the literature search.

(ORs=1.30, 95% CIs=1.08–1.57,  $P^a$  = .006) with an ASP of 98.3% (OR 1.5). In the second, the codominant outcome was preoutlier (ORs=1.43, 95% CIs=1.24–1.65) with ASPs of 99.7% and 80.7% (ORs 1.5 and 1.2), respectively. Table 3 and Table S2 (Supplemental Digital Content, <http://links.lww.com/MD2/A486>) tabulate the data for which rs1535255 and Fig. 2 shows the forest plot for this outcome. In this figure, 3 study-specific ORs were non-significant (ORs=1.20–1.31, 95% CIs=0.91–1.89) and one was (OR=1.72, 95% CI=1.37–3.14), which resulted in a significant pooled OR ( $P^a$  < .001).

**3.3.3. rs2023239.** This SNP had aggregate sample sizes of 704 cases and 681 controls (Table S2, Supplemental Digital Content, <http://links.lww.com/MD2/A486>). Of the 6 comparisons, 3 outcomes were significant, 1 of which survived the HBC. This

postoutlier effect in the codominant model (OR=1.33, 95% CI=1.13–1.56,  $P^a$  < .001) was underpowered (Table 3).

**3.3.4. rs806379.** With aggregate sample sizes of 1497 cases and 1843 controls (Table S2, Supplemental Digital Content, <http://links.lww.com/MD2/A486>), this SNP generated 12 outcomes, 4 and 8 for the overall analysis and subgroups, respectively (Table 2). The 4 overall outcomes in all genetic models were significant (ORs=1.15–1.31, 95% CIs=1.01–1.60,  $P^a$  = .007–.04) one of which survived HBC. This was the codominant pooled effect (OR 1.15, 95% CI=1.04–1.27,  $P^a$  = .007), which was statistically powered at all 3 levels of ORs (Table 3). Of the 4 significant outcomes in the Caucasian subgroup, 2 (powered at OR 1.5 and 1.2) survived HBC in the homozygous and codominant models (ORs=1.20–1.43, 95%

**Table 1**  
**Characteristics of the included studies in cannabinoid receptor 1 associations with alcohol dependency.**

First author	Bienit	Schmidt	Preuss	Marcos	Zhang	Herman	Zuo
Year	2010	2002	2003	2012	2004	2006	2007
Countries	USA and Germany	Germany	Germany	Spain	USA	USA	USA
Ethnicities	Caucasian (European descent) AA	Caucasian	Caucasian	Caucasian	Caucasian (European Americans)* AA*, Japanese	Caucasian (Non-Hispanic), AA*	Caucasian (European Americans) AA
Sample sizes (cases / controls)	1897/1932	265/136	196/210	298/155 (AD + alcohol abuse)	1161/566	769/472	550/451
Age of cases in years (mean ± standard deviation) or range	39.2 ± 9.2	39.2 ± 8.6	41.1 ± 10.0	50.8 ± 10.7	Not mentioned	43.8 ± 9.6	39.5 ± 18.0
Psychiatric diagnoses of cases	DSM-IV	CIDI†,‡	ICD-10 DSM-IV	DSM-IV No AD history	DSM-IV	DSM-III-R #	DSM-III-R, SADS
Controls screening procedure	Screened for AD	Questionnaire	Minnesota Multiphasic Personality Inventory	Examination	Not mentioned	Semi-structured assessment for drug dependence and alcoholism	DSM-III
Gender ratio (male: female)	1:1	Not mentioned	3.7:1	Male only	Not mentioned	3.4:1	2.8:1
Number of SNPs examined in the article	132	1	1	3	5	4	8
CMV1 polymorphisms examined in this meta-analysis	rs1049353	rs1049353	rs1049353	rs1049353	rs1535255 rs806379 rs2023239	rs1535255 rs806379 rs2023239	rs1049353 rs806379
Performed haplotype analysis	No	No	No	Yes	Yes	Yes	Yes
Performed linkage disequilibrium analysis	No	No	No	Yes	Yes	Yes	Yes
Addressed Hardy-Weinberg Equilibrium	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Clark-Baudouin (CB) score	9	8	9	8	8	9	8

AA = African-American, AD = alcohol dependency, CMV1 = cannabinoid receptor 1 gene, DSM = Diagnostic and Statistical Manual of Mental Disorders, ICD = International Classification of Diseases, SADS = schedule for affective disorders and schizophrenia, SCID = structured clinical interview for DSM, USA = United States of America.

\* Polysubstance cases with data for alcohol dependency.

† CIDI = Composite International Diagnostic Interview.

‡ Cases were subgrouped for clinical homogeneity.

**Table 2**  
**Summary outcomes for cannabinoid receptor 1 associations with alcohol dependency.**

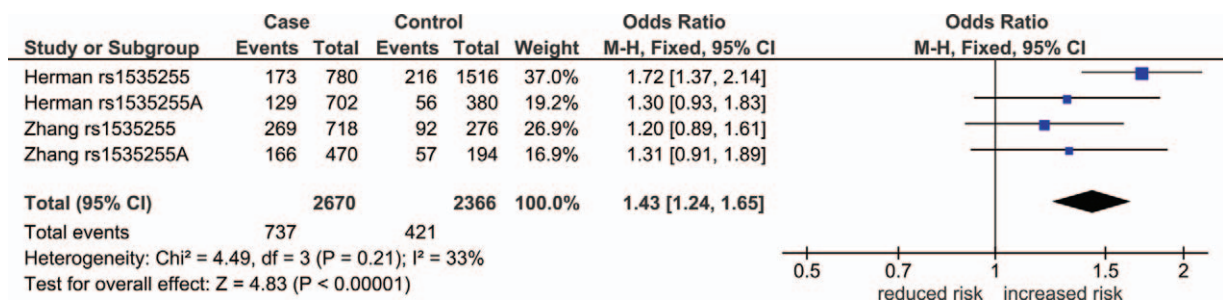
		n	Test of association			Test of heterogeneity		
			OR	95% CI	<i>P</i> <sup>a</sup>	<i>P</i> <sub>het</sub>	<i>I</i> <sup>2</sup> (%)	Analysis model
rs1049353 overall								
1	Homozygous	6	1.24	0.92–1.67	.15	.13	42	Fixed
2	Recessive	6	1.14	0.85–1.51	.38	.33	13	Fixed
3	Dominant	6	1.49	0.86–2.60	.16	<.001	92	Random
4	Dominant*	5	1.13	0.95–1.33	.16	.25	26	Fixed
5	Codominant	6	1.08	0.96–1.23	.20	.16	36	Fixed
rs1049353 Caucasian-Mixed								
6	Homozygous	4	1.31	0.94–1.82	.11	.13	46	Fixed
7	Recessive	4	1.24	0.90–1.70	.18	.26	25	Fixed
8	Dominant	4	1.14	0.94–1.37	.08	.15	44	Fixed
9	Codominant	4	1.13	0.98–1.31	.09	.10	54	Fixed
rs1535255 overall								
10	Homozygous	4	<b>1.54</b>	<b>1.05–2.26</b>	<b>.03</b>	.65	0	Fixed
11	Recessive	4	1.30	0.90–1.88	.17	.65	0	Fixed
12	Dominant	4	1.08	0.77–1.52	.64	.02	71	Random
13	Dominant*	3	<b>1.30</b>	<b>1.08–1.57</b>	<b>.006</b> <sup>†</sup>	.40	0	Fixed
14	Codominant	4	<b>1.43</b>	<b>1.24–1.65</b>	<b>&lt;.001</b> <sup>†</sup>	.21	33	Fixed
rs2023239 overall								
15	Homozygous	4	1.36	0.94–1.97	.10	.19	36	Fixed
16	Recessive	4	0.75	0.14–4.10	.74	<.001	91	Random
17	Recessive*	3	<b>1.61</b>	<b>1.03–2.52</b>	<b>.04</b>	.86	0	Fixed
18	Dominant	4	<b>1.24</b>	<b>1.04–1.48</b>	<b>.01</b>	.15	44	Fixed
19	Codominant	4	1.21	0.97–1.51	.10	.08	56	Random
20	Codominant*	3	<b>1.33</b>	<b>1.13–1.56</b>	<b>&lt;.001</b> <sup>†</sup>	.40	0	Fixed
rs806379 overall								
21	Homozygous	6	<b>1.31</b>	<b>1.07–1.60</b>	<b>.009</b>	.22	29	Fixed
22	Recessive	6	<b>1.23</b>	<b>1.03–1.46</b>	<b>.02</b>	.40	3	Fixed
23	Dominant	6	<b>1.18</b>	<b>1.01–1.38</b>	<b>.04</b>	.36	9	Fixed
24	Codominant	6	<b>1.15</b>	<b>1.04–1.27</b>	<b>.007</b> <sup>†</sup>	.18	34	Fixed
rs806379 Caucasian-Mixed								
25	Homozygous	3	<b>1.43</b>	<b>1.13–1.81</b>	<b>.003</b> <sup>†</sup>	.17	43	Fixed
26	Recessive	3	<b>1.28</b>	<b>1.05–1.57</b>	<b>.01</b>	.32	11	Fixed
27	Dominant	3	<b>1.26</b>	<b>1.05–1.52</b>	<b>.01</b>	.26	26	Fixed
28	Codominant	3	<b>1.20</b>	<b>1.07–1.35</b>	<b>.003</b> <sup>†</sup>	.15	47	Fixed
rs806379 African-American								
29	Homozygous	3	1.03	0.70–1.53	.88	.43	0	Fixed
30	Recessive	3	1.08	0.78–1.51	.64	.34	0	Fixed
31	Dominant	3	0.98	0.71–1.33	.88	.65	0	Fixed
32	Codominant	3	1.02	0.84–1.24	.85	.39	0	Fixed

CI=confidence interval, *I*<sup>2</sup>=measure of variability, n=number of studies, OR=odds ratio, *P*<sup>a</sup>=*P*-value for association, *P*<sub>het</sub>=*P*-value for heterogeneity, bold values indicate significant associations.  
<sup>\*</sup> Postoutlier.  
<sup>†</sup> Survived the Holm–Bonferroni correction.

**Table 3**  
**Power and sensitivity analyses for the main outcomes.**

Comparison	Genetic model	Outlier status	n	Sample sizes			ASP (%) α=0.05			Test of association			Test of heterogeneity		Sensitivity treatment outcome
				Cases	Controls	OR 1.5	OR 1.2	OR 1.1	OR	95% CI	<i>P</i> <sup>a</sup>	<i>P</i> <sub>het</sub>	<i>I</i> <sup>2</sup> (%)	Analysis model	
Overall															
rs1535255	Dominant	Post-	3	784	896	98.3	68.9	53.3	<b>1.30</b>	<b>1.08–1.57</b>	<b>.006</b>	.40	0	Fixed	Robust
rs1535255	Codominant	Pre-	4	1135	1,086	99.7	80.7	65.3	<b>1.43</b>	<b>1.24–1.65</b>	<b>&lt;.001</b>	.21	33	Fixed	Robust
rs2023239	Codominant	Post-	3	566	287	78.7	38.0	28.1	<b>1.33</b>	<b>1.13–1.56</b>	<b>&lt;.001</b>	.40	0	Fixed	Robust
rs806379	Codominant	Pre-	6	1497	1,843	99.9	93.2	81.9	<b>1.15</b>	<b>1.04–1.27</b>	<b>.007</b>	.18	34	Fixed	Robust
Caucasian-Mixed															
rs806379	Homozygous	Pre-	3	1045	1,316	99.8	82.5	67.5	<b>1.43</b>	<b>1.13–1.81</b>	<b>.003</b>	.17	43	Fixed	Robust
rs806379	Codominant	Pre-	3	1045	1,316	99.8	82.5	67.5	<b>1.20</b>	<b>1.07–1.35</b>	<b>.003</b>	.15	47	Fixed	Robust

Values in bold indicate significant association and in italics, statistically powered comparisons.  
 ASP=aggregate statistical power, CI=confidence interval, *I*<sup>2</sup>=measure of variability, n=number of studies, OR=odds ratio, *P*<sup>a</sup>=*P*-value for association, *P*<sub>het</sub>=*P*-value for heterogeneity.



**Figure 2.** Preoutlier forest plot in the codominant model of rs1535255. \*: Caucasian; +: African-American; CI = confidence interval; *CNR1* = cannabinoid receptor 1 gene; df = degree of freedom;  $I^2$  = measure of variability;  $P^a$  =  $P$ -value for association;  $P_{\text{het}}$  =  $P$ -value for heterogeneity.

CI = 1.07–1.81,  $P^a = .003$ ). In contrast, all 4 AA outcomes were null (ORs = 0.98–1.08,  $P^a = .64$ –.88) (Table 2).

## 4. Discussion

### 4.1. Summary of the findings

Screened for false positives (HBC), the 14 significant outcomes were reduced to 6 pooled ORs implicating 3 SNPs (rs1535255, rs2023239, and rs806379). These 6 main outcomes showed up to 1.4-fold increased risks, 67% (4/6) which had a codominant effect. This effect in rs1535255, rs2023239, and rs806379 indicated consistency of associations. The rs806379 SNP identified Caucasians as a susceptible subgroup, but not the AA. Differential outcomes between the ethnicities could have been due to a number of confounding factors that involved sex or variations in phenotype definition. Our use of HBC, power analysis, subgrouping, outlier, and sensitivity approaches were instrumental in generating strong evidence for association, delineating subgroup effects and identifying *CNR1* SNP associations with AD development. By design, such features are not present in the component single-study outcomes. Conflicting outcomes between primary studies may be attributed to small sample sizes, hence, lack of power.<sup>[30]</sup>

### 4.2. Gene–gene, gene–environment interactions

In spite of the evidence for associations, complexity of AD development involves interactions between genetic and non-genetic factors allowing for the strong likelihood of environmental and behavioral involvement. Although all the included articles focused on *CNR1*, gene–gene and gene–environment interactions have been reported to influence associations of *CNR1* SNPs with development of AD. All 6 GAS studies addressed haplotypes, with 4<sup>[22,24,36,37]</sup> performing haplotype analysis.

### 4.3. Related meta-analysis

To our knowledge, this is the first meta-analysis to focus on the associations between *CNR1* SNPs and susceptibility to AD development. We have used the multi-SNP approach of GWAS (1 *CNR1* SNP + SNPs in other genes) and GAS (several SNPs in *CNR1*) in order to unmask associations of *CNR1* SNPs with risk of developing AD. A previous meta-analysis<sup>[17]</sup> examined addictive disorders that included AD, with detailed AD results only for rs1048353. Hence, we compare outcomes of the A allele

of Benyamina (2011) with ours in the codominant model of the overall and Caucasian analyses. Thus, both meta-analyses showed non-significant associations for developing AD in rs1048353 (ORs = 1.14–1.16,  $P = .14$ –.19 vs ORs = 1.13–1.14,  $P^a = .07$ –.09). However, in the broader scheme of substance dependence (AD included), the Benyamina et al<sup>[17]</sup> findings for rs806379 were null (ORs = 1.01–1.03,  $P = .40$ –.48) while ours were significant (ORs = 1.12–1.20,  $P^a = .02$ –.003). Moreover, AA outcomes were non-significant ( $P = .20$ ) 1.1-fold association while ours were null (OR = 1.02,  $P^a = .85$ ).

### 4.4. Complex phenotype of AD

An overriding issue in the genetics of AD development is recognizing that the AD phenotype is complex, involving other dependent-prone substances.<sup>[45]</sup> These include dependency on nicotine, marijuana (cannabis), and the hard drugs (cocaine, heroin) which involve various *CNR1* SNPs. Significant influence of rs1049353 with heroin among Caucasians indicated that the AA and GG genotypes conferred protection and contributed susceptibility, respectively.<sup>[46]</sup> In a polysubstance abuse study, minor allele frequency involving rs1535255 was significantly higher in cases.<sup>[24]</sup> A significantly statistical association of impulsivity was found in several polymorphisms, which included rs1049353, rs1535255, rs2023239.<sup>[47]</sup> A significant association was found between polysubstance abuse in rs1535255, rs2023239, and rs806379.<sup>[24]</sup> The association of rs2023239 with alcohol, cannabis, and tobacco use resulted in greater activation in the nucleus accumbens and ventral medial prefrontal cortex, as shown with neuroimaging.<sup>[48]</sup> In the development of AD, C allele carriers of rs2023239 were found to modulate the effect of olanzapine,<sup>[24]</sup> but these findings were not replicated in a later study.<sup>[37]</sup> In contrast with these lack of effects, our findings for rs2023239 showed a highly significant codominant post-outlier-derived association, underpinned by robustness. More studies may be needed to confirm this finding. A 3-marker haplotype involving rs806379 had significant allelic frequency differences between various sample populations<sup>[24]</sup> but was not replicated in subsequent studies.<sup>[10,22,37]</sup>

### 4.5. Strengths and limitations

Limitations of our study include: first, insufficient number of studies precluded investigation of *CNR2*; second, most of the component studies were underpowered; third, our conclusions were limited to Caucasians and AAs. More studies may be needed

to examine other ethnicities; and fourth, the studies that presented data on polysubstance abuse subtracted from the precision of the AD phenotype. The use of AD development as the sole phenotypic end point has been proposed to be a limitation.<sup>[49]</sup> Focusing on a narrow phenotype (AD) generated limitation fourth. Broadening the phenotype (e.g., polysubstance abuse) as a countermeasure would have altered our objectives. Having defined our objectives, we were constrained at countering limitations first to third. On the other hand, strengths of this study include: first, quality (CB scores) of component studies was high; second, restricted to HW-compliant studies reduced the risk for genotyping errors and possible selection bias; third, outlier treatment was key to generating significance and eliminating heterogeneity; fourth, applying HBC minimized the risk of a Type 1 error; fifth, power analysis outcomes strengthened the evidence for associations and minimized the risk for Type 2 error.<sup>[50]</sup>

In conclusion, this meta-analysis identified 3 *CNR1* SNPs that may increase the risk for developing AD. Caucasian susceptibility differed from the lack of associations in AAs. Additional well-designed studies exploring other parameters would confirm or modify our results and add to the extant knowledge about the association of the *CNR1* polymorphism and susceptibility to the development of AD.

## Author contributions

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