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ORIGINAL ARTICLE

The associations of *CNR1* SNPs and haplotypes with vulnerability and treatment response phenotypes in Han Chinese with major depressive disorder: A case–control association study

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Abstract

Background: Understanding how genetic polymorphisms are associated with the pathophysiology of major depressive disorder (MDD) may aid in diagnosis and the development of personalized treatment strategies. *CNR1* is the gene coding Cannabinoid type 1 receptor which is highly involved in emotional processing and in regulating neurotransmitter releases. We aimed to investigate the associations of *CNR1* single-nucleotide polymorphisms (SNPs) with MDD susceptibility and treatment response.

Methods: The study reported data on 181 Han Chinese with MDD and 80 healthy controls. The associations of *CNR1* genetic polymorphisms with MDD susceptibility and treatment response were examined, wherein the MDD patients were subgrouped further by responding to antidepressant treatment, compared with healthy controls separately.

Results: The *CNR1* SNPs rs806367 and rs6454674 and haplotype C-T-T-C of rs806366, rs806367, rs806368, and rs806370 were associated with increased susceptibility for MDD and antidepressant treatment resistance, but the association was not detected in other SNPs or the haplotype block of rs806368 and rs806370.

Conclusion: The *CNR1* is a promising candidate for the genetic association study of MDD. Larger and well-characterized samples are required to confirm the genetic association of *CNR1* with MDD because of the limitations such as relatively small sample size and lack of information for correcting confounding factors.

KEYWORDS

antidepressant treatment resistance, cannabinoid receptor, *CNR1*, major depressive disorder, single-nucleotide polymorphism

Chenghao Yang and Ilja M. Nolte share the first authorship.

1 | INTRODUCTION

Major depressive disorder (MDD) is a severe mental disorder with high prevalence and disease burden (WHO, 2017). Around 30% of patients do not respond to subsequent antidepressant treatments and are characterized as treatment-resistant depression (TRD; Dunner et al., 2006). TRD represents an important clinical challenge, and there is growing interest in the development of more precise personalized diagnosis and treatment to reach higher efficacy (Jentsch et al., 2015). Pharmacogenomics is the study investigating the role of the genome in drug response, which analyzes how the individual genetic composition affects the response to drug therapeutics (Drago et al., 2009). A single gene exerts its effect on drug response through the interaction between genetic, psychological, and environmental factors (Drago et al., 2009). Therefore, it is a promising strategy to predict the response to antidepressants by identifying single-nucleotide polymorphism (SNP) in the genes involved in antidepressant response (Reynolds, 2007). Furthermore, such SNPs could also aid in the diagnosis and treatment of MDD and specifically TRD.

Endocannabinoids have the ability to regulate different physiological processes involving in mood disorders, particularly including the activity of hypothalamic–pituitary–adrenal (HPA) axis and neuro-inflammatory cytokines release (Cota et al., 2007; Yu et al., 2010), which both are deranged in MDD (Dantzer et al., 2011; Pace et al., 2007). In line with the role of dysregulated inflammation in the development of depression and antidepressant treatment resistance (Carvalho et al., 2013), endocannabinoid system also is relevant to the pathophysiology and treatment resistance for MDD (Garcia-Gutierrez et al., 2010; Kolar & Kolar, 2016). Cannabinoid type 1 (CB1) receptor is a primary mediator of endocannabinoids in the central nervous system, and is highly expressed in the amygdala, hypothalamus, prefrontal cortex, hippocampus, and basal ganglia (Mackie, 2005). Furthermore, the CB1 receptor engages in regulating neuronal activity via affecting neurotransmitter release in relation to anxiety and depression, such as glutamate and g-aminobutyric acid (GABA; Marsicano & Lutz, 2006). The expression and function of CB1 receptors in the central nervous system suggest that it may play an important role in the pathophysiology of MDD. For example, Marsicano et al. indicated that both pharmacological antagonism and genetic inactivation of the CB1 receptor can undermine the extinction of conditioned fear memories (Marsicano et al., 2002), which could be contributable to the occurrence of depression. The potential mechanisms involved could be that CB1 receptor blocking impairs neurogenesis in the hippocampus and decreases the production of a brain-derived neurotrophic factor in the brain (Aso et al., 2008). Furthermore, a mice study showed that CB1 receptor activation by repeated CB1 agonist treatments significantly reduced depressive-like symptoms (Roeckel et al., 2018). In addition, a recent study showed that chronic treatment with rimonabant, a selective CB1 receptor antagonism, induced significant elevations in the concentrations of interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) in mice, which exhibited a depressive phenotype (Beyer et al., 2010).

The *CNR1* gene (OMIM accession number: 114610), coding the CB1 receptor, has been related to MDD. For example, SNP rs1049353 of *CNR1* was associated with abnormal thalamic and striatal activity responding to emotional faces as potent environmental depression-related cues in a small study of 19 healthy probands (Chakrabarti et al., 2006). Furthermore, *CNR1* rs1049353 A allele was shown to increase the likelihood for depression in 1269 Caucasians from the UK (Juhasz et al., 2009), while its G allele increased the risk of antidepressant treatment resistance in a study of 256 Caucasian patients with MDD (Domschke et al., 2008). In contrast, a longitudinal study demonstrated that the *CNR1* rs1049353 GG genotype was associated with a better response to citalopram treatment in a relatively small male subgroup of depressive patients, but this effect was not observed in females (Mitjans et al., 2012). These findings suggest a possible involvement of the *CNR1* gene in the pathophysiology of MDD, which might be a promising genetic predictor for diagnosis of MDD and treatment response to antidepressants, although results were not unequivocal.

Very large genome-wide association studies (GWAS) have only recently begun to uncover genetic loci associated with MDD, and overall this was clearly less than originally expected (Cai et al., 2017; Flint & Kendler, 2014; Nagel et al., 2018; Van der Auwera et al., 2018). One of the factors complicating the search for the genetic underpinnings of MDD is the fact that the current diagnostic classification refers to a relatively heterogeneous group of patients in terms of symptomatology and treatment response (WHO, 2018), as well as in underlying disease mechanisms such as inflammatory dysregulation (Dantzer et al., 2011). Furthermore, study samples may be heterogeneous also in terms of cultural origin (Lin, 2001; Xu et al., 2013). The CONVERGE study detected two loci for MDD in a homogeneous population of Han Chinese through stringent criteria and deep phenotyping, with only a 10th of the estimated sample size (Cai et al., 2015), which suggested the homogeneity of the population would be critical to identify genetic effects for MDD. Following this line of thought, we focused on a similarly homogeneous group of Han Chinese, and within the MDD spectrum, we were specifically interested in the subgroup of depressive patients with antidepressant treatment resistance and increased inflammatory activity (refer this subgroup of patients to TRDI patients in the following text).

The aim of this study was to explore the impact of *CNR1* genetic polymorphisms, including allele, genotype, and haplotype distributions, on MDD susceptibility and treatment response phenotypes by comparing this subgroup of patients with non-therapy-resistant Han Chinese MDD patients (refer the major depressive patients with no treatment resistance as MDNTR in the following text) and healthy controls. As there is a range of the different alleles and possible combinations of genotypes and haplotypes, this is mainly an explorative investigation. But overall we expect that *CNR1* genetic polymorphisms (specific alleles, genotypes, or haplotypes) are associated with increased likelihood of developing MDD, and within depressed patients with a higher likelihood of antidepressant treatment resistance.

2 | EXPERIMENTAL PROCEDURES

2.1 | Sample

This study recruited three groups of participants, including TRDI patients, MDNTR patients, and healthy controls. The TRDI patients (n = 81) were recruited from the inpatient and outpatient departments of Tianjin Anding Hospital during September 2015 and October 2018, who participated in the clinical study registered on "ClinicalTrials.gov" with protocol ID "NAC-2015-TJAH" and ClinicalTrials.gov ID "NCT02972398." Inclusion criteria were: a current episode of MDD diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV-TR) diagnosed with Structured Clinical Interview for DSM-IV (SCID); age between 18 and 65 years; a total score of 17 items Hamilton Depression Rating Scale (HAMD-17) ≥17; a CRP level between 0.85 and 10 mg/L; insufficient response to one or more antidepressants given for at least 6 weeks and in an adequate dose during the current episode. More detailed information on study procedures has been described elsewhere (Yang et al., 2018).

The data of MDNTR patients (n = 100) came from inpatient and outpatient departments of Tianjin Anding Hospital and Tianjin General Hospital by our team members during November 2009 and July 2010. The inclusion criteria for MDNTR patients, in brief, were: age between 18 and 60 years, diagnosed MDD with DSM-IV, first episode or recurrent; no resistance to anti-depressant treatments, that is, defined the current episode as a relapse from the efficacious anti-depressant treatment because of drug withdrawal for first-episode patients or a recurrence with a history of effective antidepressant treatments for recurrent patients; no history of manic or hypomanic episodes; total score of HAMD-17 ≥ 17. Patients were excluded if the current depressive disorder was not idiopathic but secondary to other conditions, like substance abuse, medical diseases et al; current or historic episode of any mental disorder regardless of depressive disorders; women in menstruation, pregnancy or lactation period. The healthy controls (n = 80) were enrolled through recruitment advertisement during November 2009 and July 2010, who were matched with MDNTR patients in age and sex and were not allowed to have a history or family history of any mental disorders, interviewed with SCID.

2.2 | Genotyping and quality control

Genomic DNA was extracted from 5 ml of venous blood sample using the high-salt method, which was stored and processed at the Tianjin Anding Hospital or the Molecular or Population Genetic Center of Tianjin Medical University. For MDNTR patients and healthy controls, their samples had been storing at minus 80°C, which were unfreezed in a 4°C refrigerator before genotyping. Genotyping (in all samples) was performed by matrix-assisted laser desorption time-of-flight mass spectrometry to detect the primer extension of multiple products. Ten percentage of samples were used for re-genotyping randomly aiming for quality control, with a 100% concordance rate. Genotype calling was performed blinded to the participants' clinical data.

The quality of the SNPs was checked by determining the call rate and the Hardy–Weinberg equilibrium (HWE) *p*-value. SNPs were excluded if the call rate was <90% or the HWE *p*-value among the healthy controls was $<.05/8 = 6.3 \times 10^{-3}$.

2.3 | Candidate SNPs selection

Eight SNPs of the *CNR1* gene (NC_000006.11) were prioritized with locations, putative or known functions, based on earlier reports on their associations with clinical phenotypes as well as data from NCBI dbSNP. In addition, these SNPs occupied relatively high heterozygosity in the Han Chinese population (MAF: 0.15–0.26). These SNPs included rs806366 (chr6:88137870, MAF 0.42), rs806367 (chr6:88138697, MAF 0.43), rs806368 (chr6:88140381, MAF 0.49), rs806369 (chr6:88146459, MAF 0.44), rs806370 (chr6:88146612, MAF 0.49), rs806380 (chr6:88154934, MAF 0.19), rs6454674 (chr6:88163211, MAF 0.26), and rs2180619 (chr6:88168233, MAF 0.20).

2.4 | Statistical methods

The allele and genotype frequency, call rate, Hardy–Weinberg equilibrium (HWE), and odds ratio (ORs) were evaluated using PLINK v1.9. The chi-square test was used

to compare the genotype frequency between cases (TRDI patient and MDNTR patient groups) versus healthy controls, TRDI versus healthy controls, MDNTR versus healthy controls, and also stratified patient groups by treatment response phenotype (TRD vs. MDNTR). Analyses correcting for age and sex were performed using logistic regression with covariates. To define haplotype blocks, PLINK v1.9 was used to determine linkage disequilibrium between markers within 1Mb. For each chromosomal region, haplotype blocks were next constructed using thresholds of different LD values (strong LD, $r^2 > .8$; at least moderate LD, $r^2 > .1$). Haplotype frequencies within each haplotype block were then determined for cases and controls separately and compared using a permutation test as implemented in PHASE 2.1.1 (Stephens et al., 2001). In this permutation test, case–control status was permuted over the individuals 10,000 times and the *p*-value was determined as the proportion of tests from the permuted data with a *p*-value smaller than that when using the original case and control datasets.

To avoid false-positive findings upon the multiple testing, a multiple testing correction was applied. Spectral decomposition of the genotype data was used to determine the number of independent tests (Galwey, 2009). The significance threshold in this study was $0.05/(4 \times 6) = 0.0021$.

The power analysis was performed using "Genetic Power Calculator" online (http://zzz.bwh.harvard.edu/gpc/cc2.html).

3 | RESULTS

3.1 | Sociodemographic and clinical characteristics

In total, 261 Han Chinese participants were included, including a TRDI group (n = 81), an MDNTR group (n = 100), and healthy controls (n = 80). Three groups had significant differences in the distribution of age and sex. The detailed data are shown in Table 1.

3.2 | Individual SNP association in case–control analysis

The SNP rs806369 was out of HWE in healthy controls ($p = .0012$) and therefore not included in further analyses. The frequencies of alleles and genotypes of the *CNR1* SNPs are shown in Table 2. When comparing the allele frequency between MDD cases and healthy controls, we found that the T allele of rs806367 was more common in cases than healthy controls [$p = .00034$, odds ratio (OR; 95% confidence interval [CI]) = 4.52 (1.76, 11.61)]. This significance is still retained after adjustments for sex and age ($p = .0020$) and multiple testing correction. The rs6454674 G allele was also more frequent in cases than in healthy controls ($p = .0054$, OR [95% CI] = 1.90 [1.21, 3.00]), but this significance did not survive multiple testing correction. When comparing the allele frequency between TRDI patients and healthy controls, the T allele of rs806367 was more common in TRDI patients than in healthy controls ($p = 1.9e\text{-}006$, OR [95% CI] = 7.90 [2.99, 20.87]), and remained significant after adjustment for sex and age ($p = 4.0e\text{-}005$) and multiple testing correction. There were no significant differences between MDNTR patients and healthy controls ($p = .13$ for rs806367; $p = .051$ for rs6454674). None of the comparisons in genotype frequency of *CNR1* SNPs between cases/TRDI patients/MDNTR patients and healthy controls revealed any significant differences.

3.3 | Individual SNP association in treatment response analysis

We performed the comparison between TRDI patients and MDNTR patients to study the relation of *CNR1* SNPs

TABLE 1 Sociodemographic and clinical characteristics of patients

Characteristic	Number	Mean age (SD)	Sex (male, %)	Chi-square (sex)	*p* value	
					Age	Sex
TRDI group	81	46.0 (12.7)	47 (58.0)	23.798	.062[a]	<.001
MDNTR group	100	42.8 (10.2)	24 (24.0)		.003[b]	
HC	80	40.5 (11.6)	25 (31.3)		.183[c]	

Abbreviations: HC, healthy control; MDNTR, major depressive patient with no treatment resistance; SD, standard deviation; TRDI, treatment-resistant depression with increased inflammatory activity.

[a]Compared between TRDI and MDNTR.

[b]Compared between TRDI and HC.

[c]Compared between MDNTR and HC; chi-square and *p* value for sex are compared among three groups.

TABLE 2 Associations of *CNR1* alleles and genotypes between MDD cases and controls

SNP	Genotypes	Genotype (subject size)		Allele frequency (%)		Chi-square	OR (95%CI)	*p* value/ *p*-adjusted
		Cases (n = 181)	HC (n = 80)	Cases	HC			
rs806366	CC/CT/TT	28/86/67	10/36/34	C 39.4	34.9	0.91	1.21 (0.82, 1.80)	.37/.22
rs806367	TT/TC/CC	3/41/137	0/5/75	T 12.9	3.2	11.52	4.52 (1.76, 11.61)	**.00034/.0020**
rs806368	TT/TC/CC	41/90/50	17/40/23	T 47.8	45.6	0.20	1.09 (0.75, 1.58)	.70/.45
rs806370	CC/CT/TT	39/90/52	16/39/25	C 46.3	44.1	0.21	1.09 (0.75, 1.60)	.52/.48
rs806380	GG/GA/AA	5/49/127	1/19/60	G 16.2	13.6	0.10	0.96 (0.60, 1.53)	.77/.66
rs6454674	GG/GT/TT	19/80/82	3/26/51	G 32.8	20.4	7.90	1.90 (1.21, 2.99)	.0054/.0068
rs2180619	GG/GA/AA	9/61/111	2/20/58	G 21.6	15.0	3.08	1.56 (0.95, 2.58)	.093/.059
		MDNTR (n = 100)	**HC (n = 80)**	**MDNTR**	**HC**			
rs806366	CC/CT/TT	19/49/32	10/36/34	C 43.2	34.9	2.48	1.42 (0.92, 2.20)	.12/.090
rs806367	TT/TC/CC	0/13/87	0/5/75	T 6.7	3.2	2.25	2.20 (0.77, 6.30)	.13/.19
rs806368	TT/TC/CC	24/50/26	17/40/23	T 49.0	45.6	0.41	1.15 (0.75, 1.74)	.52/.50
rs806370	CC/CT/TT	23/50/27	16/39/25	C 48.5	43.6	0.66	1.19 (0.78, 1.83)	.42/.37
rs806380	GG/GA/AA	2/26/71	1/19/60	G 15.5	13.6	0.58	1.23 (0.80, 2.13)	.63/.55
rs6454674	GG/GT/TT	9/42/49	3/26/51	G 29.6	20.4	3.80	1.64 (1.00, 2.70)	.051/.055
rs2180619	GG/GA/AA	5/35/60	2/20/58	G 22.5	15.0	3.23	1.65 (0.95, 2.84)	.072/.076
		TRDI (n = 81)	**HC (n = 80)**	**TRDI**	**HC**			
rs806366	CC/CT/TT	10/37/34	10/36/34	C 34.6	34.9	0.0022	0.99 (0.62, 1.58)	.96/.97
rs806367	TT/TC/CC	3/27/51	0/5/75	T 20.5	3.20	22.73	7.90 (2.99, 20.87)	**1.86e-006/4.02e-005**
rs806368	TT/TC/CC	17/40/24	17/40/23	T 46.15	45.6	0.0089	1.02 (0.66, 1.59)	.92/.57
rs806370	CC/CT/TT	15/40/26	16/39/25	C 43.6	44.1	0.0075	0.98 (0.63, 1.54)	.93/.82
rs806380	GG/GA/AA	2/23/56	1/19/60	G 17.1	13.6	0.39	1.13 (0.74, 1.65)	.40/.34
rs6454674	GG/GT/TT	11/38/32	3/26/51	G 36.7	20.4	10.07	2.26 (1.36, 3.77)	**.0015/.0020**
rs2180619	GG/GA/AA	3/27/51	2/20/58	G 20.5	15.0	1.65	1.46 (0.82, 2.62)	.20/.18

CNR1 GenBank version number: NC_000006.11. All significances are shown in bold.

Abbreviations: CI, confidence interval; HC, healthy controls; MDD, major depressive disorder; MDNTR, major depressive patient with no treatment resistance; OR, odds ratio; *p*-adjusted, the *p* value after adjusting for sex and age; SNP, single-nucleotide polymorphism; TRDI, treatment-resistant depression with increased inflammatory activity.

with treatment response. We found that the T allele of rs806367 was significantly more frequent in TRDI patients than in MDNTR patients (p = 1.7e-04, OR [95% CI] = 3.60 [1.81, 7.12]), even after adjustment for sex and age (p = .000126) and multiple testing correction. No significant differences were found in the comparison of genotype frequencies. See Table 3 for detailed information.

3.4 | Haplotype association in case–control analysis

Three haplotype blocks were formed based on LD analysis: one including SNPs rs806368 and rs806370, which were in high LD with $r^2 > .8$, one including SNPs rs806366, rs806367, rs806368, and rs806370, which were

TABLE 3 Associations of *CNR1* alleles and genotypes between TRDI and MDNTR patients in relation to treatment response

SNP	Genotypes	Genotype (subject size)		Allele frequency (%)		Chi-square	OR (95% CI)	*p* value/*p*-adjusted
		TRDI (n = 81)	MDNTR (n = 100)	TRDI	MDNTR			
rs806366	CC/CT/TT	10/37/34	19/49/32	C 34.6	43.2	2.68	0.70 (0.45, 1.08)	.12/.22
rs806367	TT/TC/CC	3/27/51	0/13/87	T 20.5	6.7	14.7	3.59 (1.81, 7.12)	**.00017/.00013**
rs806368	TT/TC/CC	17/40/24	24/50/26	T 46.2	49.0	0.29	0.89 (0.59, 1.36)	.67/.83
rs806370	CC/CT/TT	15/40/26	23/50/27	C 43.6	48.5	0.82	0.82 (0.54, 1.26)	.39/.70
rs806380	GG/GA/AA	2/23/562/26/71		G 17.115.5		1.02	1.22 (0.90, 1.54)	.45/.49
rs6454674	GG/GT/TT	11/38/32	9/42/49	G 36.7	29.6	2.01	1.38 (0.88, 2.16)	.17/.41
rs2180619	GG/GA/AA	3/27/51	5/35/60	G 20.5	22.5	0.20	0.89 (0.53, 1.48)	.70/.87

Note: CNR1 GenBank version number: NC_000006.11. All significances are shown in bold.

Abbreviations: CI, confidence interval; MDNTR, major depressive patient with no treatment resistance; OR, odds ratio; *p*-adjusted, the *p* value after adjusting for sex and age; SNP, single-nucleotide polymorphism; TRDI, treatment-resistant depression with increased inflammatory activity.

in at least moderate LD with each other with a lenient r^2 threshold of 0.1, and one including SNPs rs806380 and rs6454674 that were in a moderate LD with each other ($r^2 > 0.1$). We tested the association of haplotype frequency distribution with susceptibility and treatment response.

When comparing the haplotype frequency between MDD cases/TRDI patients and healthy controls, we found differences in the two-SNP haplotype block with moderated LD ($p = .032/.027$, respectively) and the four-SNP haplotype block with at least moderate LD ($p = .027/.0001$, respectively), of which only the latter survived multiple testing correction. No significant differences were observed between MDNTR patients and healthy controls. When further examining the details of two and four-marker haplotype combinations, the C-T-T-C haplotype of rs806366, rs806367, rs806368, and rs806370, were more common in cases and TRDI patients than in healthy controls ($p = .03$, OR = 4.27, $p = .002$, OR = 6.94), while C-C-T-C haplotype was less common in TRDI patients ($p = .01$, OR = 0.38). Of these haplotypes, only the C-T-T-C in the TRDI-control comparison was still significant after multiple testing correction ($p = .002$). No significant differences were found for the haplotype block with high LD (all $p > .7$). See Table 4 for detailed information.

3.5 | Haplotype association in treatment response analysis

The association of *CNR1* SNPs with treatment response was evaluated by comparing TRDI patients and MDNTR patients. We found a significant difference in haplotype frequency distribution for the four-SNP haplotype block with at least moderate LD ($p = .002$), which was still significant after correcting for multiple testing. When analyzing the specific haplotype combinations, C-C-T-C of rs806366, rs806367, rs806368, and rs806370 was more common in MDNTR patients than in TRDI patients ($p = .003$, OR = 0.33), and C-T-T-C of rs806366, rs806367, rs806368, and rs806370, was less frequent in MDNTR patients than in TRDI patients ($p = .02$, OR = 3.11). However, both significances did not survive multiple testing correction. No significant differences were observed for specific haplotypes consisting of two SNPs in high LD or in moderate LD. See Table 5 for detailed information.

4 | DISCUSSION

In the present study, we explored the distributions of *CNR1* alleles, genotypes, and haplotypes in cases and healthy controls in relation to MDD susceptibility and

TABLE 4 Associations of *CNR1* haplotypes between MDD cases and healthy controls

Haplotype combination	HC	Cases			MDNTR			TRDI		
	Frequency %	Frequency %	OR	*p* value	Frequency %	OR	*p* value	Frequency %	OR	*p* value
C-C[a]	1.30	1.20	0.93	.95	1.60	1.21	.88	0.70	0.55	.71
C-T[a]	53.1	51.0	0.92	.76	49.4	0.86	.63	52.9	0.99	.98
T-C[a]	43.5	45.4	1.08	.78	46.9	1.14	.66	43.6	1.00	1.00
T-T[a]	2.10	2.40	1.14	.89	2.10	1.02	.99	2.80	1.38	.74
Other[a]	0.02	0.02	1.03	1.00	0.01	0.80	.98	0.04	2.30	.93
C-C-T-C[b]	31.7	26.4	0.77	.38	35.7	1.18	.60	15.0	0.38	**.01**
C-T-T-C[b]	3.30	12.6	4.27	**.03**	6.80	2.20	.29	19.5	6.94	**.002**
T-C-C-C[b]	1.20	1.00	0.85	.90	1.50	1.16	.91	0.70	0.56	.71
T-C-C-T[b]	52.9	50.1	0.89	.67	48.4	0.83	.54	51.7	0.95	.87
T-C-T-C[b]	8.60	6.20	0.70	.48	4.50	0.51	.29	8.20	0.93	.89
T-C-T-T[b]	1.90	2.10	1.11	.92	2.00	0.99	.99	2.80	1.37	.75
Other[b]	0.38	1.61	4.32	.45	1.11	0.60	.60	2.10	5.64	.38
A-G[c]	10.1	18.8	2.05	.08	16.1	1.76	.22	22.3	2.53	**.03**
A-T[c]	76.6	64.8	0.56	.06	68.2	0.64	.20	60.5	0.47	**.02**
G-G[c]	10.6	13.7	1.34	.49	13.3	1.27	.60	14.0	1.37	.49
G-T[c]	2.70	2.70	1.01	.99	2.50	0.97	.98	3.20	1.23	.81
Other[c]	0.01	0.01	0.46	.96	0.00	0.41	.96	0.02	1.98	.96

Note: CNR1 GenBank version number: NC_000006.11. All significances are shown in bold.

Abbreviations: HC, healthy control; MDNTR, major depressive patient with no treatment resistance; OR, odds ratio; OR/*p* value, compared to the healthy control, respectively; TRDI, treatment-resistant depression with increased inflammatory activity.

[a]Haplotype block of two loci with strong LD ($r^2 > 0.8$): rs806368–rs806370.

[b]Haplotype block of four loci that were in at least moderate LD ($r^2 > 0.1$): rs806366–rs806367–rs806368–rs806370.

[c]Haplotype block of two loci that were in moderate LD ($r^2 > 0.1$): rs806380–rs6454674.

treatment response. We hypothesized that *CNR1* genetic polymorphisms are associated with an increased likelihood of developing MDD, and within depressed patients with a higher likelihood of antidepressant treatment resistance. The results suggested a potential role of the *CNR1* rs806367 polymorphism in TRD susceptibility; the SNP rs6454674 polymorphism was also involved in the MDD susceptibility when the TRDI patients were considered particularly. The haplotype block of rs806368 and rs806370, with a high LD, was not involved in the MDD susceptibility or antidepressant treatment resistance, but the haplotype block of rs806366, rs806367, rs806368, and rs806370 was associated with MDD susceptibility and antidepressant treatment resistance. Haplotype C-T-T-C appeared to be a risk factor for MDD susceptibility when the TRDI patients were considered.

The CB1 receptors are very highly expressed in the brain subareas involved in motivated behavior and emotional processing (Herkenham et al., 1990). It is well-established that endocannabinoids-CB1 receptor signaling is involved in the regulation of neurotransmitter release (Hashimotodani et al., 2007). For example, CB1 receptors are present on serotonergic (Hermann et al., 2002), glutamatergic (Katona et al., 2006), noradrenergic (Oropeza et al., 2007), and GABAergic (Katona et al., 1999) axon terminals in the brain, which are known to play a role in emotional processing. Activation of the CB1 receptor could generate inhibition of transmitter release (Freund et al., 2003). The engagement of the CB1 receptor in the pathophysiology of depression had been explored earlier. For instance, animal studies illustrated that the CB1 receptor agonist exerted antidepressant effects in the forced-swimming test (Shearman et al., 2003), while rimonabant can predispose rats to depression-like behaviors by blocking CB1 receptors (Elbatsh et al., 2012). Moreover, depressive mood disorders caused by rimonabant treatment were 2.5 times higher than treatment with placebo (Christensen et al., 2007). Consistently, studies also proved the role of *CNR1* gene variants in depression vulnerability and antidepressant treatment resistance. For example, a clinical study found that male carriers with the GG genotype of rs1049353 showed a better long-term anti-depressant response to citalopram treatment (Mitjans et al., 2013), while one another study demonstrated that *CNR1* rs1049353 G allele increased the likelihood of antidepressant treatment resistance, particularly in female patients with anxious symptoms (Domschke et al., 2008). Both studies focused on Caucasians with MDD, but the former study

TABLE 5 Associations of *CNR1* haplotypes between TRDI and MDNTR patients in relation to treatment response

Haplotype combination	MDNTR frequency %	TRDI frequency %	OR	*p* value
C-C[a]	1.60	0.70	0.44	.61
C-T[a]	49.4	52.9	1.15	.64
T-C[a]	46.9	43.6	0.88	.66
T-T[a]	2.10	2.80	1.28	.80
Other[a]	0.01	0.04	2.89	.91
C-C-T-C[b]	35.7	15.0	0.33	**.003**
C-T-T-C[b]	6.80	19.5	3.11	**.02**
T-C-C-C[b]	1.50	0.70	0.20	.49
T-C-C-T[b]	48.4	51.7	1.14	.65
T-C-T-C[b]	4.50	8.20	1.88	.32
T-C-T-T[b]	2.00	2.80	1.62	.64
Other[b]	1.11	2.10	1.91	.60
A-G[c]	16.1	22.3	1.48	.30
A-T[c]	68.2	60.5	0.71	.28
G-G[c]	13.3	14.0	1.06	.89
G-T[c]	2.50	3.20	1.36	.72
Other[c]	0.00	0.02	4.88	.92

Note: CNR1 GenBank version number: NC_000006.11. The significance was in bold.

Abbreviations: HC, healthy control; MDNTR, major depressive patient with no treatment resistance; OR, odds ratio; TRDI, treatment-resistant depression with increased inflammatory activity.

[a]Haplotype block of two loci with strong LD ($r^2 > 0.8$): rs806368–rs806370.

[b]Haplotype block of four loci that were in at least moderate LD ($r^2 > 0.1$): rs806366–rs806367–rs806368–rs806370.

[c]Haplotype block of two loci that were in moderate LD ($r^2 > 0.1$): rs806380–rs6454674.

recruited participants with younger age (39.5 ± 12.19 vs. 50.4 ± 14.9), with different antidepressants (citalopram vs. more than six antidepressants, including citalopram), which could explain the contradictory results in part. Furthermore, among MDD patients TT homozygotes of rs806368, which forms a haplotype with rs1049353, were at increased risk of no remission to citalopram treatment, compared to C allele carriers (Mitjans et al., 2012). In our study, rs806368 was involved in neither MDD susceptibility nor antidepressant treatment resistance. One other study compared SNPs rs6454674 and rs806368 in relation to suicide attempters and found that both SNPs were not associated with suicidality (Murphy et al., 2011). We found that rs6454674 contributed to MDD vulnerability, but not to treatment resistance to antidepressants when the TRDI patients were considered specifically. However, our study might lack the necessary power to exclude the absence of association for these SNPs (all power <1).

To our best knowledge, this is the first time that the role of rs806367 is reported in the pathophysiology of MDD. T allele carriers presented higher risk for developing TRD. With respect to the haplotype of rs806380 and rs6454674 or haplotype of rs806368 and rs806370, there were no associations with MDD vulnerability or antidepressant treatment resistance, but the haplotype of rs806366, rs806367, rs806368, and rs806370 was significantly associated with these two phenotypes, even after correcting for multiple testing.

Genetic association studies into MDD, even genome-wide association meta-analyses with huge sample sizes, have attained far less successful results than expected (Cai et al., 2015; Mullins et al., 2019; Nagel et al., 2018; Van der Auwera et al., 2018). This is likely due to the complexity of the genetic architecture of MDD as well as the heterogeneity of depression (Cai et al., 2017; Flint & Kendler, 2014). The CONVERGE study in 11670 Han Chinese used stringent procedures to minimize misdiagnosis and biases in self-reporting and phenotyping and found two genetic associations to MDD using only one-tenth of the originally estimated sample size (Cai et al., 2017), indicating that homogeneous samples could substantially increase the power to detect genetic effects. In line with this, our findings highlight the biological heterogeneity underlying the pathophysiology of MDD in some way. Significant results were only detected in TRD patients with increased inflammatory activity for the rs6454674 polymorphism and the haplotype C-T-T-C of rs806366, rs806367, rs806368, and rs806370 in relation to the susceptibility of MDD, but not in MDNTR patients, which suggests that the higher homogeneity of TRDI patients in pathophysiology might be contributable to the positive findings.

In spite of the significant findings, the current study clearly has limitations. First, the sample size is relatively small. In the absence of a detailed understanding of genetic architecture, the sample size is one of the most important determinants for discovering reliable genetic associations (Ripke et al., 2013). Nevertheless, post hoc power analysis showed that we had sufficient power (>0.9) for detecting the effect of rs806367. Second, we estimated treatment resistance based on patients' routine treatment but not specifying any particular antidepressant drugs. Third, the data came from two separate studies lacking detailed information for adjusting findings, particularly regarding negative life events, which was an important covariate adjusting the association between *CNR1* genotype and depression in an earlier study (Juhasz et al., 2009). Fourth, we did not measure the blood levels of inflammatory markers in healthy controls or MDNTR patients, so there could still be heterogeneity in these groups regarding inflammatory dysregulation. This is clearly a limitation of our study. However, the increased levels of inflammatory markers in the TRDI group indicate a potentially relevant subtype. Current diagnostic tools in psychiatry are based on the clusters of symptoms and characteristics of clinical course rather than defining it by pathophysiological processes underlying the disease (Philip et al., 2017; WHO, 2018). We would argue that a stricter phenotype definition could increase power to detect more robust genetic effects as well as advance the reliability of findings (van der Sluis et al., 2010). Finally, three groups had significant differences in the distribution of age and sex, which could contribute to the genetic association detected in the study (Faravelli et al., 2013), although the differences were adjusted in data analysis.

In conclusion, *CNR1* SNPs and haplotypes were associated with an increased risk for developing MDD, and within depressed patients also for antidepressant treatment resistance. Larger and better-characterized samples are warranted to confirm this association, which eventually could aid in understanding the pathogenesis of MDD and developing novel pharmacological options for antidepressant treatment.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The protocols of two studies were approved by the medical ethics committee of Tianjin Anding Hospital and conformed to "Declaration of Helsinki." All participants had signed informed consent about the content and extent of the planned study prior to the participation. The patients' guardians signed the informed consent on behalf of the participants when the capacity of participants to consent was compromised.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Each author's individual contributions had been listed as following: Chenghao Yang and Jie Li contributed to the conceptualization of this study; Fokko Bosker and Jie Li contributed to the planning; Yanyan Ma, Xuguang AN, and Chenghao Yang conducted the implementation of experiments; Ilja Nolte worked out the data analysis; Chenghao Yang, Ilja Nolte, Fokko Bosker, and Jie Li contributed to the manuscript preparation.

DATA AVAILABILITY STATEMENT

We have finished the submission of original data to ClinVar (submission number: SUB6670287).

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