Further Evidence of an Association between *NCAN* rs1064395 and Bipolar Disorder

Lu Wang^a Weiqing Liu^b Xingxing Li^c Xiao Xiao^a Lingyi Li^a Fang Liu^b
Yuanfang He^b Yan Bai^b Hong Chang^a Dong-Sheng Zhou^c Ming Li^a

^aKey Laboratory of Animal Models and Human Disease Mechanisms of the Chinese Academy of Sciences and Yunnan Province, Kunming Institute of Zoology, Kunming, PR China; ^bDepartment of Psychiatry, The First Affiliated Hospital of Kunming Medical University, Kunming, PR China; ^cDepartment of Psychiatry, Ningbo Kangning Hospital, Ningbo, PR China

Keywords

NCAN · Genome-wide association studies · Bipolar disorder · Major mood disorders · Meta-analysis

Abstract

Genome-wide association studies suggest that rs1064395 in the neurocan gene (*NCAN*) is a potential risk factor for bipolar disorder (BPD), and further replication analyses in larger independent samples are needed. We herein analyzed rs1064395 in a Han Chinese sample of 1,146 BPD cases and 2,031 controls, followed by a meta-analysis of BPD samples from worldwide populations including a total of 15,318 cases and 91,990 controls. The meta-analysis found that rs1064395 showed a genome-wide significant association with BPD ($p = 4.92 \times 10^{-9}$, OR = 1.126 for the A allele), although it did not reach the significance level in the Han Chinese sample ($p = 0.415$, OR = 1.070 for the A allele). We also examined the association between the single nucleotide polymorphisms and major depressive disorder (MDD)

given the presumed genetic overlap between BPD and MDD, and rs1064395 was also associated with MDD ($p = 0.0068$, OR = 1.067 for the A allele) in a meta-analysis of 14,543 cases and 14,856 controls. Our data provide further evidence for the involvement of *NCAN* in the genetic susceptibility to BPD and also implicate its broader role in major mood disorders.

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Introduction

Bipolar disorder (BPD) is a severe mental illness with a lifetime prevalence of up to 1.6% [1, 2]. Previous studies showed that the child of an affected parent has about a ten-fold increased risk of developing BPD, and twin studies of this illness has revealed an estimated heritability of 0.7–0.8 [3, 4]. Interestingly, previous analyses have found increased morbidity of major depressive disorder (MDD) within family members of a proband with BPD [5]. This

L. Wang and W. Liu contributed equally to this work.

Dong-Sheng Zhou, MD
Department of Psychiatry, Ningbo Kangning Hospital
1 Zhuang-yu Nanlu
Ningbo, Zhejiang 315201 (PR China)
E-Mail wyzhouds@sina.com

Ming Li, PhD
Kunming Institute of Zoology, Chinese Academy of Sciences
32 Jiao-chang Donglu
Kunming, Yunnan 650223 (PR China)
E-Mail limingkiz@mail.kiz.ac.cn

leads to the idea that BPD and MDD may have common genetic risk features. In fact, clinical, epidemiological, and genetic studies have revealed similar phenotypic characteristics between BPD and MDD [3, 6], and there are also overlapping risk genes, such as *CACNA1C* [7], *CREB1* [8], and *AS3MT* [9–11]. It is thus proposed that genetic analyses of these two illnesses could uncover important genes underlying the symptoms observed in both diseases.

To uncover the BPD susceptibility genes, several genome-wide association studies (GWAS) have been conducted in multiple case-control samples, and a number of such genes (*CACNA1C*, *ANKK1*, *TRANK1*, etc.) have been reported [12–22]. Among those genes, the neurocan gene (*NCAN*) (encodes neurocan, containing 15 exons and spanning 40.3 kb in the genome, and locates in 19p13.11) was one of the first genes showing genome-wide significant associations with BPD and caught wide attention from the community [13]. The single nucleotide polymorphism (SNP) rs1064395 in *NCAN* was found to be genome-wide significantly associated with BPD in a GWAS plus replication analysis in a total of 8,441 cases and 35,362 controls by Cichon et al. [13] ($p = 2.14 \times 10^{-9}$). However, this SNP did not reach genome-wide significance in other BPD GWAS [15, 17–19], which may be the result of genetic background heterogeneity among different samples. However, a potential impact of rs1064395 on the risk of BPD was supported by its associations with brain function, cognitive performance, and risk of other psychiatric disorder (i.e., schizophrenia) [23–27]. In addition, a previous study found that the rs1064395 risk allele was significantly associated with the “mania” factor, which was confirmed by the discovery that compared to wild-type mice, the homozygous *Ncan* knockout mice were more hyperactive and exhibited more frequent risk-taking and repetitive behaviors, less depression-like conduct, plus impaired prepulse inhibition, amphetamine hypersensitivity, as well as increased saccharin preference [28]. All these lines of evidence support the link between *NCAN* and BPD susceptibility.

In the present study, to further investigate whether *NCAN* confers risk of BPD, we conducted a replication analysis of rs1064395 in a Han Chinese sample of 1,146 BPD cases and 2,031 controls. We then collected the published data of 15,318 cases and 91,990 controls worldwide and conducted a meta-analysis to further verify the replication results. In addition, considering the substantial genetic overlap between BPD and MDD [6, 29], we also examined rs1064395 in two MDD GWAS samples.

Materials and Methods

Han Chinese BPD Case-Control Sample

A total of 1,146 BPD cases and 2,031 controls of Han Chinese origin were included in the present study. We collected BPD patients from seven mental health centers and psychiatric departments of general medical hospitals in five provinces from the Southern China area. Each patient was independently interviewed and diagnosed as having BPD by a consensus of at least two experienced psychiatrists. Diagnoses were further confirmed with an Extensive Clinical Interview and a Structured Clinical Interview for DSM-IV Axis/Disorders, Patient Version given by a research psychiatrist. Cases were excluded if they had a preexisting history of other psychiatric disorders, mental retardation, or drug/alcohol addiction. Mentally healthy controls of Han Chinese origin were also collected from Southern China. Subjects with any history of major psychiatric or neurological disorders, drug abuse, or any family history of severe forms of psychiatric disorders were excluded. All participants provided written informed consent before any study-related procedures were performed.

SNP Genotyping and Statistical Analysis

Genomic DNA was isolated from blood samples using the standard phenol-chloroform. Each sample was genotyped on a custom array using the Sequenom MassARRAY system according to the manufacturer's instructions. MassARRAY TYPER 4.0 was used to analyze the mass spectrograms and to generate the final calling of genotypes.

Allele-specific logistic p values, ORs, and 95% CIs were calculated with PLINK assuming an additive genetic model [30]. In addition to our Chinese sample, we also collected the statistical data (OR and p value) of rs1064395 from two independent Japanese samples (Japan 01: 1,545 cases and 7,408 controls; Japan 02: 1,419 cases and 54,479 controls) [20] and six European datasets (MooDS GWAS, PGC1 GWAS, Iceland, France, Bosnia and Herzegovina/Serbia, and Romania) from previously published studies [13, 17, 18, 31]. The PGC1 GWAS statistical results were obtained from the Psychiatric Genomics Consortium public website (<https://www.med.unc.edu/pgc>). Table 1 presents a list of these studies and their information such as sample area, definition criteria for psychiatric disorders, sample size, etc. The “metafor” package in R was used to perform the meta-analysis to calculate the pooled ORs and 95% CIs across different samples [32]. The heterogeneity between individual studies was estimated using the Cochran (Q) χ^2 test through calculating the weighted sum of squares of deviations of individual OR estimates from the overall estimate [33, 34]. The pooled ORs and the 95% CIs were graphically presented in a forest plot, in which squares represented different studies and sizes of the squares denoted their respective weights.

Results

Association of NCAN rs1064395 with BPD

rs1064395 is a SNP in the 3' untranslated region of *NCAN* and was initially implicated in a GWAS of BPD in Europeans [13]. This SNP did not show genome-wide significance in the following GWAS [12, 15, 17–19], rais-

Table 1. Characteristics of included BPD samples in this meta-analysis

Sample	Pheno-type	Diagnostic criteria	Cases, <i>n</i>	Controls, <i>n</i>	Genotyping method	rs1064395		Data source
						OR	95% CI	
MooDS GWAS	BPD	DSM-IV, RDC	2,266	5,028	Illumina Human610-Quad, HumanOmni1-Quad	1.25	1.13–1.39	17
PGC1 GWAS	BPD	DSM-IIR, DSM-IV, RDC	7,481	9,250	Illumina and Affymetrix platforms	1.10	1.04–1.17	18
Iceland	BPD	DSM-III, DSM-IV, ICD10, RDC	422	11,487	Illumina HumanHap300	1.10	0.91–1.34	13
France	BPD	DSM-IV	471	1,758	Illumina HumanHap300, 550, 610-Quad	1.28	1.07–1.53	13
Bosnia and Herzegovina/Serbia	BPD	DSM-IV	107	113	Sequenom iPLEX Gold assay	1.20	0.75–1.93	13
Romania	BPD	DSM-IV	461	436	Sequenom iPLEX Gold assay	1.22	0.95–1.54	31
Japan 01	BPD	DSM-IV-TR	1,545	7,408	Illumina HumanOmniExpress v1	1.09	0.97–1.23	20
Japan 02	BPD	DSM-IV-TR	1,419	54,479	Illumina HumanOmniExpressExome v1.2	1.07	0.95–1.19	20
China	BPD	DSM-IV	1,146	2,031	Sequenom MassARRAY	1.07	0.91–1.26	current

BPD, bipolar disorder; GWAS, genome-wide association study.

ing the need for further replication analyses. In our Han Chinese case-control sample, while rs1064395 did not achieve the conventional nominal significance ($p = 0.415$, OR = 1.070 for the A allele, Table 1), the direction of allelic effect was the same as those in European studies.

We then retrieved the statistical data of rs1064395 from previously published GWAS and candidate gene studies (see detailed information in Table 1) and conducted a meta-analysis using these available data. A total of nine case-control datasets were included in the meta-analysis, yielding 15,318 cases and 91,990 controls. The full results of the meta-analysis are shown in Figure 1. We did not observe significant heterogeneity between samples ($p = 0.387$, $I^2 = 5.8\%$) and thus applied a fixed-effect model for the analyses. Interestingly, rs1064395 showed genome-wide significance ($p = 4.92 \times 10^{-9}$, OR = 1.126 for the A allele, Fig. 1). A post hoc analysis stratified by ethnic groups showed that rs1064395 was associated with BPD in both European ($p = 1.35 \times 10^{-8}$, OR = 1.149 for the A allele) and East Asian ($p = 0.0418$, OR = 1.077 for the A allele) populations.

Association of NCAN rs1064395 with MDD

We also examined the association of rs1064395 in two datasets of MDD GWAS. In the PGC1 GWAS including

9,240 MDD cases and 9,519 controls of European ancestry [35], rs1064395 was associated with MDD ($p = 0.0127$, OR = 1.075 for the A allele); in the CONVERGE GWAS of 5,303 cases and 5,337 controls in a Chinese population [36], the association of rs1064395 with MDD did not achieve conventional nominal significance ($p = 0.339$, OR = 1.049 for the A allele), but was in the same direction as that reported by PGC1 GWAS [35]. A meta-analysis of two MDD GWAS including 14,543 cases and 14,856 controls in total found that rs1064395 was also nominally associated with MDD ($p = 0.0068$, OR = 1.067 for the A allele). The allelic effects of rs1064395 in BPD and MDD analyses were in the same direction.

Discussion

NCAN was one of the first genes showing genome-wide significant association with BPD in general populations. In 2011, Cichon et al. [13] conducted a GWAS of 682 BPD cases and 1,300 controls in tandem with a follow-up replication analysis of an additional 7,759 cases and 34,062 controls, and found that rs1064395 in NCAN was significantly associated with BPD ($p = 2.14 \times 10^{-9}$, OR = 1.17).

Fig. 1. Forest plot of meta-analyses for rs1064395 combined with bipolar disorder. GWAS, genome-wide association study.

To further understand the genetic susceptibility of BPD conferred by this SNP, we carried out a case-control analysis in a Han Chinese population and a follow-up meta-analysis combining all available samples, and observed a genome-wide significant association between rs1064395 and BPD in this large-scale sample. Based on the hypothesized genetic overlap between BPD and MDD, we then took one step further to show that rs1064395 was also associated with MDD, providing important evidence supporting the shared genetic risk components between these two illnesses. These data indicate that rs1064395 is likely a true risk variant for a broader spectrum of major mood disorders. More importantly, our meta-analysis included samples from both European and Asian populations, providing essential information regarding the genetic susceptibility conferred by this locus across different ethnic samples. Indeed, the observed consistent effect sizes of allelic risk at rs1064395 in different populations makes it compelling to further study the association of rs1064395 with major mood disorders in Asians.

The discovered association between rs1064395 and major mood disorders appeals studies revealing the underlying biological mechanisms of this GWAS locus on the pathogenesis of these diseases [37]. Although the precise function of this SNP remains unclear, as it locates in the 3' untranslated region of the *NCAN* gene, previous studies have implicated its regulative effects on the mRNA expression of *NCAN* in the frontal and cerebellar cortex [27]. As stated in a previous study [28], *NCAN* preferentially affected mania symptoms in humans, and homozygous *Ncan* knockout mice showed behavioral abnormalities that were strikingly similar to those of the human

mania phenotype; neurobiological studies from this perspective are necessary.

In summary, the present study strongly suggests that *NCAN* is a susceptibility gene for BPD in general populations. These data also provide useful insights for a better understanding of the genetic mechanism by which *NCAN* affects the risk of major mood disorders.

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Statement of Ethics

All the protocols and methods used in this study were approved by the institutional review board of the Kunming Institute of Zoology, Chinese Academy of Sciences.

Disclosure Statement

The authors declare no conflict of interest.

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