Journal of Affective Disorders 162 (2014) 81–88

Contents lists available at ScienceDirect

Journal of Affective Disorders

journal homepage: www.elsevier.com/locate/jad

Research report

Association of the 3′ region of the neuregulin 1 gene with bipolar I disorder in the Chinese Han population

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ARTICLE INFO

Article history:
Received 14 November 2013
Received in revised form
17 March 2014
Accepted 18 March 2014
Available online 1 April 2014

Keywords:
Bipolar disorder
Neuregulin 1 gene
Psychotic features

ABSTRACT

Background: Based on the function of neuregulin 1 (NRG1) in neurodevelopment, susceptibility to bipolar disorder presumably involves this gene. The 3′ region of NRG1 contains the majority of the coding exons, and transcripts from this region encode 8 of the 9 known NRG1 isoforms; therefore, this region is likely to be predominant versus the 5′ region in terms of their relative contributions to NRG1 function. We investigated the association between the 3′ region of the NRG1 gene and bipolar I disorder (BPI) in the Chinese Han population and performed further analyses depending on the presence or absence of psychotic features.

Methods: A total of 385 BPI patients and 475 healthy controls were recruited for this study. Thirty tag single nucleotide polymorphisms (SNPs) across the 3′ region of the NRG1 gene were genotyped for allelic and haplotypic associations with BPI and subgroups with psychotic features (BPI-P) or without psychotic features (BPI-NP).

Results: Individual marker analysis showed that 2 SNPs (rs12547858 and rs6468121) in this region were significantly associated with BPI. Moreover, subgroup analyses showed significant but marginal associations of rs6468121 with BPI-P and rs3757933 with BPI-NP. Haplotype analyses showed that 6 haplotypes were associated with BPI only.

Limitations: The sample size was relatively small. The investigated tag SNPs only represented 83% of the information on the targeted region. There might be a retrospective bias in the subgroup analyses.

Conclusion: The results suggest that the 3′ region of the NRG1 gene plays a role in BPI susceptibility in the Chinese Han population. In addition, the preliminary results show that BPI with psychotic features and BPI without psychotic features may constitute different sub-phenotypes; however, this finding should be confirmed in a larger population sample.

1. Introduction

Bipolar disorder (BP) is a severe psychiatric disorder with profound symptoms of mood instability; it is largely divided into types I and II. Bipolar I disorder (BPI) is mainly characterized by episodes of typical mania, whereas bipolar II disorder is predominantly characterized by episodes of depression and hypomania. BPI is thought to affect approximately 1.0% of the US population (Merikangas et al., 2007), and the life time prevalence of this disorder in China is approximately 0.1% (Phillips et al., 2009). Although the etiological mechanisms of bipolar disorder are still not fully understood, studies of families, twins and adopted individuals indicate strong evidence of genetic predisposition; the heritability of BPI can reach up to 93% (Kieseppa et al., 2004).

In recent decades, it has been widely accepted that neurodevelopmental disruptions are implicated in the pathophysiology of schizophrenia (SP) (Murray and Lewis, 1987; Weinberger, 1987). Based on the clinical features and susceptibility genes that are common to SP and BP, some investigators have hypothesized that there are similar neurodevelopmental alterations in BP as well (Nasrallah, 1991; Sanches et al., 2008). Currently, there is some evidence to suggest

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http://dx.doi.org/10.1016/j.jad.2014.03.037
0165-0327/

that the risk of BP may be increased by the harmful effects of the prenatal and peri-natal environment on neurodevelopment (Torrey et al., 1997; Kinney et al., 1998). Minor physical anomalies (Trixler et al., 2001) and abnormal patterns of brain anatomical symmetry (Swayze et al., 1992) have been reported in BP patients. The impairment of social functions (Cannon et al., 2002) and cognitive functions (Barrett et al., 2009; Torres et al., 2011) among BP patients at the premorbid stage or first episode also indicates that abnormal neurodevelopment exists in BP. Apart from environment effects, neurodevelopment is also regulated by genes such as *neuregulin 1* (*NRG1*), *brain-derived neurotrophic factor* (*BDNF*), *nerve growth factor* (*NGF*), and *reelin* (*RELN*) (Andreasen, 2010). One in particular, *NRG1*, has been intensively studied. Several protein isoforms encoded by this gene play many roles in neural development, including in radial neuron migration, axon guidance, myelination, oligodendrocyte development, and synapse formation, and these roles are mediated by Neuregulin 1/V-erb-b2 avian erythroblastic leukemia viral oncogene homolog 4 (NRG1/ErbB4) signaling (Falls, 2003; Mei and Xiong, 2008). So far, some studies have suggested that *NRG1* is the susceptibility gene in SP; in addition, NRG1 has been proposed as a promising candidate gene underlying BP.

The association between the *NRG1* gene and BPI was first reported in the UK (Green et al., 2005). Since then, more evidence has emerged from Bulgarian, Scottish, European Caucasian and American populations (Georgieva et al., 2008; Prata et al., 2009; Goes et al., 2009). However, it was difficult to find repeated SNP associations in different studies; the relationship between this gene and BP was not found in Ireland or the Central Valley region of Costa Rica (CVCR) (Cassidy et al., 2006; Moon et al., 2011). Moreover, several genome-wide association studies (GWASs) of BPI in European populations found no significant associations with the chromosome region carrying the *NRG1* gene (PGC-CD, 2013; Green et al., 2013; PGC-BD, 2011; Cichon et al., 2011; Scott et al., 2009; Ferreira et al., 2008; WTCCC, 2007). These studies indicate that the relationship between the *NRG1* gene and BPI is still ambiguous; furthermore, these types of studies have not been carried out in the Chinese Han population. The largest GWAS of psychiatric illness performed so far has reported that some genetic risk is shared between SP and BP (PGC-CD, 2013); the research group that performed this GWAS further reported that 15% of susceptibility genes are shared by these two disorders (Lee et al., 2013). Therefore, given that psychotic symptoms are regarded as the possible link between BPI and SP, investigators hypothesize that BPI with psychotic features might show a stronger association with the *NRG1* gene. A significant genome-wide linkage has been shown between BPI with psychotic symptoms and the chromosome 8p12 carrying the *NRG1* gene (Park et al., 2004). Several subsequent studies also reported that *NRG1* single nucleotide polymorphisms (SNPs) are correlated to BPI subtypes with psychotic or mood-incongruent features (Green et al., 2005; Prata et al., 2009; Goes et al., 2009). Additionally, a new marker, rs7014762, has been associated with "typical" BPI phenotypes, which are characterized by an excellent recovery between episodes and the absence of mood-incongruent features (Georgieva et al., 2008). The studies above suggest that the clinical features that indicate the presence or absence of psychotic symptoms might be the criteria that divide BP subtypes.

The 3′ region of the *NRG1* gene spans the majority of exons that encode 9 isoforms: Heregulin (HRG)-alpha, HRG-beta 1/2/3, HRG-gamma, glial growth factor (GGF), GGF2, neutrophil diffraction factor 43 (NDF43), and sensory and motor neuron-derived factor (SMDF). Therefore, variations in this region might be more directly involved in the functions of this gene; however, recent studies focusing on the 3′ region of *NRG1* are still rare in BPI. Therefore, in this study, we hypothesized that the 3′ region of the *NRG1* gene might be associated with BPI, especially the BPI subtype with psychotic features. To increase the homogeneity of the diagnosis, our study recruited only BPI patients with clinical phenotypes that are easy to identify. In this case-control association study, we primarily investigated the relationship between 30 tag SNPs covering the 3′ region of the *NRG1* gene and BPI in the Chinese Han population.

2. Methods

2.1. Participants

The protocol was carried out in accordance with the Declaration of Helsinki and was approved by the Institutional Review Boards at Affiliated Brain Hospital of Guangzhou Medical University (Guangzhou Brain Hospital) and West China Hospital, Sichuan University. Written informed consent was obtained from all participants prior to enrollment in the study. Inpatients and outpatients ($N=390$) from Guangzhou Brain Hospital were included in the study, and 477 healthy volunteers were recruited as controls. All participants were screened for BPI diagnoses based on the Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV) using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID), which was administered by trained postgraduates or psychiatrists.

Inclusion criteria for individuals with BPI were: (a) a diagnosis of BPI based on DSM-IV criteria, and (b) Han Chinese ethnicity. Healthy controls were included if they: (a) lacked a lifetime personal history of Axis I or Axis II psychiatric disorders, (b) had no family history (first- or second-degree relative) of mental disorders, and (c) were of Han Chinese ethnicity.

Participants were excluded if they had: (a) any current Axis I diagnosis other than bipolar affective disorder, (b) neurological diseases, (c) obvious somatic illnesses, or (d) a history of disturbance in consciousness after traumatic brain injury.

Based on the clinical manifestations of BPI, the entire BPI patient group was divided into subgroups of patients: those patients with psychotic symptoms (BPI-P) and those without psychotic symptoms (BPI-NP). Psychotic symptoms were defined as: (a) the criteria for hallucinations and/or delusions as described in the DSM-IV, and (b) sustained episodes lasting no less than 1 day or intermittent episodes lasting no less than 3 days. Thirty-three patients with ambiguous psychotic symptoms were not included in subgroup analyses.

2.2. Genotyping

Peripheral blood (5 ml) was collected from all participants, and genomic DNA was extracted according to the standard phenol-chloroform procedure (Sambrook and Russell, 2001). A total of 31 SNPs within the region of the *NRG1* gene between 32530235 and 32734640 were selected based on SNP tagging of the Han Chinese population in Beijing through the HapMap database (http://hapmap.ncbi.nlm.nih.gov). Next, a total of 250 ng of DNA was genotyped using the GoldenGate genotyping assay according to the manufacturer's instructions (Illumina Bead Station 500, Illumina Inc., San Diego, USA).

2.3. Data analysis

Demographic and clinical data analyses were performed using the PASW 18.0 statistical software package (SPSS Inc., Chicago, IL, USA) (Cronk, 2010). Student's *t*-test and the χ^2 test were used for continuous and categorical variables when appropriate.

The genetic data analyses were performed using the Plink 1.07 program (http://pngu.mgh.harvard.edu/purcell/plink). Prior to the