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LETTER TO THE EDITOR

Association of Genetic Polymorphisms with Age at Onset in Han Chinese Patients with Bipolar Disorder

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Dear Editor,

Bipolar disorder (BD) is a chronic and disabling disorder characterized by manic, hypomanic, or depressive episodes, with an estimated lifetime prevalence ranging from 1% to 5%. Globally, it is a leading cause of disability and a socioeconomic burden. Gene-environment interactions are thought to be involved in the neurobiology of BD, with genetic factors contributing to 60%–85% of BD patients. Twin studies have shown a heritability of 59%, and an increased risk of BD has been found in first-degree relatives of probands [1]. Currently, many genes have been reported as possible risk factors for BD, including *PBRM1*, *CACNA1C*, *ANK3*, *ODZ4*, *SYNE1*, *ITIH1*, *GABRB1*, *DAOA*, *NCAN*, and *TRANK1*. Environmental factors such as maternal stress, prenatal malnutrition, preterm birth, childhood abuse, stressful life events, and cannabis use can also contribute to the development of BD.

Early recognition and treatment of BD can improve treatment responses and the prognosis [2]. However, there is always a long delay from the onset of disease to a definite diagnosis. Nearly half of BD patients develop their first mood episode at puberty. Kataoka *et al*. have found a significant association of early disease onset in BD probands with *de novo* protein-altering mutations when compared with non-carriers [3]. Therefore, age at onset (AAO) may be a potential clinical marker for genetic susceptibility to BD. The relationship between risk genes for BD and AAO, however, has not been fully investigated.

In this study, we aimed to explore the correlation between high-risk single-nucleotide polymorphism (SNP) loci and AAO in Chinese patients with BD. A total of 224 Han Chinese participants with BD were consecutively recruited from the First Affiliated Hospital of Zhejiang University School of Medicine from August 2011 to March 2016. The diagnosis was made according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) and further confirmed by the Structural Clinical Interview for DSM-IV. All patients were diagnosed with either type I or II BD. The exclusion criteria included prior or current comorbidity of other psychiatric diseases, alcohol or other psychoactive substance abuse, poisoning, or other physical conditions that could cause emotional lability. After a search of gene linkage analysis or genome-wide association studies in databases (PubMed, EMBASE, and the Cochrane Library) up to February 2016, 35 SNP loci were screened as candidates. The genotypes were determined by the MassArray system (Agena iPLEX assay, San Diego, CA), with a 384-element SpectroCHIP gene array. Given our small sample size, the minor allele frequency of each SNP locus was required to be > 0.05, and 26 SNP loci were finally included in the analysis. To determine the relationship between SNP loci and AAO of BD, Plink and Haploview statistical software was used and logistic regression analysis was performed. All study

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procedures were approved by the Hospital Ethics Committee of the First Affiliated Hospital of Zhejiang University School of Medicine and were in accordance with the Helsinki Declaration of 1975. Informed consent was given by all participants.

The participants were predominantly female (59.8%, 134/224), BD type II (62.1%, 139/224), and the mean AAO was 23.5 ± 11.2 years. Genotyping was carried out in all participants, and distribution vector maps were drawn for each SNP. The detection rate of *rs1935058* was 94.7%, and the genotypic vector maps of *rs1935058* and *rs9311149* showed unsatisfactory clustering performance (Fig. 1). No significant correlation was found between SNP loci and AAO, except for a positive correlation for the *rs12899449* locus (P = 0.026; BETA = 8.024) (Table 1). In subgroup analysis, the impact of gender and type of BD on AAO was further analyzed. The results showed no significant association between SNP loci and female or BD type I. *rs12899449* was a significant locus in males (P = 0.018), and *rs12899449*, *rs1064395*, and *rs6046396* were significant loci in BD type II (P = 0.002, 0.018, and 0.040, respectively) (Table 1). For *rs12899449*, however, the numbers of AA, GA, and GG genotypes were 210, 6, and 1, respectively, in all participants; and 133, 2, and 1 in the BD type II group. Similarly, the numbers of AA, GA, and GG genotypes for *rs1064395* were 110, 26, and 1 respectively in the BD type II group. Therefore, due to the genotype distribution bias, no firm conclusion could be drawn for *rs12899449* and *rs1064395*, so the findings in this study only suggested a significant correlation between *rs6046396* and AAO in individuals with BD type II. The ages of patients (mean ± standard deviation) with the *rs6046396* AA, GA, and GG genotypes were 20.2 ± 10.5, 23.6 ± 11.1, and 26.0 ± 14.2, respectively. Therefore, the A allele was considered to be the risk allele type.

Here, we explored the correlation between high-risk SNP loci and AAO in Han Chinese BD patients. We identified a significant association between *rs6046396* and AAO in BD type II individuals. Although *rs6046396* has been reported to be associated with BD in previous studies [4], its relationship with AAO in BD patients had not yet been investigated. Therefore, the above finding is new and worth further verification.

The *rs6046396* A/G single-nucleotide variation is upstream of *RIN2* (Ras and Rab interactor 2) at 20p11.23 [4]. However, the mechanism by which this SNP locus functions to facilitate the onset of BD is still unknown. The protein encoded by *RIN2* binds preferentially to the GTP-bound form of RAB5 protein over the GDP-bound form, acting as a guanine nucleotide exchange factor and activating RAB5 [5, 6]. RAB5 protein is a small GTPase involved in membrane trafficking and is a crucial regulatory component of the endocytic pathway and cellular signaling cascades [7, 8]. In the presynaptic terminal, RAB5 is required for maintaining endosome integrity [9]. At synapses, endocytosed vesicular membrane may be reused for a new round of neurotransmitter release [9]. Therefore, RAB5 dysfunction impairs the release efficacy of neurotransmitters such as serotonin, noradrenaline, dopamine, and gamma-aminobutyric acid, which are involved in the pathogenesis of affective disorders.

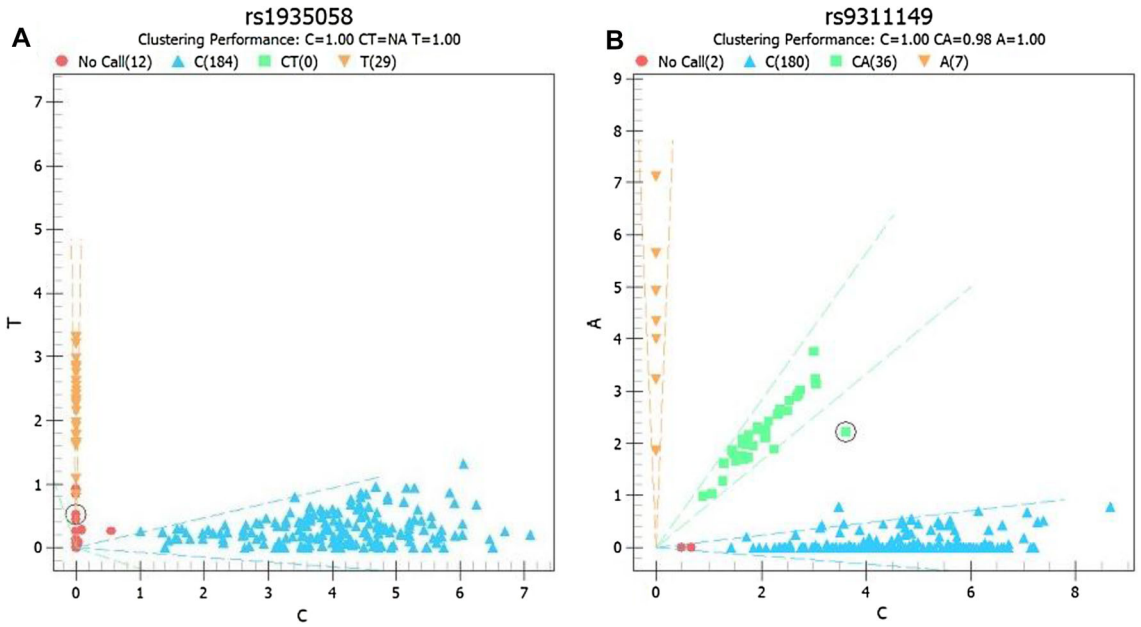

Fig. 1 Genotype vector maps for *rs1935058* and *rs9311149*.

Table 1 Genotype frequencies of *rs12899449*, *rs1064395*, and *rs6046396*, and their relationships with age of onset.

SNP	Genotype	Total				Male				Female				Bipolar type I				Bipolar type II			
		N (%)	BETA	SE	*P**	*N* (%)	BETA	SE	*P*	*N* (%)	BETA	SE	*P*	*N* (%)	BETA	SE	*P*	*N* (%)	BETA	SE	*P*
rs12899449	AA	210 (93.8)	8.024	3.575	**0.026**	82 (91.1)	13.780	5.686	**0.018**	128 (95.5)	2.521	4.610	0.585	77 (90.6)	−2.276	5.482	0.679	133 (95.7)	14.09	4.562	**0.002**
	GA	6 (2.7)				5 (5.6)				1 (0.7)				4 (4.7)				2 (1.4)			
	GG	1 (0.4)				0 (0.0)				1 (0.7)				0 (0.0)				1 (0.7)			
	Missing	7 (3.1)				3 (3.3)				4 (3.0)				4 (4.7)				3 (2.2)			
rs1064395	GG	176 (78.6)	2.183	1.716	0.205	73 (81.1)	3.218	3.565	0.369	103 (76.9)	2.058	1.773	0.248	66 (77.6)	−1.552	2.267	0.496	110 (79.1)	5.439	2.27	**0.018**
	GA	41 (18.3)				15 (16.7)				26 (19.4)				15 (17.6)				26 (18.7)			
	AA	4 (1.8)				0 (0.0)				4 (3.0)				3 (3.5)				1 (0.7)			
	Missing	3 (1.3)				2 (2.2)				1 (0.7)				1 (1.2)				2 (1.4)			
rs6046396	AA	102 (45.5)	1.303	1.188	0.274	43 (47.8)	3.392	1.932	0.083	59 (44.0)	−0.558	1.463	0.704	35 (41.2)	−2.282	1.801	0.209	67 (48.2)	3.122	1.502	**0.040**
	GA	103 (46.0)				37 (41.1)				66 (49.3)				42 (49.4)				61 (43.9)			
	GG	19 (8.5)				10 (11.1)				9 (6.7)				8 (9.4)				11 (7.9)			

*$P < 0.05$ (two-tailed).

In addition, RAB5 protein has been reported to participate in various immune processes, including T cell migration, phagolysosome formation, and cytokine secretion [10–12]. The role of immune dysfunction in affective disorders has emerged as a popular research focus in recent years [13, 14]. The immune system plays an important role in brain development during early life [15]. Therefore, the impact of the *rs6046396* genotype on AAO in the BD population may be affected by RAB5-associated immune activity.

Given the study limitations, the results should be prudently interpreted. First, the sample size was small, which may weaken the statistical power. Second, we did not analyze the effects of environment on AAO, which could also result in the early onset of BD. Third, no healthy controls were recruited. The correlation between the high-risk SNPs included and Han Chinese should be determined before further investigations. Fourth, multiple testing correction was not carried out in the analysis, and this may lead to false-positives. However, the findings in this study were predominantly negative.

In summary, we investigated the relationship between high-risk SNPs and AAO in Han Chinese individuals with BD and revealed that the AA genotype of *rs6046396* was associated with the early onset of BD type II. This finding needs to be further verified in large samples.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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