Progress in Neuro-Psychopharmacology & Biological Psychiatry 34 (2010) 1196–1201

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

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

Association between *CLOCK* 3111T/C and preferred circadian phase in Korean patients with bipolar disorder

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ARTICLE INFO

Article history:
Received 23 March 2010
Received in revised form 8 June 2010
Accepted 14 June 2010
Available online 21 June 2010

Keywords:
Age at onset
Bipolar disorder
CLOCK
Korean
Morningness/eveningness

ABSTRACT

Genes associated with circadian rhythms have been suggested to play an important role in the pathophysiology of bipolar disorder. A single nucleotide polymorphism (SNP) in the 3′-flanking region of *CLOCK* (3111T/C; rs1801260) has been reported to be associated with sleep disturbances and an increased recurrence rate in patients with bipolar disorder. We examined the association of *CLOCK* 3111T/C with bipolar disorder in 260 patients and 350 controls in a Korean population. *CLOCK* 3111T/C showed significant allelic and genotypic associations with bipolar disorder ($P=0.012$, $P=0.033$, respectively). Morningness/eveningness (M/E) was evaluated using the Composite Scale of Morningness (CSM) in 108 patients with bipolar disorder. In the subgroup analysis of the highest and lowest 25th percentile of M/E scores, significantly more C allele carriers were found among extreme evening types than among extreme morning types ($P=0.041$). After correcting for age, C allele carriers had lower M/E scores than those carrying the T/T genotype, but the association was not statistically significant. We also analyzed the association between age at onset (AAO) and *CLOCK* 3111T/C in the bipolar disorder group, and no association was found. Despite the relatively small sample sizes, these results support a possible role of the *CLOCK* 3111T/C SNP in bipolar disorder. Further studies with larger samples and more polymorphisms are necessary.

1. Introduction

Disruption of circadian rhythms has been a focus in studies on the pathogenesis of bipolar disorder. The association of bipolar disorder with genes related to the regulation of circadian rhythms has been suggested by many researchers. Marked alterations in circadian function, such as in sleep–wake cycles, appetite, and in hormone levels were reported among patients with bipolar disorder during relapse and even during remission (Bauer et al., 2006; Le-Niculescu et al., 2009; Leibenluft et al., 1996; Linkowski et al., 1994; Schreiner et al., 2001). Furthermore, medications used in the treatment of bipolar disorder, such as mood stabilizers and antidepressants, are known to exert their action via molecules associated with the regulation of circadian rhythms (Abe et al., 2000; Lamont et al., 2007; Manji et al., 2001; McClung, 2007).

The *CLOCK* gene, located on chromosome 4q12, is expressed in the suprachiasmatic nucleus (SCN) of the hypothalamus, hippocampus, pyriform cortex, and cerebellum. The *CLOCK* gene encodes a basic helix–loop–helix (bHLH) transcription factor that forms a complex with BMAL1, a key element of the circadian clock system in the SCN (Reppert and Weaver, 2002; Turek and Kolker, 2001). It has been reported that the *CLOCK* gene expressed in brain areas other than the SCN plays a mood-regulatory role (Roybal et al., 2007). The *CLOCK* gene mutation in mice led to a lengthening of the circadian period (Vitaterna et al., 1994). Moreover, mice carrying a mutation in the *CLOCK* gene showed mania-like behaviors, such as hyperactivity, reduced sleep, reduced depression-like behaviors, and less anxiety; these mania-like behaviors could be reversed with chronic lithium treatment (Roybal et al., 2007). Furthermore, the *CLOCK* mutant mice were reported to show an increase in dopaminergic activity in the ventral tegmental area (Roybal et al., 2007), which seems consistent

Abbreviations: AAO, age at onset; ANCOVA, analysis of covariance; ANOVA, analysis of variance; BD, bipolar disorder; BMAL, brain and muscle arnt-like; CI, confidence interval; CLOCK, circadian locomotor output cycles kaput; CSM, composite scale of morningness; *df*, degrees of freedom; DIGS, diagnostic interview for genetic studies; DSM-IV, diagnostic and statistical manual of mental disorders, 4th edition; FE, fixed effect; GSK, glycogen synthase kinase; M/E, morningness/eveningness; OR, odds ratio; PER, period; SD, standard deviation; SNP, single nucleotide polymorphism; SPSS, Statistical Package for the Social Sciences; VNTR, variable number of tandem repeats.

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doi:10.1016/j.pnpbp.2010.06.010

with the clinical response to antipsychotic drugs in bipolar disorder (Perlis et al., 2006).

The effects of *CLOCK* mutations in humans have not yet been confirmed. Among many polymorphisms of the *CLOCK* gene, the *CLOCK* 3111T/C single nucleotide polymorphism (SNP; rs1801260) seems to affect the stability and translatability of the mRNA (Mignone et al., 2002; Steeves et al., 1999). Initially, the *CLOCK* 3111T/C SNP was suggested to be associated with human diurnal preference. Katzenberg et al. (1998) reported that the *CLOCK* 3111C allele was associated with evening preference in healthy Caucasians, although subsequent studies were unable to replicate the association (Pedrazzoli et al., 2007; Robilliard et al., 2002). A study of a Japanese population sample also found a positive association between the *CLOCK* 3111C allele and evening preference in healthy adults (Mishima et al., 2005). However, a recent study in healthy Korean college students showed no direct correlation between the 3111T/C SNP in the *CLOCK* gene and diurnal preference (Lee et al., 2007). The same group reported that the *CLOCK* 3111T/C SNP does not seem to play a major role in susceptibility to seasonal variations (Paik et al., 2007).

CLOCK 3111T/C has been reported to show significant associations with clinical features of mood disorders. *CLOCK* 3111T/C was associated with a higher rate of recurrence of bipolar disorder (Benedetti et al., 2003; Serretti et al., 2003), sleep disturbances in patients with major depression or bipolar disorder (Benedetti et al., 2007; Serretti et al., 2003), and improved insomnia in patients with bipolar disorder during antidepressant treatment (Serretti et al., 2005). However, many studies have found no association between *CLOCK* 3111T/C and bipolar or mood disorder itself (Bailer et al., 2005; Johansson et al., 2003; Kishi et al., 2009; Mansour et al., 2006; Nievergelt et al., 2006).

A finer classification of bipolar disorder phenotypes is necessary to facilitate the identification of susceptibility genes. In particular, it has been suggested that quantitative phenotypes might be correlated with a genetic liability to bipolar disorder. The genetic analysis of quantitative phenotypes could be more straightforward, more informative, and more powerful than the diagnosis itself in the search for genetic underpinnings of bipolar disorder. Although quantitative phenotypes or endophenotypes are largely unknown for bipolar disorder, circadian variations appear to be excellent candidates. M/E (morningness/eveningness), a stable and quantifiable measure reflecting diurnal preference, could be regarded as a quantitative phenotype associated with bipolar disorder. We previously reported that patients with bipolar I disorder showed a significant evening preference (including delayed sleep timing) compared with control individuals using CSM (Composite Scale of Morningness) scores (Ahn et al., 2008). The aforementioned study in healthy Korean students showed no direct association between *CLOCK* 3111T/C and M/E (Lee et al., 2007). We investigated this relationship in patients with bipolar disorder. We studied whether the 3111T/C SNP in the *CLOCK* gene influenced preferred circadian phase, as measured by CSM, in patients with bipolar disorder.

In addition to diurnal variation, age at onset (AAO) has been viewed as important in the study of bipolar disorder. Although bipolar disorder shows wide variations in severity and clinical features, AAO variation has been assumed to reflect underlying genetic heterogeneity. For example, bipolar disorder patients with early AAO may represent greater involvement of genetic risk factors relative to environmental factors (Leboyer et al., 2005; Lin et al., 2006; Manchia et al., 2008). It was reported that the GSK3β-50 SNP was associated with AAO of bipolar disorder and improvement under lithium therapy (Benedetti et al., 2004, 2005).

In the present study, we examined whether the *CLOCK* 3111T/C SNP correlated with circadian preference in Korean patients with bipolar disorder. We examined a possible association between the *CLOCK* 3111T/C polymorphism and bipolar disorder in several ways. We used M/E as well as the diagnosis of bipolar disorder, and we also explored the association with AAO of bipolar disorder.

2. Methods

2.1. Subjects

Patients with bipolar disorder were recruited from the Seoul National University Hospital and several psychiatric clinics in Korea. All satisfied the diagnostic criteria of DSM-IV for bipolar disorder. They were individually interviewed by trained nurses using the Korean-translated version of the Diagnostic Interview for Genetic Studies (Joo et al., 2004; Nurnberger et al., 1994). Consensus diagnostic meetings of three or more psychiatrists were held regularly to evaluate the participants' final diagnoses. Subjects with a history of any kind of organic abnormality of the brain, substance dependence, drug abuse, or other physical conditions possibly manifesting as psychiatric symptoms were excluded from this study. The final analyses were thus based on 260 patients with bipolar disorder (111 men and 149 women; average age, 35.5 ± 12.4). Among them, 198 met the DSM-IV diagnostic criteria for bipolar I disorder, and 62 for bipolar II disorder. Healthy controls were free from present, past, and family history (first-degree relatives) of psychiatric illness or substance abuse diagnoses. The total number of control subjects was 350 (174 men and 176 women; average age, 25.9 ± 6.6).

All subjects participating in this study provided written informed consent. The study protocol was approved by the Ethics Committee of Seoul National University Hospital.

2.2. DNA analysis

DNA was extracted from blood samples using a DNA isolation kit (Roche). Genotyping was performed using the TaqMan method (Applied Biosystems, Foster City, CA, USA) (McGuigan and Ralston, 2002).

2.3. Age at onset

Age at onset (AAO) was defined as the first occurrence of a manic, hypomanic, or depressive episode in the patients' clinical course using the DIGS interview, the National Institute of Mental Health (NIMH) retrospective life chart method, and all available medical records (Roh et al., 2007; Roy-Byrne et al., 1985). A consensus meeting with three independent psychiatrists who were blind to patient identity was carried out to determine reliable AAOs.

2.4. Morningness/eveningness (M/E)

M/E (morningness/eveningness) was evaluated using the Composite Scale of Morningness (CSM) (Smith et al., 1989), which is derived from the widely used Horne-Ostberg scale (Horne and Ostberg, 1976). The CSM has been translated into Korean, and the Korean translation (KtCS) has been shown to have acceptable psychometric properties (Kook et al., 1999; Yoon et al., 1997). The CSM measures three intercorrelated factors according to previous Korean studies (Kook et al., 1999; Lee et al., 2007): time of rising (items 1, 6, 10, and 11; e.g., "Considering only your own "feeling best" rhythm, at what time would you get up if you were entirely free to plan your day?"), performance (items 2, 7-9, and 13; e.g., "You wish to be at your peak performance for a test which you know is going to be mentally exhausting and lasting for 2 h. Which ONE of the four testing times would you choose?"), and morning alertness (items 3-5, 12; e.g., "Assuming normal circumstances, how easy do you find getting up in the morning?"). Three items are scored on a five-point scale from 1 to 5, and the remaining 10 items are scored on a four-point scale, from 1 to 4. Lower scores indicate evening preference. Volunteers among the patients with bipolar disorder were asked to complete the CSM. The total number of the patients with bipolar disorder who completed the CSM was 108. We analyzed the