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Mutation screening of the human *Clock* gene in circadian rhythm sleep disorders

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Abstract

We tested whether the human *Clock* (*hClock*) gene, one of the essential components of the circadian oscillator, is implicated in the vulnerability to delayed sleep phase syndrome (DSPS) and non-24-hour sleep–wake syndrome (N-24). Screening in the entire coding region of the *hClock* gene with PCR amplification revealed three polymorphisms, of which two predicted the amino acid substitutions R533Q and H542R. The frequencies of the R533Q and H542R alleles in patients with DSPS or N-24 were very low and not significantly different from those in control subjects. A T3111C polymorphism in the 3′-untranslated region of *hClock*, which had been reportedly associated with morning or evening preference for activity, was also investigated; the results showed that the 3111C allele frequency decreased in DSPS. Polymorphisms in the coding region of the *hClock* gene are unlikely to play an important role in the development of DSPS or N-24. The possible contribution of the T3111C polymorphism to DSPS susceptibility should be studied further.

Keywords: Delayed sleep phase syndrome; Non-24-hour sleep–wake syndrome; Single-strand conformation polymorphism; Transcription factors; Missense mutation; Genetic screening

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1. Introduction

Circadian (~24 h) rhythmicity of biological processes is a conserved feature of most organisms, and is controlled by endogenous self-sustaining oscillators (Hastings, 1997). Recent genetic studies revealed that the circadian clock system consists of transcriptional/translational feedback loops, of which the molecular components have been isolated from bacteria, plants, insects, and mammals, with circadian rhythm mutants (Dunlap, 1999). The mammalian circadian clock is located principally in the suprachiasmatic nuclei in the hypothalamus. Mutations in clock-relevant genes, such as *Clock*, *BMALI* (*Mop3*), *Period2* (*Per2*), *Per3*, *Cry1/2*, and *Casein KinaseIepsilon* (*CKIε*), alter or abolish the circadian rhythmicity in mice and hamsters (Bunger et al., 2000; King and Takahashi, 2000; Lowrey et al., 2000; Shearman et al., 2000).

In humans, sleep and wakefulness, cognitive function, body temperature, and hormonal secretion cycles are regulated by an endogenous circadian clock, which is synchronized to the 24-h day by environmental stimuli, notably light (Hastings, 1997). However, patients with circadian rhythm sleep disorders, such as delayed sleep phase syndrome (DSPS) and non-24-hour sleep–wake syndrome (N-24), fail to adapt their sleep–wake cycle to the environmental time cues (Regestein and Monk, 1995). Patients with DSPS show sleep-onset insomnia and difficulties in awaking at the desired time in the morning. Patients with N-24 exhibit daily delay of the sleep–wake phase, which apparently is not entrained to the environmental light–dark cycles. Although melatonin administration and chronological treatment, such as morning bright light exposure or a daily 3-h delay of bedtimes for several nights, sometimes improve symptoms, patients often suffer from difficulties in social and family life and a high incidence of depression (Czeisler et al., 1981; Dahlitz et al., 1991; Regestein and Monk, 1995). Because some of the circadian rhythm sleep disorders occur in families (Fink and Ancoli-Israel, 1997; Jones et al., 1999), polymorphisms in the human clock genes may lead to the development of DSPS and N-24. A recent study reported that a polymorphism in the human *Per2* gene, one of the three human homologs of the *Drosophila* Clock gene *Per*, causes another type of circadian rhythm sleep disorder, advanced sleep phase syndrome, in which both sleep onset and offset occur much earlier than the desired time (Toh et al., 2001). We have already reported that one of the haplotypes of the *hPer3* gene, another human homolog of the *Drosophila Per*, is significantly associated with the susceptibility to DSPS (Ebisawa et al., 2001).

The *Clock* gene is the first essential component of the mammalian clock. A point mutation in the mouse *Clock* gene, which causes skipping of exon 19, reduces the transcriptional activity of the CLOCK protein and lengthens the period of the locomotor activity rhythm in a dominant-negative fashion (King et al., 1997; Gekakis et al., 1998). These results suggest that the human Clock *hClock* gene is another potential candidate for susceptibility to circadian rhythm sleep disorders.

In this study, mutation screening was performed in the complete coding region of the *hClock* gene in circadian rhythm sleep disorders and controls. The distribution of the T3111C polymorphism in the 3′-untranslated region (3′-UTR) of the gene, which is reportedly associated with morning or evening preference for activity, was also investigated (Katzenberg et al., 1998).

2. Methods

2.1. Subjects

We screened 96 patients with circadian rhythm sleep disorders (59 with DSPS, 37 with N-24) diagnosed according to the International Classification of Sleep Disorders (ICSD, 1990, 1997) criteria and 109 healthy controls.

Brief descriptions of the diagnostic criteria are as follows.

DSPS: (1) inability to fall asleep and wake up spontaneously at the desired time; (2) persistent phase delay of the major sleep episode in relation to the desired time for sleep; (3) symptoms present for at least 1 month; and (4) sleep of normal quality and duration when not required to maintain a conventional sleep–wake schedule.

N-24: (1) difficulty either in falling asleep or in awaking; (2) progressive delay of sleep–wake