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BRIEF REPORT

# A human period gene (HPER1) polymorphism is not associated with diurnal preference in normal adults

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**Mammalian circadian rhythmicity has recently been shown to be regulated at the genetic level by transcription–translation feed-back loops. Key molecular components such as Clock, Bmal-1, Timeless and three Period proteins have been isolated in mammals. In this study, we hypothesized that polymorphisms at the level of one of these genes – HPER1 – could be associated with differential morningness–eveningness tendencies. The sample comprised 463 middle-aged participants enrolled in the Wisconsin Sleep Cohort Study. Diurnal preferences were evaluated using the Horne-Ostberg questionnaire. An *A* to *G* synonymous substitution at position 2548 was identified in HPER1 c-DNA sequence by comparing available sequence data. This polymorphism was verified by sequencing and typed using established oligotyping techniques in all subjects, yielding allele frequencies of 0.85 and 0.15 for HPER1 2548G and HPER1 2548A, respectively. Morningness–eveningness scores were then compared between genotype groups. In contrast to data previously published using a Clock polymorphism, scores did not differ significantly across HPER1 groups. These results suggest that polymorphism at the level of HPER1 does not significantly modulate morningness–eveningness tendencies in the general population.** 

**Keywords: circadian rhythm, morningness–eveningness, Horne-Ostberg, HPER1, single nucleotide polymorphism**

## INTRODUCTION

Circadian regulation is an almost universal function in physiology. In constant environmental conditions, behavioral and metabolic activities usually maintain endogenous fluctuations with a free-running period close to 24 h. Studies in organisms as diverse as plants, insects and mammals have demonstrated the importance of genetic factors in generating this phenomenon (Dunlap, 1996). In Drosophila, mutations in the period (per), dClock, timeless (tim) or cycle (cyc) genes significantly affect the endogenous free-running circadian period. These genes are part of a regulatory feed-back loop system involving Clock-cyc (Ruttila *et al.*, 1998; Darlington *et al.*, 1998) and Per-tim (Gekakis *et al.*, 1995; Sangoram *et al.*, 1998) heterodimers generating circadian rhythmicity. In the mouse, a mutation in the Clock gene dramatically prolongs the endogenous period under free-running conditions (Vitaterna *et al.*, 1994). These alterations are also associated with changes in the phase angle of entrainment under light/dark conditions. Typically, animals with short free-running periods increase their activity earlier with respect to light/dark transitions than animals with long free-running periods. These relationships between free-running period and phase angle of entrainment also hold in normal animals and can be considered as reflecting genetic control in morningness–eveningness tendencies (see Katzenberg *et al.*, 1998 for review). In humans, the intrinsic period of the circadian pacemaker is related to the entrained phase of the core body temperature cycle, which in turn is coupled to morningness–eveningness tendencies (Czeisler and Khalsa, 1999).

In a recent study (Katzenberg *et al.*, 1998), we have tested the hypothesis that genetic variation in the human Clock gene could modulate diurnal preferences using a polymorphism located in the 3′ untranslated region of the human gene. Carriers of the rarer Clock allele were found to have significantly higher Horne-Ostberg scores, thus indicating increased eveningness tendencies. In this study, another candidate circadian gene is similarly tested. A silent single nucleotide polymorphism (SNP) was identified in the human HPER1 gene, typed in 463

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unrelated individuals, and correlated with Horne-Ostberg scores.

## SUBJECTS AND METHODS

A population-based random sample of middle-aged adults enrolled in the Wisconsin Sleep Cohort Study (Young *et al.*, 1993) was used in this analysis. All subjects had undergone nocturnal polysomnography as part of a longitudinal study on the natural history of sleep-disordered breathing. Subjects ($n$ = 463) were asked to complete the 19-item Horne-Ostberg questionnaire (Horne and Ostberg, 1976). Mean age ± SE was 46.3 ± 0.4 years, 264 subjects (57.0%) were males. Although most subjects were American born, primary ethnic heritage was ascertained. Most subjects (97%) were Caucasian and the most frequent primary heritage recorded was German (39%).

An SNP located within the human HPER1 gene was identified by comparing four human c-DNA sequences available in National Center for Biotechnology Information (NCBI) databases (reference position 2548G in AF022991 and AA085381, versus *A* in AB002107 and AA421103). This SNP is a synonymous substitution located within the coding sequence (Thr 787). DNA extracted from white blood cells was amplified in the region of interest using PER F (AGACGCCTACCGTCCAGTG) and PER R (GAAGAGCTGTCGAGTCCACG) with five cycles at 95°C 1 min, 60°C 1 min, 72°C 1 min followed by 30 cycles of 95°C 1 min, 55°C 1 min, 72°C 1 min followed by 5 min at 72°C. The resulting polymerase chain reaction (PCR) product was dot blotted onto nylon membranes, hybridized at 42°C in a standard solution (6X SSPE, 5X Denhardt's, 0.1% N-lauroylsarcosine and 0.02% SDS) with either of two labeled sequence-specific oligonucleotides (HPER1A: CTCCACACACAGAAGGAA and HPER1G: CTGCACACGCAGAAGGAA, labeled with Digoxin-11-ddUTP) and washed at 55°C in TMAC solution (50mM Tris, pH8, 3M tetramethylammonium chloride, 2mM EDTA, 0.1% SDS). Hybridization signals were detected by chemiluminescence after application of anti-digoxin antibodies according to the manufacturer's recommendations (Boehringer-Mannheim). Subjects were then categorized into three groups on the basis of their HPER1 genotypes (HPER1 2548 A/A, HPER1 2548 A/G and HPER1 2548 G/G). PCR products from 43 randomly selected individuals were also directly sequenced to confirm the oligotyping data.

Allele frequencies were derived as described in Ott (1985). Observed genotype frequencies were compared with expected Hardy-Weinberg equilibrium values using $\chi^2$ analysis. Horne-Ostberg scores were calculated in all subjects using pre-established values for each question as previously described (Horne and Ostberg, 1976). Linear regression modeling was used to assess differences between groups for the Horne-Ostberg scores unadjusted and adjusted for the potential confounding factors of age, sex, and ethnic heritage. The statistical significance of regression coefficients was assessed using $t$-tests. An interaction between the HPER1 polymorphism and the previously typed Clock 3111 polymorphism was also examined using linear regression modeling. The SAS statistical package (SAS User's Manual, 1990) was used for all analysis, and $P$-values of less than 0.05 were considered to indicate statistical significance.

## RESULTS

Overall allele frequencies for HPER1 2548A and HPER1 2548G alleles in this sample were 0.15 and 0.85, respectively. Observed frequencies for A/A, A/G and G/G genotypes were 6/463, 126/463 and 331/463 respectively and did not differ significantly from derived Hardy-Weinberg equilibrium values. Mean Horne-Ostberg scores were then compared between genotypes (Table 1). The mean Horne-Ostberg scores, unadjusted or adjusted for age, sex, and ethnic heritage, were not significantly different between the HPER genotype groups. As previously reported using a largely overlapping sample (Katzenberg *et al.*, 1998), the 3111 Clock polymorphism significantly affected the Horne-Ostberg scores ($P < 0.01$). No interaction was found between this Clock polymorphism and the HPER1 polymorphism in relation to the Horne-Ostberg scores (data not shown).

## DISCUSSION

In this study, an informative but silent HPER1 *G* to *A* transition located in the coding region of the gene is

TABLE 1. Horne-Ostberg score by HPER1 genotypes

| HPER1 genotypes | Number of subjects | Horne-Ostberg score (unadjusted) | Horne-Ostberg score (adjusted[a]) |
|---|---|---|---|
| 2548 A/A | 6 | 62.0 ± 4.2 | 61.8 ± 4.2 |
| 2548 A/G | 126 | 61.3 ± 0.9 | 60.57 ± 1.0 |
| 2548 G/G | 331 | 61.1 ± 0.6 | 60.5 ± 0.7 |

Data are means ± SEM. [a]Adjusted for age, sex, and ethnic heritage. None of the values differ significantly between groups.

reported (2548A/G) and is found not to be associated with morningness–eveningness tendencies. Although very suggestive, this result does not entirely exclude the involvement of other undetected functional HPER1 polymorphisms on diurnal preferences. The report of this HPER1 SNP may also be useful to study rare circadian disorders such as delayed or advanced sleep phase syndrome, or to explore the possible role of the HPER1 in modulating circadian functions other than morningness–eveningness tendencies. The transcription of *per-1* is known to be regulated by the binding of Clock-Bmal-1 heterodimer (*bmal-1* is the mammalian orthologue of cycle) to an E-Box (Gekakis *et al.*, 1998) and *per-1* transcription is triggered by light exposure (Albrecht *et al.*, 1997). Polymorphisms at the level of HPER1 may thus be involved, for example, in regulating light transduction effects to the suprachiasmatic nuclei, rather than in regulating endogenous circadian rhythmicity *per se*. Another possible explanation for this negative result may be the redundancy of the *per* genes in mammals. Three *per* genes have been cloned in mammals, whereas only one *per* gene is known to exist in the fruit fly. Mutations within *per* in Drosophila produce strong circadian alterations and a natural polymorphism modulates temperature compensation of the circadian clock, resulting in natural selection and a north–south gradient of allele frequencies (Sawyer *et al.*, 1997). In mammals however, *per-1* knock-out mouse models are not yet available to test if functional alterations in *per-1* result in circadian phenotypes. It is thus possible that any HPER1 alteration may be compensated by the other two HPER genes.

The observation that some individuals prefer evening or morning hours is likely to have a genetic basis. Data from a study in 238 twin pairs indicated substantial heritability ($H$ = 0.48–0.56) for morningness–eveningness tendencies as measured using the Horne-Ostberg questionnaire (Drennan *et al.*, 1992). Our data gathered with Clock also suggest that at least one of the five currently identified key circadian genes is involved (Katzenberg *et al.*, 1998). Additional studies using these and other circadian gene polymorphisms in normal subjects and human circadian disorders are awaited to further this line of investigation.

## REFERENCES


Albrecht U, Sun ZS, Eichele G, Lee CC (1997). A differential response of two putative mammalian circadian regulators, mper1 and mper2, to light. *Cell* **91**:1055–1064.

Czeisler CA, Khalsa SBS (1999). The human circadian timing system and sleep/wake regulation. In: *Principles and practice of sleep medicine*, Kryger MH, Roth T, Dement WC (editors). Philadelphia: WB Saunders (in press).

Darlington TK, Wager-Smith K, Ceriani MF, Staknis D, Gekakis N, Steeves TDL *et al.* (1998). Closing the circadian loop: clock-induced transcription of its own inhibitors per and tim. *Science* **280**:1599–1603.

Drennan MD, Shelby J, Kripke DF, Kelsoe J, Gillin JC (1992). Morningness/eveningness is heritable. *Soc Neurosci Abstr* **18**:196.

Dunlap, JC(1996). Genetic and molecular analysis of circadian rhythms. *Ann Rev Gen* **30**:579–601.

Gekakis N, Saez L, Delahaye-Brown A-M, Myers MP, Sehgal A, Young MW (1995). Isolation of timeless by PER protein interaction: defective interaction between timeless protein and long-period mutant PER-L. *Science* **270**:811–815.

Gekakis N, Staknis D, Nguyen HB, Davis FC, Wilsbacher LD, King DP *et al.* (1998). Role of the clock protein in the mammalian circadian mechanism. *Science* **280**:1564–1569.

Horne JA, Ostberg O (1976). A self assessment questionnaire to determine morningness–eveningness in human circadian rhythms. *Int J Chronobiol* **4**:97–110.

Katzenberg D, Young T, Finn L, Lin L, King DP, Takahashi JS *et al.* (1998). A clock polymorphism associated with human diurnal preference. *Sleep* **21**:569–576.

Ott J (1985). *Analysis of human genetic linkage*. Baltimore, MD: Johns Hopkins University Press. pp. 1–223.

Ruttila JE, Suri V, Le M, So WV, Rosbash M, Hall JC (1998). CYCLE is a second bHLH-PAS clock protein essential for circadian rhythmicity and transcription of Drosophila period and timeless. *Cell* **93**:805–814.

SAS Institute (1990). *SAS User's Manual, Release 6.08*. Cary, NC: SAS Institute.

Sangoram AM, Saez L, Antoch MP, Gekakis N, Staknis D, Whiteley A *et al.* (1998). Mammalian circadian autoregulatory loop: a timeless ortholog and mPer1 interact and negatively regulate CLOCK-BMAL-1 induced transcription. *Neuron* **21**:1101–1113.

Sawyer LA, Hennessy JM, Peixoto AA, Rosato E, Parkinson H, Costa R *et al.* (1997). Natural variation in a Drosophila clock gene and temperature compensation. *Science* **278**:2117–2120.

Vitaterna MH, King DP, Chang A-M, Kornhauser JM, Lowrey PL, McDonald JD *et al.* (1994). Mutagenesis and mapping of a mouse gene, clock, essential for circadian behavior. *Science* **264**:19–725.

Young TB, Palta M, Dempsey J, Skatrud J, Weber S, Badr S (1993). The occurrence of sleep-disordered breathing among middle-aged adults. *New Engl J Med* **328**:1230–1235.