LETTER

Human Diurnal Preference and Circadian Rhythmicity Are Not Associated with the *CLOCK* 3111C/T Gene Polymorphism

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Abstract Genetic association studies of the *CLOCK* 3111C/T polymorphism and diurnal preference have yielded conflicting results since the first report that the 3111C allele was associated with eveningness. The goal of the present study was to investigate the association of this polymorphism with diurnal preference and circadian physiology in a group of 179 individuals, by comparing the frequency of the 3111C allele to diurnal preference, habitual sleep timing, circadian phase markers, and circadian period. We did not find a significant association between this allele and morningness/eveningness or any circadian marker.

Key words chronotype, human circadian rhythms, circadian phase, circadian period, melatonin

Diurnal preference refers to an individual's preference for timing daily activities. Diurnal preference is an individual trait that is normally distributed and may be assessed subjectively through questionnaires such as the Horne-Östberg Morningness-Eveningness Questionnaire (MEQ) (Horne and Östberg, 1976). Based on the idea that the circadian timing system has a strong influence on diurnal preference, a series of recent investigations have studied the association of morningness/eveningness with circadian clock–related genes. The first such report (Katzenberg et al., 1998) described a single nucleotide polymorphism (SNP) in the human *CLOCK* gene (3111C/T) that was associated with diurnal preference, finding that the 3111C allele correlated with eveningness (Katzenberg et al., 1998). While one subsequent report replicated that finding (Mishima et al., 2005), several others have not (Johansson et al., 2003; Pedrazzoli et al., 2007; Robilliard et al., 2002). The purpose of the current study was to examine the association between this SNP and diurnal preference in a group of individuals who underwent circadian rhythm phenotyping in our laboratory and to further examine whether there were differences in circadian phenotypic measures (circadian phase, phase angle, or period) between individuals of different *CLOCK* 3111C/T genotypes.

This prospective study included 179 healthy individuals who had previously completed a physiological study in the Division of Sleep Medicine at Brigham and Women's Hospital across all seasons from 2001 to 2010.

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Table 1. Demographic data, Morningness-Eveningness Questionnaire (MEQ) score, habitual sleep timing, and chronobiological measures for the 2 allelic groups: C and non-C carriers; and the 3 genotypic groups: T/T, C/T , and C/C.

	*p*1	*n*	C Carriers C/T and C/C	*n*	Non-C Carriers T/T	*n*	C/T	*n*	C/C	*p*2
Age (y)	0.911	74	27.60 ± 12.60	105	27.40 ± 11.20	67	27.70 ± 13.00	7	26.30 ± 8.50	0.949
Male:female (*n*)	NA	74	51:23	105	73:32	67	46:21	7	5:2	NA
Race[a] (%)	NA	74	77	105	73	67	73	7	100	NA
MEQ	0.902	74	51.95 ± 12.80	105	52.20 ± 12.50	67	51.95 ± 13.00	7	52.00 ± 16.40	0.992
Bed time (h)	0.887	74	2356 ± 0130	105	2354 ± 0142	67	2355 ± 0130	7	2411 ± 0130	0.898
Wake time (h)	0.543	74	0815 ± 0124	105	0823 ± 0130	67	0818 ± 0124	7	0747 ± 0112	0.557
CBT phase (h)	0.334	42	0519 ± 0148	57	0454 ± 0218	37	0528 ± 0148	5	0409 ± 0147	0.263
CBT phase angle	0.174	42	2.90 ± 1.20	57	3.28 ± 1.50	37	2.80 ± 1.20	5	3.64 ± 0.55	0.171
CBT period	0.262	30	24.18 ± 0.20	39	24.13 ± 0.20	28	24.18 ± 0.20	2	24.21 ± 0.21	NA
Melatonin phase (h)	0.752	40	2254 ± 0142	48	2230 ± 0200	35	2257 ± 0142	5	2235 ± 0159	0.925
Melatonin phase angle	0.053	40	1.28 ± 0.80	48	1.72 ± 1.10	35	1.37 ± 0.80	5	1.78 ± 1.10	0.149
Melatonin period	0.203	29	24.21 ± 0.20	39	24.15 ± 0.20	27	24.21 ± 0.20	2	24.16 ± 0.20	NA

Mean ± standard deviation. Bed times, wake times, and phase measures for both core body temperature (CBT) and melatonin are listed as clock hour. CBT phase angle is calculated as the difference between CBT nadir and habitual wake time/lights-on; melatonin phase angle is the difference between $DLMO_{25\%}$ and habitual bed time/lights-off. *p*1 values denote comparisons between allelic groups (C v. non-C carriers), and *p*2 values are from comparisons among the genotypes (T/T, C/T, and C/C).
a. Data are given as percentage of whites.

The study was approved by the Partners Health Care Human Research Committee and conducted according to the principles stated in the Declaration of Helsinki. Subjects were recruited for participation if they had completed an in-patient phenotyping protocol, and separate written informed consent was obtained for enrollment in the genetic study. Each completed the MEQ, which was scored according to the categories defined in the original publication (Horne and Östberg, 1976), and donated a blood sample from which DNA was extracted and genotyped for the *CLOCK* 3111T/C polymorphism (rs10801260). In a subset of 41 subjects, the MEQ score was used as selection criterion for enrollment into the in-patient study, and those individuals were all morning or evening types. For 2 to 3 weeks prior to admission, subjects were required to maintain a stable sleep schedule, which was self-selected and based on their habitual sleep times. Subjects who participated in studies where circadian phase was measured were required to keep an 8-hour sleep schedule. Compliance was verified with daily time-stamped calls and confirmed with wrist actigraphy for at least 1 week. Habitual bed times and wake times were calculated from the mean call-ins of the week prior to admission.

Some of the protocols included a constant routine procedure during which circadian phase was assessed using the core body temperature (CBT) nadir (Brown and Czeisler, 1992) and/or the dim-light melatonin onset ($DLMO_{25\%}$) as described elsewhere (Cain et al., 2010). Phase angle of entrainment was defined as the interval between CBT nadir and wake time/lights-on and/or $DLMO_{25\%}$ and bed time/lights-off. For 35 subjects, their study also included a forced desynchrony procedure from which circadian period was determined using CBT and/or melatonin (Czeisler et al., 1999). For an additional 33 subjects, we could assess circadian period but not circadian phase (Suppl. Fig. S1). Mean age ± SD of the group was 27.46 ± 11.80 years (range = 18-70) and was 27.78 ± 12.15 years in the 55 females and 27.32 ± 11.69 years in the 124 males. The racial composition was 74% white, 12% Asian, 7% black/African American, and 7% reporting more than 1 or unknown race. The frequencies of the 3111C (23%) and T (77%) alleles did not deviate significantly from the Hardy-Weinberg equilibrium calculated for this allele (χ^2, $p = 0.85$). Frequencies of the C/C, C/T, and T/T genotypes were 3.9% ($n = 7$), 37.4% ($n = 70$), and 58.7% ($n = 105$), respectively, similar to a previous study with a similar racial composition (Pedrazzoli et al., 2007).

We compared MEQ score, age, habitual bed time and wake time, circadian phase, phase angle of entrainment, and circadian period, where available, among genotypes (C/C, C/T, and T/T) and between allelic (C allele and non-C allele) groups using mixed model analysis of variance (ANOVA) (SAS 9.1, SAS Institute, Cary, NC). Due to the small number of individuals with the C/C genotype, our analysis focused on comparing the 2 allelic groups, as was done in the original publication of this polymorphism (Katzenberg et al., 1998).

MEQ score, sleep timing, and circadian measures are shown in Table 1. MEQ scores were normally distributed when grouped by C allele carriers (Fig. 1, top panel) and by genotype (Fig. 1, bottom panel) and did not vary among allelic ($p = 0.902$) or genotypic ($p = 0.992$) groups. Habitual bed time and wake time did not differ between allelic groups (Table 1 and Fig. 2) or between

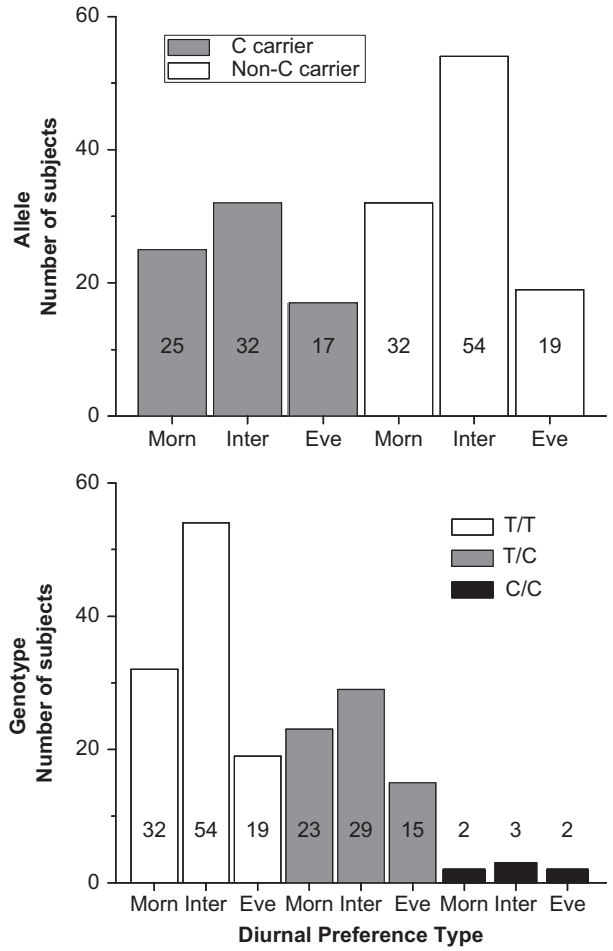

Figure 1. Distribution of diurnal preference in *CLOCK* 3111 C and non-C allele carriers and genotypes. The upper panel shows the distribution of morning, intermediate, and evening types by allelic group (C allele [gray bars] and non-C allele [open bars] carriers) and by genotype in the lower panel (T/T [open bars], T/C [gray bars], and C/C [black bars]. Numbers within/above the bars indicate number of subjects.

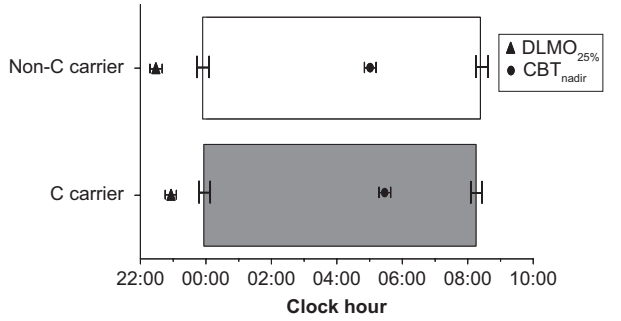

Figure 2. Habitual sleep times and timing of circadian phases for *CLOCK* 3111 C allele and non-C allele carriers. Data are shown as mean ± SEM. Horizontal bars represent the timing of the habitual sleep episode, triangles represent the timing of the circadian phase of melatonin onset ($DLMO_{25\%}$), and circles represent the timing of the circadian phase of the core body temperature nadir (CBT_{nadir}).

genotypes (Table 1). There were no differences in the timing of circadian phase measures between C allele and non-C allele carriers nor between genotypes (Table 1). The phase angle between CBT nadir and habitual wake time/lights-on was not different between allelic groups or by genotype (Table 1). The phase angle between $DLMO_{25\%}$ and bed time/lights-off suggested a trend for a shorter interval in C allele carriers ($p = 0.053$), but when the factors age and sex were included in our statistical model, this was no longer the case ($p = 0.463$). The phase angle between $DLMO_{25\%}$ and bed time/lights-off was not different among the genotypes (Table 1). Circadian period did not differ between allelic groups (Table 1). We did not compare circadian period by genotype due to the small number in the C/C group from whom circadian period was collected ($n = 2$).

The goal of the current study was not simply to examine the association of the *CLOCK* 3111C allele with diurnal preference but to extend previous findings by investigating the influence of this polymorphism on sleep timing and chronobiological measures of circadian phase, phase angle of entrainment, and period. In our study population, MEQ score did not associate with the *CLOCK* 3111T/C genotype, and the frequency of the 3111C allele was not significantly different between morning and evening types. We found no association between the 3111C allele and habitual sleep timing, circadian phase, phase angle of entrainment, or circadian period. Given that none of the measures showed differences between the genotypic or allelic groups, this polymorphism does not appear to be a strong determinant of human diurnal preference.

We did not replicate the original finding of an association between the 3111C/C genotype with evening preference (Katzenberg et al., 1998), which has been replicated in only one subsequent report in a study of 421 Japanese subjects (Mishima et al., 2005). This may be related to differences in the study populations, selection criteria, and/or analytic methods. While our sample size was relatively small for a genetic study, it was similar to or larger than the other studies of this SNP (Johansson et al., 2003; Mishima et al., 2005; Pedrazzoli et al., 2007; Robilliard et al., 2002). Furthermore, our finding of no association between diurnal preference and the 3111C/T SNP is consistent with several other studies (Johansson et al., 2003; Lee et al., 2007; Pedrazzoli et al., 2007; Robilliard et al., 2002), and our phenotyping (which included documented sleep timing and circadian parameters) was more extensive than reliance on a one-time self-report of diurnal preference. Given the increasing number of studies seeking to find a genetic

basis for individual differences in human sleep and circadian rhythmicity, our findings suggest that caution should be used when inferring phenotypic information from a questionnaire.

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NOTE

Supplementary material for this article is available on the *Journal of Biological Rhythms* website: http://jbr.sagepub.com/supplemental.

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