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ORIGINAL ARTICLE

A variable-number tandem repeat polymorphism in *PER3* is not associated with chronotype in a population with self-reported sleep problems

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Abstract

We examined whether a variable-number tandem repeat (VNTR) polymorphism in the circadian clock gene *PER3* was associated with subjective ratings of sleep and diurnal preference in a Romanian population with high levels of self-reported sleep problems. Individuals, self-reporting to their GP for sleep disturbances, completed a battery of validated scales that assess the presence of insomnia, sleep quality and diurnal preference and had their PER3 VNTR genotype determined. We found no significant differences in chronotype, sleep quality or other psychometric measures according to PER3 VNTR and conclude that diurnal preference or self-reported sleep measures are not associated with PER3 genotype in this population.

Key words: circadian gene, diurnal preference, insomnia, sleep.

INTRODUCTION

Circadian clock genes, which underpin the functioning of the circadian timekeeping system, have associations with diurnal preference, sleep and several psychiatric, neurological and metabolic diseases.[1,2] Some of the strongest evidence for such links comes from *PER3*, a member of the *PERIOD* clock gene family, identified as a predictor of diurnal preference according to the genotype of the variable-number tandem repeat (VNTR; short version with 4 repeats, long version with 5 repeats) present in the human version of this gene.[3] In a British sample the shorter allele (*PER3*^{4}) was associated with evening preference and delayed-phase sleep syndrome, whereas the longer one (*PER3*^{5}) was associated with morning preference.[4] In contrast, other studies have found that the frequency of the longer allele was higher in Brazilians with delayed-phase sleep syndrome[5] or that there was no link between *PER3* and chronotype in North Americans.[6] Associations of the *PER3* VNTR polymorphism with sleep homeostasis in differences of sleep–wake structure, sleep propensity, and cognitive performance after sleep loss have also been noted.[7] The polymorphism has also been proposed as a predictor for therapeutic response in insomnia[6] and as a marker for individual items on diurnal preference instruments.[8]

Sleep parameters and diurnal preference may be assessed with both objective and subjective methods. There are a number of well-validated psychometric instruments that assess such factors including the Pittsburgh Sleep Quality Index[9] and the Composite Scale of Morningness.[10] In the current study we set out to ascertain whether the VNTR polymorphism in *PER3* was associated with such subjective ratings of sleep and diurnal preference in a Romanian population with high levels of self-reported sleep problems. To date no other

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study has examined the potential links between diurnal preference and *PER3* VNTR specifically in populations with sleep problems, an issue that may be of consequence given the interplay between sleep disturbance and chronotype.[11]

METHODS

Participants were recruited from those with sleep problems that presented at their general practitioner in Baia Mare, Romania, and from their acquaintances with self-reported sleep problems. Those suffering from major conditions that affect sleep (e.g., severe heart failure, psoriasis, stroke, Parkinson's disease, epilepsy, chronic obstructive pulmonary disease, chronic or acute psychosis or dementia) were excluded. The study was approved by the Ethics Committee of "Iuliu Haţieganu" Medicine and Pharmacy University, Cluj-Napoca. Participation was voluntary and anonymous. Patients received a letter from their GP inviting participation and explaining the nature of the research and ethical requirements for confidentiality. Once participants agreed to take part in the study, they were given a battery of questionnaires (Romanian translations of the Composite Scale of Morningness (CSM),[10] the Sleep Disorders Questionnaire (SDQ),[12] the Pittsburgh Sleep Quality Index (PSQI)[9] and demographic questions). All the participants were also asked to recruit adults from their acquaintances using snowball sampling. When the questionnaire was returned, once informed consent was obtained, blood for genotyping was collected by finger-sticking with a lancet, then dropped and dried on FTA cards (Whatman). A 2-mm punch from the card was used for DNA extraction according to the manufacturer's instructions. The DNA was amplified using Taq polymerase (Qiagen, UK), using primers described by Ebisawa *et al*.[3] and amplification conditions as in Goel *et al*.[6] Fragments were visualized on 2% agarose gel stained with ethidium bromide, following separation at 100 V in tris-borate electrophoresis buffer. The gels were viewed under a *trans*-illuminator.

To examine the statistical significance of the differences, the likelihood ratio and Kruskal–Wallis test were used, with $P < 0.05$ considered significant. Co-variate analysis was by means of ANCOVA. Data analysis was performed using SPSS (version 16.0.2). Allele frequencies of the group were calculated by direct counting from the genotypes observed and tested for the Hardy–Weinberg equilibrium (HWE) with HWSIM software (available at http://krunch.med.yale.edu/hwsim/) and 100 000 simulations.

RESULTS

One hundred and fifty-four adults were given the questionnaires, but only seventy-two (46.7%) agreed to provide blood for genotyping. Forty (55.5%) were men and thirty-two (44.5%) women, with a mean age of 48.6 ± 12.4 years. Most of the participants were homozygotes for the longer allele ($n = 31$, 43.1%) and the *PER3*^{5} allele frequency was 0.61. There was no statistical deviation from the HWE in the sample. Chi-square analysis did not reveal any alterations in the distribution of *PER3* genotypes between insomnia subtype or chronotype (details in Table 1) or between genotype and classification according to the PSQI (poor/good sleeper).

Table 1 Basic demographics, insomnia type and diurnal preference according to *PER3* VNTR genotype

		PER$^{4/4}$	*PER*$^{4/5}$	*PER*$^{5/5}$	Total
Sex	Female	5 (15.6%)	13 (40.6%)	14 (43.8%)	32 (44.5%)
	Male	10 (25.0%)	13 (32.5%)	17 (42.5%)	40 (55.5%)
Age		49.5 ± 10.2	47.0 ± 14.5	49.5 ± 11.6	48.6 ± 12.4
Insomnia†	Chronic insomnia	4 (16.7%)	9 (37.5%)	11 (45.8%)	24 (33.3%)
	Acute insomnia	3 (21.4%)	6 (42.8%)	5 (35.8%)	14 (19.4%)
	Symptoms	8 (23.5%)	11 (32.4%)	15 (44.1%)	34 (47.2%)
Diurnal preference‡ (25/75 percentile)	Eveningness	3 (18.8%)	3 (18.8%)	10 (62.5%)	16 (22.3%)
	Neither	8 (21.1%)	16 (42.1%)	14 (36.8%)	38 (52.7%)
	Morningness	4 (22.2%)	7 (38.9%)	7 (38.9%)	18 (25.0%)
Total		15 (20.8%)	26 (36.1%)	31 (43.1%)	72 (100%)

Values for age and BMI represent means ± SD; other values are total number of participants in each group with the percentage of the study sample represented. †Chronic and acute insomnia as defined by International Classification of Sleep Disorders, 2nd ed. ‡The cut-off scores were determined by three age groups: under 30, 30–45 and above 45, as set in the validation study of the CSM: 29/39, 34/43 and 39/46, respectively.

Long-allele homozygotes scored lower in each of these scales, but there were no significant differences. When analyzing individual items in these scales, we found that homozygotes for the longer allele reported difficulties in getting up ($n = 13$, 18.1%) more frequently than those of the shorter one ($n = 2$, 2.7%) or heterozygotes ($n = 3$, 4.2%), and the differences reached the significance level ($X = 17.4$, $P = 0.008$).

To address potential attenuating effects of age on *PER3* genotype influence on diurnal preference, an analysis of co-variance was run with CSM score as the dependent variable, *PER3* as the independent variable and age and sex as co-variates. This indicated no effect of genotype on CSM score ($F_{(2, 67)} = 1.01$, $P = 0.37$). Further, when chi-square analysis was conducted for genotype versus chronotype in three age groupings (<30, 30–45, >45) there were no significant associations. To further examine whether the effects of *PER3* VNTR on diurnal preference may be masked by the influence of sleep problems, we controlled for insomnia group and PSQI scores as co-variates in an ANCOVA with CSM score as the dependent variable and genotype as the independent variable, and no significant effect was seen ($F_{(2, 67)} = 0.46$, $P = 0.63$).

DISCUSSION

These preliminary findings of this pilot study do not support the hypothesis that *PER3* genotype is associated with diurnal preference or self-reported sleep disturbance in a sample selected due to sleep problems. Despite prior UK[4] and Brazilian[5] studies showing that the *PER3* VNTR polymorphism is associated with diurnal preference, we found no such association, although this may well be due to the particular nature of the study sample in the present work. Further, a previous study on a US sample also failed to find an association between diurnal preference and *PER3* genotype.[6] Other possible explanations are small sample size or ethnic differences in study populations, as well as different instruments used for assessing the diurnal preference: both Goel[6] and ourselves used the CSM and smaller samples. There is also the possibility that sleep problems mask effects of genotype on chronotype, although previous findings in Romanian populations do not report significant associations between measures of sleep quality or disturbance and CSM scores,[13] nor are there effects of genotype on CSM score when sleep disturbance is controlled for in the present study. We have also controlled for the potential attenuating effects of age on *PER3* genotype effects on diurnal preference,[14] and do not find significant differences. We report a significant effect of genotype on self-reported difficulty getting up in the morning, an item on the Horne–Östberg Questionnaire that did not significantly discriminate between *PER3* genotypes in a previous study.[8] The study of Ellis *et al*.[8] did find a strong discrimination for *PER3* genotype according to whether an alarm clock was required in the morning, and seeing as this item is not on the CSM, perhaps there is a common factor between these findings and the difficulty getting up reported here.

Although the *PER3*^{4} allele frequency reported here is unusually low compared to interpolated results for this geographic area, there has been considerable variation in *PER3* VNTR reported among global populations,[15] with the lowest frequency in Papua New Guineans (0.17) and highest in Mongolians (0.89). It is hypothesized that differences in latitude could contribute in an important manner to the selection of these polymorphisms in different geographical zones, but no conclusive evidence was found.[15,16] After performing a complex analysis on the distributions of the *PER2*, *PER3*, *CLOCK* and *ARNTL1* and *ARNTL2* polymorphisms worldwide, it has been concluded that is unlikely that a pattern of natural selection has shaped the evolutionary process of these genes, suggesting that a random process is involved, possibly genetic drift.[17]

It is worth noting a number of limitations of the present pilot study. First is the small number of participants, and the fact that this was a self-selected study population. Further study will need to be undertaken to corroborate these findings in larger samples of those with sleep problems, and compared to study samples without significant sleep disturbances. Alongside, as noted above, the facts that sleep disturbance and diurnal preference were self-assessed with psychological instruments that are not as reliable as objective measures, and that the specific causes of sleep disturbances were not delineated, point to directions in which future studies may go. However, given that this is the first study of the *PER3* VNTR in a population with high levels of sleep complaints, we feel that the findings are novel and indicate that pre-existing sleep complaints may impact on the relationship between *PER3* VNTR genotype and diurnal preference.

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