Study of a Functional Polymorphism in the PER3 Gene and Diurnal Preference in a Colombian Sample

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Abstract: Polymorphisms in human clock genes have been evaluated as potential factors influencing circadian phenotypes in several populations. There are conflicting results for the association of a VNTR in the PER3 gene and diurnal preference in different studies. The objective of this study was to investigate the association between diurnal preference and daytime somnolence with the PER3 VNTR polymorphism (rs57875989) in healthy subjects from Colombia, a Latin American population.A total of 294 undergraduate university students from Bogotá, Colombia participated in this study. Two validated self-report questionnaires, the Composite Scale of Morningness (CSM) and the Epworth Sleep Scale (ESS) were used to assess diurnal preference and daytime somnolence, respectively. Individuals were genotyped for the PER3 VNTR using conventional PCR. Statistical comparisons were carried out with PLINK and SNPStats programs.

The PER3 VNTR polymorphism was not associated with either diurnal preference or daytime somnolence in this population. No significant differences in mean scores for those scales were found between PER3 VNTR genotypes. In addition, there were no differences in allelic or genotypic frequencies between chronotype categories. This is consistent with several negative findings in other populations, indicating that the proposed influence of this polymorphism in diurnal preference, and related endophenotypes of neuropsychiatric importance, needs further clarification. This is the first report of molecular genetics of human circadian phenotypes in a Spanish-speaking population.

Keywords: Chronobiology, endophenotypes, genetics, Latin America, molecular genetics, neuropsychiatric neurogenetics, sleep.

INTRODUCTION

Diurnal preference is a well-known circadian phenotype based on the favorite timing of daily activities [1]. There is a high variability for this preference among the general population, which has been the basis for a further categorization of this characteristic in a phenotype known as chronotype [1]. Several polymorphisms in classical "clock" genes, such as Period homolog 3 (*PER3*) or Clock homologue (CLOCK), have been studied as possible genetic correlates of chronotypes and other circadian phenotypes in healthy humans [2-7].

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A variable-number tandem repeat (VNTR) polymorphism (rs57875989) in the *PER3* gene (located on chromosome 1p36.23), consisting of two alleles of four or five tandem 54 bp repeats (coding for a region of 18 amino acids in exon 18), has been evaluated as a potential genetic factor for chronotypes and other circadian phenotypes [2, 5]. However, previous findings in several populations have been inconsistent [2, 6, 7] and it has been hypothesized that intrinsic factors of the populations, such as latitude and genetic background, may have an effect on these associations [1, 6].

The aim of this study was to perform a replication of the previous studies of the association of VNTR polymorphism in *PER3* and diurnal preference in a sample of healthy young subjects from Bogotá, Colombia, a South American population.

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MATERIALS AND METHODOLOGY

Subjects

A total of 294 healthy undergraduate students (66.6% female, mean age [SD] was 20.5 [2.7] years, range: 18-30 years) of two private universities in the capital city of Colombia (Bogotá) (04º 38′ N, 74°05′ W) participated voluntarily in the study. Additional 24 samples (7.5% of the total sample) were excluded from the final analysis due to incomplete phenotypic or genetic data. The population living in Bogotá is composed of a European genetic background with some historical admixture with Amerindians [8]. Subjects with self-report of neuropsychiatric diseases, including sleep disor-ders, were excluded. This study was approved by the Institutional Ethics Committee of each participant institution and all subjects provided written informed consent [8].

Phenotypic Evaluations

To measure and characterize the circadian phenotypes each participant filled out two self-report scales, previously validated and widely used in other populations [9]: Composite Scale of Morningness (CSM) and Epworth Sleep Scale (ESS) [10, 11].

Composite Scale of Morningness (CSM)

The CSM scale consists of 13 questions regarding the preferred times for mental and physical activity as well as the subjective alertness of the individuals. The total score of the CSM questionnaire is obtained by adding the individual scores of all the items, and it ranges from 13 (extreme eveningness) to 55 (extreme morningness). CSM has shown a strong correlation with other commonly used scales, such as the Horne-Östberg Morningness-Eveningness Questionnaire (given the fact that some of the scales consist of similar items), and with objective measures derived from actigraphy [12]. In this study, we have used the validated version in Spanish of the CSM [13], that has been previously used in a Colombian sample [11]. The internal consistency of this scale (Cronbach's alpha) for the present sample was 0.722.

Epworth Sleep Scale (ESS)

The ESS is a widely validated tool to quantify the general level of daytime sleepiness in general population) [10, 11]. It is a self-administrated questionnaire composed by eight situations of daily life that subjects have to range from 0 to 3 on their propensity to fall asleep. It ranges from 0 to 24, with higher scores related to more sleepiness. This scale has been used in different studies of genetics of circadian phenotypes [14-16]. We used the validated version in Spanish of the ESS [11], that has also been used before in a Colombian sample [17]. The internal consistency (Cronbach's alpha) in this sample was 0.716.

Additionally all participants completed a self-administered general questionnaire that was used to collect socio-demographic variables (age, gender and personal history of neuropsychiatric disorders) and additional sleep parameters, such as self-report number of Hours of Sleep during Working Days (HSWD) and number of Hours of Sleep during Weekends (HSW).

DNA Extraction and Genotyping

Genomic DNA was isolated from peripheral blood of participants using a standard salting-out method [8]. Genotyping of the *PER3* VNTR (rs57875989) was performed using a touchdown PCR with a final annealing temperature of 56ºC, using the primers and conditions previously described [7]. Two primers were used, which amplify fragments of 310 bp and 364 bp for 4 and 5 alleles, respectively. PCR products were separated in a 2% agarose gel at 140V and visualized with ultraviolet light after SYBR Safe (Invitrogen, Carlsbad, CA, USA) staining. A random subsample (10% of subjects) was reanalyzed for the *PER3* polymorphism to verify consistency in the genetic results. Additionally, all genotypes were checked by two different researchers in order to confirm and validate the results.

Statistical Analysis

Allele and genotype frequencies and Hardy-Weinberg Equilibrium (HWE) were calculated with the SNPStats software [18]. SNPStats was also used for the analysis of the association of *PER3* genotypes with quantitative measures of circadian phenotypes (CSM and ESS scores, HSWD and HSW), using a linear regression model, corrected by sex and age. Codominant, dominant, recessive, overdominant, and log-additive models were tested.

In order to compare the extreme groups of diurnal preferences, the 25^{th} and 75^{th} percentiles of the CSM score distribution were used to define the cut off points to classify the individuals in Evening (values ≤36), Neither (values from 37 to 42), or Morning type (values ≥43) [13]. Similarly, the 75^{th} percentile of the ESE distribution was used to determine the cut-off point (14 points) to classify the population in Normal (values ≤14) or High (values ≥15) categories for diurnal somnolence. The 25^{th} and 75^{th} percentiles were used as cut off points in order to have categories with sample sizes that are suitable for carrying out statistical comparisons of genotype and allele frequencies.

PLINK sofeware (Version 1.01) [19] was used to evaluate the association of *PER3* polymorphism with categories of morning preference and diurnal somnolence, using a $\chi 2$ test (comparisons of genotype and allele frequencies between categories). For the comparison of genotype frequencies, several models were tested (dominant, recessive and genotypic). A nominal value of $p<0.05$ was considered as statistically significant.

RESULTS

In this study, we found an allele frequency of 0.8 for the 4 allele of the PER3 VNTR, with the most common genotype being the homozygous 4/4 (0.65). The frequencies of the genotypes for this polymorphism were in Hardy–Weinberg equilibrium (p=0.36). In our sample, the scores for the CSM ranged from 24 to 54, with a mean of 39.74 (SE: 0.32) and the ESS had scores from 0 to 21 with a mean of 10.89 (SE: 0.25). In our sample, the scores for the CSM ranged from 24 to 54, with a mean of 39.76 (SE:0.33) and

Table 1. Association of the *PER3*-VNTR with (A) Scores of the Composite Scale of Morningness (CSM) and its categorization in morning/neutral/evening chronotypes and (B) Scores of the Epworth Sleep Scale (ESS) and its categorization in High/ Low somnolence.

(A)

***PER3*-VNTR**		**Chronotypes**			***p value***	**Composite Scale of Morningness Scores** Mean (SE)	***p value***
		Morning Type n (%)	**Neutral Type** n (%)	**Evening Type** n (%)			
Genotypes	4/4	41 (60.3)	108 (65.9)	43 (69.4)	0.58[c]	39.55 (0.38)	0.37[a]
	4/5	25 (36.8)	48 (29.3)	15 (24.2)		40.48 (0.63)	
	5/5	2 (2.9)	8 (4.9)	4 (6.5)		38.79 (1.56)	
	Total	**68 (100)**	**164 (100)**	**62 (100)**		39.74 (0.32)	
Alleles	4	0.81	0.80	0.79	0.58[c]		
	5	0.19	0.20	0.21			

(B)

***PER3*-VNTR**		**Diurnal Somnolence**		***p value***	**Epworth Sleep Scale Scores** Mean (SE)	***p value***
		Normal n (%)	**High** n (%)			
Genotypes	4/4	148 (64.6)	44 (67.7)	0.15[b]	10.94 (0.32)	0.32[a]
	4/5	69 (30.1)	19 (29.2)		11.02 (0.42)	
	5/5	12 (5.2)	2 (3.1)		9.43 (1.05)	
	Total	**229 (100)**	**65 (100)**		10.89 (0.25)	
Alleles	4	0.80	0.82	0.13[c]		
	5	0.22	0.18			

a. *p value* derived from a linear regression model (codominant model)
b. *p value* for differences in genotype frequencies between categories (trend model)
c. *p value* for differences in allele frequencies between categories

the ESS had scores from 0 to 21 with a mean of 10.93 (SE:0.26).

The linear regression model, corrected by sex and age, showed no differences in scores for the CSM or ESS scales between carriers of the *PER3* genotypes (Table **1**), under several genotypic models. Self-reported hours of sleep (HSWD and HSW) were not associated with the *PER3* VNTR polymorphism in our sample (p= 0.25 and p=0.49).

Moreover, the distribution of the genotypes and alleles was not significant between the extreme morning preference and somnolence categories (Table **1**), under several genotypic models. Inclusion of additional covariates, such as sex, did not help to identify positive associations (data not shown).

DISCUSSION

This is the first study carried out in the Colombian population regarding the association of a variant in a clock gene (*PER3*) and circadian phenotypes (diurnal preference and diurnal somnolence). In this study we did not confirm the association between the *PER3* VNTR and neither of the phenotypes measured, using different types of analysis and genetic models.

Both positive and negative results have been previously reported for this association. Few studies in Caucasian populations have confirmed this association [2, 6, 16] while several others have failed to replicate it [3, 20]. Colombia is a South American country that lies near to the Equator. These geographic characteristics make it different from the previous populations studied; there are no major seasonal changes through the entire year. It may explain, in addition to differences in genetic background, the lack of association with morning preference that we found here.

This is the first report of circadian phenotypes related to clock genes in a Spanish speaking population and in subjects living in the equatorial region. In addition, it highlights the importance of analyzing South American populations as a complement to the European populations commonly studied, to generate a better understanding of their role in circadian phenotypes [6]. A meta-analysis on this association should be carried out to have a real quantitative measure of the effect of this polymorphism in different populations. Future analysis of other candidate functional polymorphisms on the *PER3* gene and other clock genes could be helpful for the genetic analysis of circadian phenotypes [1], and related endophenotypes of neuropsy-chiatric importance, in healthy humans.

CONCLUSION

The PER3 VNTR polymorphism was not associated with either diurnal preference or daytime somnolence in a sample from the Colombian population. No significant differences in mean scores for those scales were found between PER3 VNTR genotypes. In addition, there were no differences in allelic or genotypic frequencies between chronotype categories. This is consistent with several negative findings in other populations, indicating that the proposed influence of this polymorphism in diurnal preference needs further clarification. This is one of the first reports of molecular genetics of human circadian phenotypes in a Spanish-speaking population and in subjects living in the equatorial region.

CONFLICT OF INTEREST

The authors confirm that this article's content has no conflicts of interest.

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