Association between specific diurnal preference questionnaire items and *PER3* VNTR genotype

Jason Ellis[1]

Malcolm von Schantz[2]

Kay H S Jones[2]

Simon N Archer[2]

1) Division of Community Based Sciences, Section of Psychological Medicine, University of Glasgow, Glasgow G12 0XH, UK

2) Faculty of Health and Medical Sciences and Surrey Sleep Research Centre, University of Surrey, Guildford GU2 7XH, UK

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Corresponding author:

Dr. Jason Ellis

Division of Community Based Sciences

Section of Psychological Medicine

University of Glasgow

Glasgow

G12 0XH

UK

Telephone: +44 141 2327700

Telefacsimile: +44 141 2327697

E-mail: j.ellis@clinmed.gla.ac.uk

Abstract:

Although significant intraindividual differences in self-reported diurnal preference, as measured by validated questionnaires, exist, the relative contribution of exogenous and endogenous factors to self-reported diurnal preference largely remains to be investigated. The present study examined which items from the Horne-Östberg (HÖ) questionnaire of diurnal preference were better at predicting genotypes in the variable number tandem polymorphism (VNTR) in the coding region of the gene *PER3*. This polymorphism has previously been reported to associate with diurnal preference, sleep parameters, and cognitive performance markers following sleep deprivation. Participants (n = 240, selected from a previously studied population) had completed the HÖ questionnaire and provided a DNA sample, which was genotyped with regard to the *PER3* VNTR. A multinomial logistic regression showed that four items significantly increased prediction accuracy between the two homozygotic genotypes, with homozygotes for the longer variant of the gene ($PER3^{5/5}$) associated with answers indicating a stronger morning preference than those chosen by homozygotes for the shorter variant ($PER3^{4/4}$). Only one item, the question of whether the respondent required an alarm clock, discriminated between all three genotypes. Moreover, when the items were divided into those with the strongest genetic association and those with the weakest genetic, there was a significant relationship between age and the questions not predicting genotype, but not between age and genotype-predictive questions. This may explain previous findings regarding age-related differences in self-reported diurnal preference. These findings could facilitate the future development of diurnal preference scales specifically tailored to the study of specific biological parameters.

Key Words: Behavioural genetics; Clock genes; Diurnal preference; Genetic polymorphism

Introduction

Diurnal preference refers to the preferred timing of sleep and waking activities and individual differences in this domain have been shown to result in task-related variations in psychophysiological responses including cognitive failures, heart rate variability, and mood regulation (Hirata et al., 2007; Matthews, 2006; Willis et al., 2005). The most widely used self-reported measure of diurnal preference, the Horne-Östberg morningness/eveningness questionnaire (Horne and Östberg, 1976), has also been shown to correlate with several mechanisms involved in the regulation, and timing, of the sleep-wake cycle, including the secretion of melatonin (Duffy et al., 1999; Taillard et al., 2003), providing further validation of its use.

Whilst intrinsic circadian period is not believed to change with age (Czeisler et al., 1999, Duffy and Czeisler, 2002), the phase relationship with the sleep-wake cycle does, so that with increasing age, sleep onset and offset occur earlier (Duffy et al., 1999). A corresponding increase in morningness with age has been reported in studies of diurnal preference (Carrier et al., 1997). Additionally, a gender difference has been reported, with women tending more towards morningness (Vink et al., 2001).

However, where associations between the HÖ and endogenous markers of sleep-wake timing have been consistently demonstrated, these associations have tended to be modest, indicating either that other factors, not considered within the parameters of the HÖ, are related to endogenous timing (i.e. low sensitivity within the HÖ), or that the HÖ contains items unrelated to endogenous timing (low specificity). In terms of

sensitivity, theoretical saturation of the HÖ was reached during its development (Horne and Östberg, 1976), thus suggesting that the issue may not be under-inclusion.

Diurnal preference, both extreme (Toh et al., 2001) and within the normal range (Katzenberg et al., 1998), has been reported to associate with polymorphism in the circadian clock genes that create daily oscillations within the cells of humans and other animals. We have studied a polymorphism in the *PER3* gene in this context. This polymorphism is a variable number tandem repeat within the coding region, which is repeated either four (*PER3*^{4}) or five (*PER3*^{5}) times, encoding proteins of different lengths (Ebisawa et al., 2001). We found an association between the *PER3*^{5} allele and morningness and between the *PER3*^{4} allele and eveningness (Archer et al., 2003), a finding that was replicated in a Brazilian population (Pereira et al., 2005). More recent data from a prospective study of homozygotes for the two genotypes indicate that the function of *PER3* may be more closely related to sleep and cognitive performance parameters than to the circadian pacemaker (Viola et al., 2007). In spite of there being no significant difference in circadian parameters such as melatonin and clock gene transcript levels in peripheral leukocytes (Archer et al., 2008), *PER3*$^{5/5}$ homozygotes had a significantly shorter sleep latency. EEG theta activity was elevated both during wakefulness and sleep in this group, whereas REM sleep was decreased and slow-wave sleep increased. Following sleep deprivation, they showed a considerable greater decline in cognitive performance tests during the latter part of the night (Groeger et al., 2008). These findings suggested the possibility that the association between the *PER3* VNTR and diurnal preference, as determined by the HÖ score, may reflect differences in sleep homeostasis more than differences within the circadian oscillator. In other words, the effects of the *PER3*^{5} allele may be

described as a higher sleep pressure. The significant but moderate association between extreme diurnal preference and *PER3* VNTR genotype found by us (Archer et al, 2003; Jones et al, 2006) and others (Pereira et al, 2005) may be entirely or in part due to this, rather than a difference in parameters such as circadian period length. The present study seeks to define further exactly which part of the HÖ questionnaire related most strongly to *PER3* VNTR genotype.

Materials and Methods

This study conformed to the standards outlined in the Declaration of Helsinki and international ethical standards for chronobiological research (Touitou et al., 2006), and was granted a favourable opinion by the University of Surrey Ethics Committee. Participants had previously been recruited at two separate studies in 2001 and 2004 (see Jones et al., 2007, for full methodology, including exclusion criteria). After completing questions regarding demographic details, participants completed the HÖ questionnaire of diurnal preference and provided a buccal swab for DNA extraction and genotyping (Archer et al., 2003). Out of a total 1,594 participants, 240 were selected, representing equally sized groups of extreme morning and evening preference, as well as intermediates.

Statistical Analysis

A multinomial logistic regression was used to examine the predictors of genotype grouping. This method allows a simultaneous analysis of categorical variables where there are at least three groups. The *PER3*$^{5/5}$ variant was chosen as the reference category (i.e. the comparison group) as it is the rarest of the three genotypes. Pearson product-moment correlations were used to examine associations between the HÖ sub-dimensions and age, and chi-square analyses were used to examine differences between scoring groups on genotype. All analyses were conducted using SPSS-v15 (SPSS, Chicago, IL).

Results

The final study group comprised 127 females (52.9%) and 113 males with a mean age of 40.2 (SD = 14.4) and a mean HÖ score of 51.8 (SD = 17.8). A multinomial logistic regression was performed in order to determine which items from the HÖ predicted group membership, with genotype (*PER3*$^{4/4}$, *PER3*$^{4/5}$, *PER3*$^{5/5}$) as the dependent variable, and *PER3*$^{5/5}$ as the reference category. In addition to each individual item on the HÖ, age and gender were also entered into the regression, to examine their prediction accuracy. Whereas the multinomial regression makes no assumptions about linearity and normality, it is sensitive to outliers. Therefore, two binary logistic regressions were conducted to test for outliers. The removal of cases with standardised residuals greater than 3, or Cook's distances greater than 1.0, did not significantly increase the overall model's prediction accuracy. Therefore, all cases were used in the final analysis.

The overall model was significant (chi-square 72.94, df = 42, $p<0.002$), with the accuracy of correctly predicting which variation of the *PER3* genotype the individual had increasing from 50.9% at chance level to 61.5% with the inclusion of the independent variables. Overall, five items from the HÖ were identified as significant predictors of genotype grouping (Items 2, 3, 9, 11, and 19); however, only Item 3 was a significant predictor of all three genotypes (i.e. whether someone would possess the *PER3*$^{4/4}$, *PER3*$^{4/5}$, or *PER3*$^{5/5}$). Furthermore, items 9, 11, and 19 significantly distinguished between *PER3*$^{4/4}$ and *PER3*$^{5/5}$, and item 2 significantly distinguished between *PER3*$^{4/5}$ and *PER3*$^{5/5}$ (Table 1). Demographic factors (i.e., age and gender)

did not significantly increase the detection accuracy of the model, nor were they independent predictors of genotype group membership.

Insert Table 1 here

When the items that had the strongest genetic component (i.e. 2, 3, 9, 11, and 19) were summed to form a single dimension, labelled *PER3* genotype-dependent diurnal preference ($\alpha = 0.85$), this did not correlate significantly with age ($r = 0.03$, $n = 240$, n.s.), whereas genotype-independent diurnal preference ($\alpha = 0.93$) — i.e. the summed scores of all the items which had the weakest genetic component — did ($r = 0.14$, $n = 240$, $p<0.05$). There were no gender differences in either the *PER3* genotypic-dependent ($t = -0.13$) or *PER3* genotypic-independent diurnal preference sub-scales ($t = 0.51$).

Splitting the *PER3* genotype-dependent diurnal preference dimension into low (range 3-11), mid (range 12—18), and high scores (range 19—25) showed that higher scorers were more likely to be $PER3^{5/5}$ (59.3% vs. 30.2% and 31.1%), mid scorers were more likely to be $PER3^{4/5}$ (43.4% vs. 25.5% and 22.2%), and lower scorers were more likely to be $PER3^{4/4}$ (43.4% vs. 26.4% and 18.5%) (Figure 1). Overall, a significant difference in the distribution of genotypes by scoring group (high, mid, or low) was observed (chi-square 18.60, df = 4, $p<0.001$).

Insert Figure 1 here

Discussion

The aim of the present study was to determine the relationship between the HÖ and *PER3* VNTR genotype. The results demonstrate that although five items significantly predicted genotype, only one item discriminated between all of them. This item relates to the need for an alarm clock to awaken in the morning, with more extreme scores increasing the chance of discriminating which genotype the individual possessed. The findings indicate that $PER3^{4/4}$ homozygotes are most likely to need an alarm clock in order to wake, and $PER3^{5/5}$ homozygotes least likely, the heterozygotes being intermediate. A closer examination of the items in the HÖ suggests that this is the only one that asks the participant to rate their reliance on an external time-giving cue. One item, number 2, which pertains to preferred bedtime, was the only one to discriminate exclusively between heterozygotes and $PER3^{5/5}$ homozygotes, with the latter preferring an earlier bedtime. An additional three items, numbers 9, 11, and 19, discriminated between the two homozygotic groups. Question 9 asks for preferred timing of physical exercise, with $PER3^{5/5}$ homozygotes favouring an earlier time for optimal physical performance. Question 11 presents the same query but pertaining to a mentally exhausting task, again with $PER3^{5/5}$ homozygotes preferring an earlier schedule. In item 19, where the participants are directly asked to define their own diurnal preference, $PER3^{5/5}$ homozygotes again indicated a stronger morning preference.

These findings are of considerable interest when viewed in relation to the recently published reports of the phenotypic correlates of the *PER3* VNTR, with $PER3^{5/5}$ homozygotes apparently living under a higher sleep pressure (Viola et al., 2007) and

suffering more from sleep deprivation in terms of their cognitive performance (Groeger et al., 2008). The findings reported in the present communication might either represent a morning preference in order to compensate for the effects of a higher sleep pressure, or an overt advantage during the earlier part of the day. In other words, it remains to be determined whether *PER*$^{5/5}$ homozygotes perform better in the morning following a normal night's sleep only relatively to other times of the day, or whether they have specific advantages relative to the other genotypes. It is of interest to note that the *PER3*^{4} allele dominates in the majority of ethnic populations studied to date, with the exception of Papua New Guinea, where the distribution is the opposite (Nadkarni et al., 2005). No significant selection pressure was found in favour of either of these alleles.

At best, only five items relate to genotype-dependent factors and the other 14 to factors independent of *PER3* genotype. This agrees with our previous research, which demonstrated age-related differences in the relationship between overall HÖ scoring and the *PER*3 VNTR (Jones et al., 2007). As age and gender are not predictors of genotype, it could be tentatively suggested that the previously observed age differences are more expressive of genotype-independent changes in timing preferences over the life span. This argument is further advanced by the finding that after splitting the HÖ into the strongest genetic components and the weakest ones, *PER3* genotype-independent diurnal preference was associated with age, whereas *PER3* genotype-dependent diurnal preference was not. Furthermore, when examining the distribution of genotypes, based on the range of scores from the *PER3* genotype-dependent dimension, a clear association between the scoring group (high, mid, or low) and the likelihood that the individual will have a specific genotype can be seen.

These associations are of moderate strength, as one would expect given that the traits examined by the selected questions are bound to be polygenic. Nonetheless, this finding may help in future research and practice when attempting to locate a sample with a specific genotype, as using the reduced-item questionnaire would double the odds of identifying the rarer *PER3*$^{5/5}$ homozygotes. This may be particularly useful in studies targeting effects of the polymorphism not related to diurnal or sleep parameters, such as the reported increased risk of breast cancer in premenstrual women associated with the *PER3*^{5} allele. It also indicates the possibility of designing a modified diurnal preference scale that, similarly to intrinsic circadian period (Czeisler et al., 1999), would be independent of age. Interestingly, gender differences have been reported in circadian period (Wever, 1984).

The data shown here indicate that the endogenous nature of most of the questions of the HÖ score make it robust enough to correlate selected questions more directly to genotype. They provide an interesting facet of the phenotypic effects of *PER3* VNTR genotype reported by Viola et al. (2007), and indicate the possibility of identifying the rare *PER3*^{5} homozygotes more efficiently for other studies. A similar approach may be used in the future to study the specific effects of other polymorphisms in genes related to circadian rhythms and sleep on specific aspects of diurnal preference.

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Table 1: Results from the Multinomial Logistic Regression

Item from the HO	Predicting differences between the *PER3*$^{4/4}$ and *PER3*$^{5/5}$		Predicting differences between the *PER3*$^{4/5}$ and *PER3*$^{5/5}$	
	Exp(B)	95% Confidence Interval	Exp(B)	95% Confidence Interval
1) Considering your own feelings, at what time would you get up if you were entirely free to plan your day?	0.70	0.31 - 1.56	1.27	0.58 - 2.80
2) Considering only your own feelings, at what time would you go to bed if you were entirely free to plan your day?	1.88	0.77 - 4.60	2.48*	1.01 - 6.10
3) If there is a specific time you have to get up in the morning, to what extent are you dependent on being woken up by an alarm clock?	2.73**	1.31 - 5.67	2.24*	1.09 - 4.62
4) Assuming adequate environmental conditions, how easy do you find getting up in the morning?	0.71	0.29 - 1.73	0.61	0.25 - 1.46
5) How alert do you feel during the first half hour after having woken in the morning?	0.92	0.41 - 2.06	0.97	0.44 - 2.14
6) How is your appetite during the first half hour after having woken in the morning?	0.71	0.41 - 1.23	0.99	0.57 - 1.71
7) During the first half hour after having woken in the morning, how tired do you feel?	1.34	0.60 - 3.01	0.68	0.31 - 1.49
8) When you have no commitments the next day, at what time do you go to bed compared to your usual bedtime?	1.12	0.58 - 2.18	1.55	0.80 - 3.01
9) You have decided to engage in some physical exercise. A friend suggests that you do this one hour twice a week and the best time for him is between 0700 and 0800h. Bearing in mind nothing else but your own inclinations, how do you think you would perform?	0.41*	0.18 - 0.96	0.63	0.28 - 1.45
10) At what time in the evening do you feel tired and in need of sleep?	0.78	0.36 - 1.70	0.80	0.37 - 1.71
11) You wish to be at your peak for a test	0.57*	0.34 - 0.93	0.72	0.44 - 1.19

which you know is going to be mentally exhausting and lasting for two hours. You are entirely free to plan your day, when would you do this task?				
12) If you went to bed at 2300h at what level of tiredness would you be?	1.05	0.68 - 1.63	1.29	0.83 - 2.01
13) For some reason you have gone to bed several hours later than usual, but there is no need to get up at any particular time the next morning. Will you:	0.67	0.36 - 1.23	0.87	0.47 - 1.58
14) One night you have to remain awake between 0400 and 0600h. You have no commitments the next day. Which suits you best:	1.28	0.74 - 2.22	1.12	0.65 - 1.92
15) You have to do hours physical work. Which hours would you prefer to do it between:	0.55	0.21 - 1.48	0.39	0.15 - 1.05
16) You have decided to engage in some physical exercise. A friend suggests that you do this between 2200 and 2300h twice a week. How do you think you would perform:	1.20	0.69 - 2.11	0.80	0.47 - 1.38
17) Suppose that you can choose your own work hours, but had to work five hours in the day. Which five consecutive hours would you choose:	1.61	0.74 - 3.53	1.28	0.59 - 2.78
18) At what time of day do you feel your best?	0.73	0.37 - 1.46	0.51	0.26 - 1.02
19) One hears of “morning” and “evening” types. Which do you consider yourself to be?	1.70*	1.04 - 2.78	1.45	0.90 - 2.34
Age	0.99	0.95 - 1.03	0.98	0.94 - 1.01
Gender	0.75	0.26 - 2.17	0.91	0.32 - 2.56

The reference category is: *PER3*$^{5/5}$

* = p<0.05

** = p<0.01

Figure 1: Percentage of sample, by genotype, on scores on the endogenous diurnal preference dimension. White bar = *PER3*$^{4/4}$, grey bar = *PER3*$^{4/5}$, black bar = *PER3*$^{5/5}$.