Novel Potential Link between Sleep Pattern Per3 Gene and those with Insomnia in the Bermuda Population

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Abstract

The objective of this study was to assess the correlation of the Per3 gene VNTR polymorphism to insomnia patients in Bermuda. Buccal swabs were taken, and DNA was extracted, after which the genotypes of volunteers were characterised by using polymerase chain reaction. There were 25 total volunteers (21 females, 4 males, aged 20-79) that participated in the pilot study. 15 control volunteers and 10 insomniac volunteers. All volunteers with insomnia were classified by a pre-determined ICD-10 classification. Controls were those without any ICD-10 insomnia diagnosis.

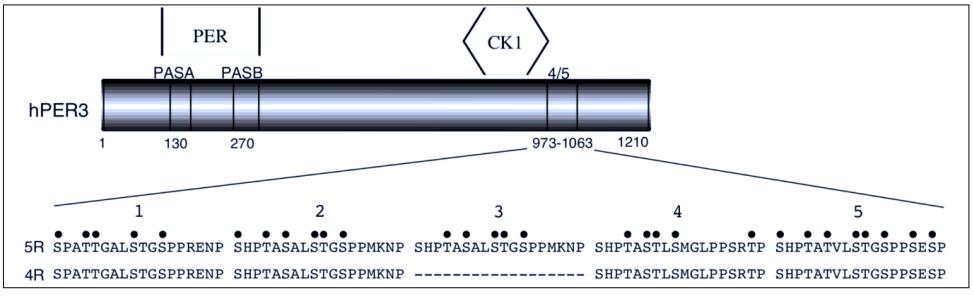
The frequency of the 4-repeat allele appears three times lower in insomniacs compared to the control group. However, this is not statistically significant in our sample size. When comparing our p-value of the Fisher's Exact Test to the cut off value of 5%, we see that there is not much difference, suggesting that a larger sample size could result in a significant result. When comparing the allele frequency for Bermudian insomniacs to the British patients with delayed sleep phase syndromes (DSPS), the 5-repeat allele is significantly higher (16 times greater) in Bermudian insomniacs. Thus, a larger sample size would distinguish if there is a statistical significance between those with insomnia and those without. Bermudian insomniacs vary distinctly in the Per3 allele frequencies to those in Britain with DSPS, suggesting a potential geographical or ethnic distinction.

KEY WORDS: *Per3 gene, insomniacs*

Introduction

Dijk and Czeisler (1995) indicate that the Per3 gene, or 'Period Circadian Regulator 3', codes for a protein that is involved in the regulation of the circadian clock, the body's internal time-keeping system. Per3 has a variable number of tandem repeats (VNTR) downstream of its CK1 domain (Figure 1). This yields two main Per3 alleles: the 4-repeat allele and 5-repeat allele. If a person is carrying two copies of the allele 4-repeat allele they may be classified as a night person as they may tend to have a higher preference for evening activities and stay up later. Those who carry the allele with 5 repeats may be classified as morning people as they tend to have a higher preference for morning activities and wake up earlier (Archer, Robilliard, Skene, Smits, Williams, Arendt, & von Schantz, 2003). The same study showed there was a differing genotype distribution in those who suffered from sleep disorders than control patients as noted in Figure 1.

Figure 1: **Structure of PER3 Protein (adapted from Archer et al., 2003)**



Insomnia is generally when a person has difficulty falling asleep or staying asleep, even when they have the chance to do so (https://www.sleepfoundation.org/insomnia/what-insomnia, 2019). This pilot will be the first if its kind in Bermuda and may shed light onto any genetic basis for insomnia.

This report suggests that there may be a novel link between the Per3 and those with insomnia in the Bermuda population. The Per3 genotype was studied both in control volunteers and those with insomnia.

Method

Insomnia patients were determined by ICD-10 testing at a local physician's office prior to the pilot study. 25 total volunteers (21 females, 4 males, aged 20-79) participated in the pilot study. 15 control volunteers and 10 insomniac volunteers provided buccal swab samples after informed consent was obtained.

The study was granted approval by the Bermuda Hospitals Board Research Ethics Committee. DNA was extracted from each sample to determine the presence of the two alleles via a Per3 specific polymerase chain reaction (PCR) provided by an online lab (the miniPCR Sleep Lab Kit). Agarose gel electrophoresis was used to determine if volunteers were homozygous for the 4-repeat allele (night person), heterozygous (no preference) or homozygous for the 5-repeat allele (morning person).

Results

Demographics of our volunteers showed the majority to be female (84%), suggesting that perhaps male participation needs to be more encouraged in the future. The highest participation (32%) came from the 60-69 age range, with the lowest participation (4%) from extreme age groups (20-29 and 70-79). The sample also included 28% in the 50-59 age range, 20% in the 40-49 age range, and 12% in the 30-39 age range.

All 9 parishes were represented in the data, with Devonshire and Warwick having the highest portion at 20% each. Based on this, we consider our results to be representative of the island, however skewed towards the female population. The results of the study is also limited due to the small size. Specifically, the small cell size by age which would preclude comments on the relation between per3, age, and insomnia.

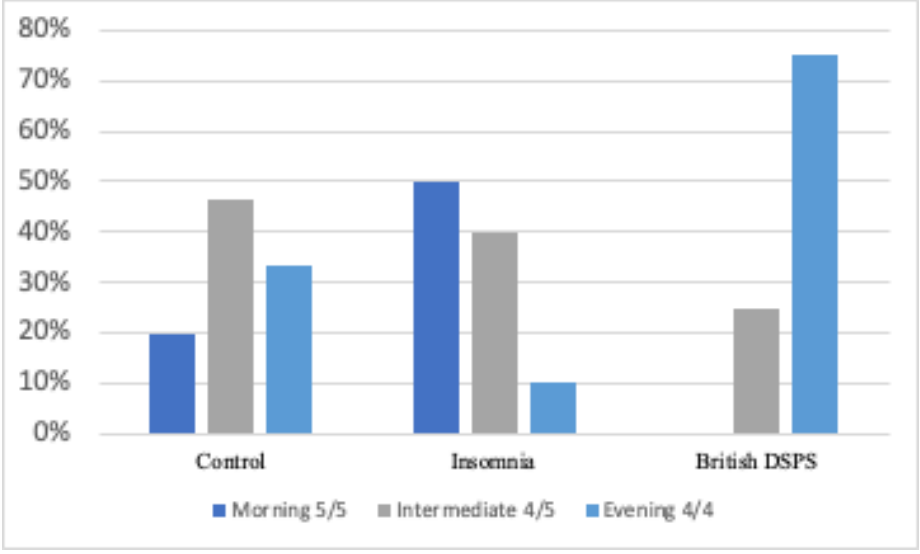
Each participant genotype was clear to determine via gel electrophoresis and the frequency of each was recorded below in Table 1. From first observation, it is clear to see that the distribution of the genotypes is different between the two groups, which presents a clear distinction in the two homozygous genotypes.

Table 1: **Genotype Frequencies for Control and Insomnia Groups**

Genotype	**4/4**	**4/5**	**5/5**	**Total**
Control	5 (33.3%)	7 (46.7%)	3 (20%)	15
Insomnia	1 (10%)	4 (40%)	5 (50%)	10

As expected, genotype frequencies fell into the Hardy-Weinberg equilibrium for both the control (p-value=0.982) and insomniac groups (p-value=0.989). When graphing these data to include the British sleep disorder patients, it is even more striking that there is a drastic difference between the Bermuda insomniacs and British with DSPS as noted in Figure 2.

Figure 2: **Percentage of participants with each of the Per3 repeat genotypes in the control insomniac and British DSPS groups**



As each participant has two copies of an allele, that would mean that if they have 4/4 (or 5/5), that is 2 copies of the 4-repeat (or 5-repeat) allele, and if they have 4/5 that is one copy of both the 4-repeat and 5-repeat alleles. Following this logic of Mendelian inheritance, the expected percentage of each allele present in the population would be 50-50. However, the following observed results were achieved.

Table 2: **Statistical ratios of the different repeat alleles**

	4-repeat	5-repeat
Expected	0.5	0.5
Control	0.567	0.433
Insomnia	0.3	0.7
British DSPS	0.88	0.12

When conducting a Fisher's exact test, the frequency of the 4-repeat allele appears three times lower in insomniacs compared to the control group. However, this is not statistically significant in our sample size (n=25, Fisher's Exact Test, p=0.086, odd ratio=0.3277). When comparing our calculated value of the Fisher's Exact Test to the threshold value of <5%, we can see that there is not much a difference, suggesting that a larger sample size could result in a significant result.

Discussion

When comparing the allele frequency for Bermudian insomniacs to the British patients (4-repeat: 0.88, 5-repeat: 0.12) with delayed sleep phase syndromes (DSPS) in the Archer study, it resulted in the 5-repeat allele being extremely significantly higher (16 times greater) in Bermudian insomniacs (Fisher's Exact Test, p=0.0000345, odd ratio=16.3).

There has been a suggestion that geographical location may play a role in Per3 allele frequencies in a population but this was not conducted for those with sleeping disorders (Nadkarni, Weale, von Schantz, Thomas, 2005). To date only the British and Japanese populations (Nadkarni, Weale, von Schantz, Thomas, Hida, Kitamura, Kadotani, Uchiyama, Ebisawa, Inoue, Kamei, and Mishima, 2018) have done a correlation study, with the Japanese finding no correlation at all with Per3 and sleep disorder.

Conclusion

Our results show that there seems to be a very strong correlation between those who are homozygous for the 5-repeat allele (morning preference) and those with insomnia in Bermuda. A larger sample size would distinguish if there is a statistical significance between those with insomnia and those without. Bermudian insomniacs vary distinctly in the Per3 allele frequencies to those in Britain with DSPS, suggesting a potential geographical or ethnic distinction. Overall, further study is warranted to determine if these suggested links indeed exist.

References

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