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Common Polymorphisms for the Time of Living and Death?

All organisms on Earth live with the daily light–dark cycle and adapt their physiology and behavior to environmental circadian rhythm factors. Many cellular mechanisms and physiological functions are known to exhibit circadian rhythmicity, from regulation of the cell cycle to metabolism.[1] Elegant genetic studies in model organisms revealed a molecular clock conserved from invertebrates to humans for circadian regulation. At the core of the molecular clock that drives the daily rhythm is an interlocking, positive and negative, transcriptional–translational feedback loop comprised of *CLOCK*, *BMAL1*, *PER1-3*, *CRY1-2*, and other genes.[2,3] For humans living under culturally enforced time schedules and artificial illumination, there are expected interactions between the body clock (endogenous rhythm) and the social clock.[4] Many measurable human-specific biorhythmic behaviors may exist due to these interactions. Furthermore, as the human population has expanded explosively in recent history, a large number of genetic variations deposited in human genomes on a population level may exert subtle influences on human biorhythmic behaviors. Defining more measurable biorhythmic behaviors and identifying the genetic basis for interpersonal differences may represent the next exciting frontier in human circadian research.

The report by Lim et al in this issue is an example of research in this direction.[5] They carried out a candidate gene association study with 135 tagging single nucleotide polymorphisms (SNPs) covering 18 candidate genes within circadian pathways for normal participants from an unrelated aging study. They determined a common SNP, rs7221412, in the adjacent region downstream of *PER1*, to be associated with 2 traits, the activity acrophase from actigraph (midpoint of active hours) and the time of death. Subjects with the rs7221412^{GG} genotype on average were found to have an activity acrophase 67 minutes later than subjects with the rs7221412^{AA} genotype. Subjects with rs7221412^{GG} were also found on average to have a time of death 7 hours later than subjects with rs7221412^{AG} or rs7221412^{AA} in participants from a nonoverlapping cohort mostly from the same study. These findings were replicated in a small independent cohort with participants of similar ethnic background and of a much younger age. In addition, they validated the activity acrophase measures using cosinor analysis, the time of dim light melatonin onset, and body temperature nadir in the replicate cohort, further strengthening the value of using activity acrophase as a real world marker of circadian rhythm. Interestingly, the rs7221412 genotype did not show association with the period length of intrinsic biological rhythm, although it had a clear influence on entrained phase, supporting separate regulatory mechanisms for period length and phase of entrainment.

In many ways, this study is innovative and reflects many difficulties in identifying common polymorphisms for circadian rhythm in human populations. First of all, it is always more difficult to work with human subjects for studying circadian rhythm compared with other model organisms. In classical studies,[6] subjects were put under controlled lighting conditions to block environmental time cues. However, using actigraph, as Lim et al did for their discovery cohort, has the benefit of being convenient for recording activity for days with minimum interruption in the normal lives of a large number of subjects. This method also gives an objective measure of activity instead of relying on self-reporting. However, this approach inevitably increases the variance of measurements, as many unexpected and unrecorded factors may influence results. For example, differences in active hours between weekdays and weekends are expected, although they may be smaller for retired people or inpatient subjects. This may have contributed to the effect size observed in the Lim et al paper. Sophisticated statistical treatments are needed for repeated measure data from circadian studies to correct for these factors. Validation of novel measures of biorhythmic activities in additional cohorts is also necessary.

One intrinsic difficulty in association studies with common SNP markers is demonstrating biological relevance. The Lim et al paper shows suggestive differences in daytime *PER1* expression between subjects with different genotypes at rs7221412. For circadian genes with oscillating expression patterns, comparison of expression can be skewed by the time of sampling. Expression profiling at precise timing or multiple standardized time points is necessary to compare circadian gene expression, which is often not feasible when using human subjects.

Conversely, the finding that subjects with different genotypes at rs7221412 have significantly different time of death is intriguing. The data presented by Lim et al further suggest that time of death is also likely to be under circadian modulation. The correlation between time of death and time of day was first reported in 1987,[7] and this connection is further corroborated by the polymorphic marker. Together, these findings suggest circadian rhythm regulation to be among the most globally influential regulatory networks on an organismal level.

The Lim et al study raises several issues to ponder in designing association studies for circadian behaviors. First, what circadian behavior should we measure? The classical variables, such as sleep time, sleep duration, daytime sleepiness, and responses to sleep deprivation, are theoretically and experimentally better established. However, they require either inpatient study in special sleep facilities or data collection from self-reporting questionnaires. Conversely, measurements such as activity acrophase can use directly collected biorhythmic data from large cohorts under real world conditions. Although the validity of this method needs further replication, Lim et al provide the first example of using this measurement as a circadian behavioral marker. Second, which genotyping method should we use? Most current studies use SNP chips to examine common polymorphisms. Yet, assessing the functional causality (if any) of common variants remains daunting. Using whole exome sequencing or whole genome sequencing to identify rare variants that contribute to biorhythmic differences is a promising alternative or complementary method. However, specific rare variants may exist in a very small fraction of the population, and each individual harbors numerous common and rare mutations, so finding the causal rare variants is challenging.[8] Nonetheless, the timing has so matured from intense investigation over the past 2 decades with these complementary approaches that a cohesive analysis of results derived from them can pave the way for understanding the genetic basis of human behavioral traits.

Potential Conflicts of Interest

Nothing to report.

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