A Common Polymorphism Near *PER1* and the Timing of Human Behavioral Rhythms

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Objective: Circadian rhythms influence the timing of behavior, neurological diseases, and even death. Rare mutations in homologs of evolutionarily conserved clock genes are found in select pedigrees with extreme sleep timing, and there is suggestive evidence that certain common polymorphisms may be associated with self-reported day/night preference. However, no common polymorphism has been associated with the timing of directly observed human behavioral rhythms or other physiological markers of circadian timing at the population level.

Methods: We performed a candidate gene association study with replication, evaluating associations between polymorphisms in homologs of evolutionarily conserved clock genes and the timing of behavioral rhythms measured by actigraphy. For validated polymorphisms, we evaluated associations with transcript expression and time of death in additional cohorts.

Results: rs7221412, a common polymorphism near period homolog 1 (*PER1*), was associated with the timing of activity rhythms in both the discovery and replication cohorts (joint $p = 2.1 \times 10^{-7}$). Mean activity timing was delayed by 67 minutes in rs7221412^{GG} versus rs7221412^{AA} homozygotes. rs7221412 also showed a suggestive time-dependent relationship with both cerebral cortex ($p = 0.05$) and CD14$^+$CD16$^-$ monocyte ($p = 0.02$) *PER1* expression and an interesting association with time of death ($p = 0.015$) in which rs7221412^{GG} individuals had a mean time of death nearly 7 hours later than rs7221412$^{AA/AG}$.

Interpretation: A common polymorphism near *PER1* is associated with the timing of human behavioral rhythms, and shows evidence of association with time of death. This may be mediated by differential *PER1* expression. These results may facilitate individualized scheduling of shift work, medical treatments, or monitoring of vulnerable patient populations.

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Circadian rhythms modulate human behavior and physiology, including cognitive function,[1] as well as the timing and treatment of several neurological and medical disorders, including hemorrhagic[2] and ischemic[3] stroke, amyloid-β secretion,[4] epilepsy,[5] myocardial infarction,[6] ventricular arrhythmias,[7] cancer,[8] and even time of death.[9] Moreover, circadian misalignment, as seen in shift work, predisposes not only to accidents and lost productivity, but also to common diseases, such as diabetes.[10] Circadian traits are heritable,[11–14] and understanding their genetic architecture may facilitate individualization of the timing of school or work schedules, as well as medical investigations and therapies.

In animals, a conserved genetic network regulates circadian rhythms. At its core is a transcription–translation feedback loop formed by the genes *PER1-3*, *CRY1-2*, *BMAL1*, and *CLOCK* that generates near 24-hour rhythmicity.[10] In animals, mutations in these genes can lead to alterations in or loss of circadian rhythmicity.[10]

There is some genetic evidence for the role of homologs of these genes in the regulation of human circadian rhythms. Mutations in *PER2* and *CSNK1D*, a *PER2* kinase, have been implicated in select pedigrees with extreme circadian timing,[15,16] but these are rare, and their significance at the population level is unclear. More common variants in *PER1*, *PER3*, and *CLOCK* have evidence of association with self-reported day/night preference,[17–20] but these have not been shown to be associated with differences in the timing of directly observed human behavior. To our knowledge, no common genetic variant has been associated with the timing of directly observed human behavior or any other physiological marker of circadian timing in a real world setting at the population level.

To identify and characterize such variants, we performed a candidate gene association study, evaluating associations between polymorphisms in homologs of evolutionarily conserved clock genes and the timing of behavioral rhythms measured by actigraphy as a marker of circadian phase. For validated polymorphisms, we evaluated associations with intrinsic circadian period, transcript expression in cerebral cortex and peripheral blood mononuclear cells (PBMCs), and time of death.

Subjects and Methods

Participants

Characteristics of the study participants are shown in the Table. Inclusion and exclusion criteria are described in the Supplementary Material. The discovery cohort consisted of 537 participants of European ancestry from the Rush Memory and Aging Project (MAP cohort), a community-based study of aging.[21–23] The replication cohort consisted of 38 individuals of European ancestry who had participated in detailed inpatient circadian period phenotyping studies at Brigham and Women's Hospital from 2001 to 2010 (BWH Circadian cohort). Transcript expression was examined in 3 data sets: 1 derived from postmortem cerebral cortical samples from 193 individuals without dementia[24] (National Institute on Aging [NIA] cohort), another derived from PBMCs from 228 individuals with multiple sclerosis[25] (BWH MS cohort), and a third derived from $CD14^+CD16^-$ monocytes from 69 healthy subjects without inflammatory or metabolic disease (PhenoGenetic cohort). Time of death was examined in 687 deceased participants of European ancestry from the MAP and the Religious Orders Study (ROS/MAP cohort). The ROS/MAP cohort excluded any MAP participants who were part of the primary discovery cohort.

All protocols were approved by the institutional review board at each institution. All participants provided written informed consent.

Phenotyping

As described in the Supplementary Material, we recorded 1 week of actigraphy from participants in the MAP and BWH Circadian cohorts. Our primary actigraphic phenotype was the timing of the acrophase of activity, defined as the midpoint of the 8 consecutive hours of each day with the greatest activity (Fig 1). The timing of the nadir and acrophase of activity calculated using cosinor analysis[26] provided secondary actigraphic phenotypes.

To validate activity acrophase as a marker of circadian phase, we compared acrophase timing with that of dim-light melatonin onset (DLMO) and the core body temperature nadir under conditions of constant routine[27] in a subset of 16 BWH Circadian participants closely following their actigraphy recordings.

To determine the intrinsic periods of the temperature and melatonin rhythms, each participant in the BWH Circadian cohort was studied in an inpatient forced desynchrony protocol as previously described.[28]

Time of death in the ROS/MAP cohort was determined from clinical records. Time of death is well captured, because all participants are organ donors. Information about cause of death was not available for the majority of the ROS/MAP subjects.

Genotyping and Expression Data

A detailed description of genotyping procedures is found in the Supplementary Material.

Eighteen candidate genes were selected based on involvement in rare human pedigrees with extreme circadian phenotypes or functional importance in circadian rhythms in animals (Supplementary Table 1). After linkage disequilibrium-based pruning to reduce the multiple testing burden, and additional quality control, 135 tagging polymorphisms within these 18 loci were selected for our primary analysis (Supplementary Table 2).

Genotypes at all 135 polymorphisms were determined in the MAP cohort. For the BWH Circadian cohort, polymorphisms significant in the MAP cohort along with flanking variants (Supplementary Table 3) were genotyped. For the PhenoGenetic, NIA, BWH MS, and ROS/MAP cohorts, genotypes for polymorphisms significant in both the MAP and BWH Circadian cohorts were extracted from pre-existing genome-wide data sets[22,24,25] or genotyped as described in the Supplementary Material.

RNA data for the NIA and BWH MS cohorts were obtained from the National Center for Biotechnology Information Gene Expression Omnibus database (GSE8919 and GSE16412).[24,25,29] RNA data for the PhenoGenetic cohort were obtained from $CD14^+CD16^-$ monocytes as described in the Supplementary Material.

Analyses

A description of the statistical analyses, along with a comprehensive report on all methods, is available in the Supplementary Material.

TABLE. Characteristics of Study Participants and Phenotyping

Characteristic	Cohort					
	MAP	BWH Circadian	NIA	BWH MS	PhenoGenetic	ROS/MAP
No.	537	38	193	228	69	687
Age, yr, mean (SD) [Range]	82.9 (6.6) [59–100]	32.9 (17.6) [18–70]	80.9 (9.2) [65–100]	40.9 (9.4) [21–68]	32.4 (9.5) [19–51]	88.3 (6.4) [67–109]
F, No. {%}	412 {76}	14 {37}	86 {46}	183 {80}	41 {56}	426 {62}
% European ethnic ancestry	100%	100%	100%	100%	100%	100%
Dementia, No. {%}	55 {10}	0 {0}	0 {0}	0 {0}	0 {0}	289 {42}
Genotyping platform	Affymetrix 6.0	Sequenom MassARRAY	Affymetrix 500k	Affymetrix 6.0	Sequenom MassARRAY	Affymetrix 6.0
Actigraphy	7 days with Phillips Respironics Actical	7 days with Phillips Respironics Actiwatch-L	NA	NA	NA	NA
Expression profiling platform	NA	NA	Illumina Human Refseq-8 BeadChip	Affymetrix Human Genome U133 Plus 2.0	Affymetrix Human Genome U133 Plus 2.0	NA
Comments		All without medical comorbidities		All with relapsing–remitting multiple sclerosis	Healthy volunteers without inflammatory or metabolic disease	No overlap with MAP participants used for primary association analysis

BWH Circadian = Brigham and Women's Hospital Division of Sleep Medicine; BWH MS = Brigham and Women's Hospital Comprehensive Longitudinal Investigation of Multiple Sclerosis; MAP = Memory and Aging Project; NA = not applicable; NIA = National Institute of Aging Laboratory of Neurogenetics; ROS/MAP = Religious Orders Study and Memory and Aging Project deceased participants.
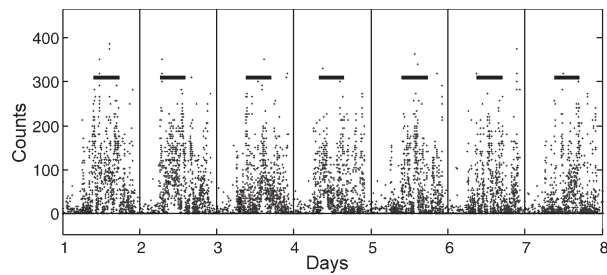
FIGURE 1: Example actigraphic record. The activity acrophase for each day, defined as the midpoint of the 8 consecutive hours of each day with the greatest activity, is indicated by a horizontal black bar. The vertical divisions indicate midnight.

Results

We determined the average timing of the daily activity peak (the acrophase) in 537 participants from the MAP using 1 week of actigraphic data (see Fig 1). Demographic and other characteristics of the participants are presented in the Table. As expected, age and absence of dementia were associated with earlier timing of the activity peak (Supplementary Table 4).[27,30,31]

We identified 135 tagging polymorphisms in 18 candidate genes known to be functionally important for circadian rhythms in animals (Supplementary Tables 1 and 2). These polymorphisms capture common genetic variation among our subjects of European ancestry. Each polymorphism was evaluated for association with acrophase timing in the MAP cohort. Of these, only rs7221412, 2.8kb downstream of *PER1*, was associated with acrophase timing after Bonferroni correction for 135 tests (Supplementary Table 5 and Fig 2A). Under our default additive model, each additional minor allele at rs7221412 was associated with a further delay in acrophase timing ($p = 6.0 \times 10^{-7}$; Bonferroni corrected $p = 8.1 \times 10^{-5}$), with 67 minutes separating the 2 homozygote genotype classes. Genotype at rs7221412 was not associated with the amplitude of the activity rhythm (Supplementary Fig 2) and controlling for amplitude in the model relating genotype at rs7221412 to acrophase timing did not attenuate this relationship (unadjusted $p = 5.9 \times 10^{-7}$). Similar results were seen in subjects with and without dementia (Supplementary Fig 3). Results were similar with the cosinor-determined activity nadir and acrophase as the phenotypes (Supplementary Figs 4 and 5), indicating that the results are not specific to the method used to analyze the activity data.

Characterization of the local association structure by testing all available polymorphisms (HapMap release 22) within 20kb of rs7221412 for association with activity acrophase timing in the MAP cohort revealed a 14kb association peak overlapping with the 3′ end of the *PER1*
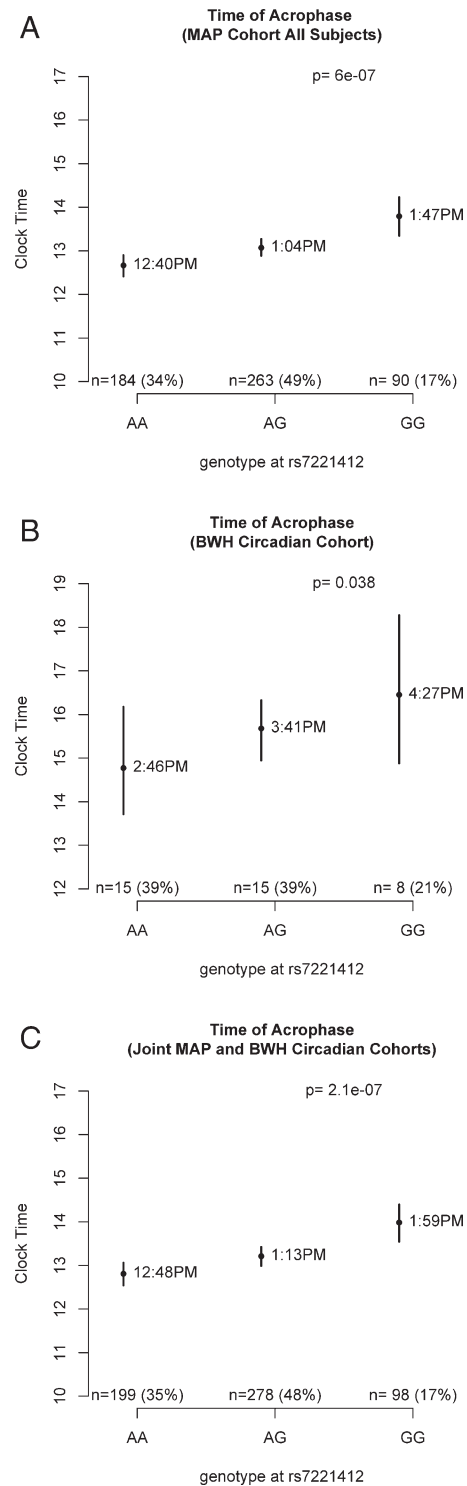

FIGURE 2: Association between the average timing of the acrophase of activity and genotype at rs7221412. Dots indicate angular means, and bars indicate 95% confidence intervals of the mean. All significance values were calculated using Fisher–Lee regression using an additive model, and adjusted for age, sex, and presence or absence of dementia. In the joint analysis, source cohort was added as an additional covariate. (A) Memory and Aging Project (MAP) cohort. (B) Brigham and Women's Hospital (BWH) Circadian cohort. (C) Joint analysis.

gene as well as downstream regions and containing rs7221412 at its center (Fig 3).

To confirm these observations, we genotyped rs7221412 and flanking polymorphisms in an independent cohort (the BWH Circadian cohort) and tested for associations with acrophase timing. rs7221412 remained significantly associated with acrophase timing ($p = 0.038$; see Fig 2B). In a combined analysis of both the MAP and BWH Circadian cohorts (n = 575; see Fig 2C), the strength of the association between rs7221412 and acrophase timing was enhanced ($p = 2.1 \times 10^{-7}$).

To validate activity acrophase as a marker of circadian phase, we compared the timing of the activity acrophase to that of DLMO or of the temperature nadir under conditions of constant routine in a subset of the BWH Circadian cohort. In this validation subset, activity acrophase timing was strongly correlated with the timing of both DLMO and of the temperature nadir ($r = 0.7$, $p = 0.0026$ and $r = 0.75$, $p = 0.009$, respectively; Supplementary Fig 6), indicating that it was a reasonable marker of phase in this study. The small number of individuals in whom DLMO was measured (n = 16) precluded meaningful direct assessment of the relationship between genotype at rs7221412 and DLMO.

Because differences in phase can reflect differences in the length of the period of individuals' intrinsic biological rhythms,[31,32] we tested for associations between rs7221412 genotype and intrinsic circadian period in the BWH Circadian cohort. rs7221412 genotype was not associated with the period of either the temperature or melatonin rhythms (Supplementary Fig 7), suggesting that it does not alter the length of the period of the intrinsic biological rhythm but rather displaces its entrained phase.

Alterations in *PER1* expression can affect circadian phase[33] and may be a mechanism mediating the association between rs7221412 and phase. Therefore, we assessed for associations between rs7221412 genotype and *PER1* expression. In cerebral cortex expression data from the NIA cohort, rs7221412 genotype was not significantly associated with overall *PER1* expression ($p = 0.29$; Fig 4). However, when the analysis was stratified by the inferred time of death (see Supplemental Material for details), a suggestive relationship emerged; rs7221412 genotype was associated with *PER1* expression in samples from individuals inferred to have died during the day ($p = 0.05$), but not from those inferred to have died during the night ($p > 0.99$). A similar pattern was not seen for *PER2* expression in cerebral cortex, suggesting that the association between rs7221412 and daytime *PER1* expression was not merely attributable to overall phase changes in the molecular circadian clock
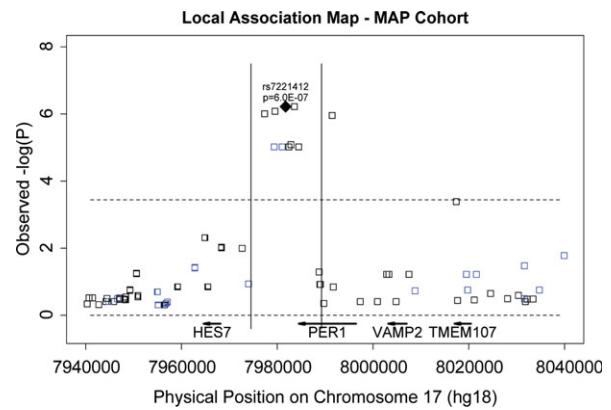

FIGURE 3: Local association plot in the *PER1* locus. The plot displays the significance, as indicated by the $-\log_{10}(p$ value), of the associations between timing of the circadian acrophase and genotype at polymorphisms flanking rs7221412. All significance values were calculated with Fisher–Lee regression using an additive model, and adjusted for age, sex, and presence or absence of dementia. Blue squares indicate directly genotyped polymorphisms; black square indicate polymorphisms whose genotypes were imputed (imputation quality score >0.90 for all imputed polymorphisms). Vertical black bars indicate the extent of the association peak. The black diamond indicates the index single nucleotide polymorphism rs7221412. The horizontal dotted line indicates the Bonferroni-corrected threshold for significance. MAP = Memory and Aging Project.

(Supplementary Fig 8). In PBMCs from the PhenoGenetic and BWH MS cohorts, all of which were collected during the day, similar associations were seen ($p = 0.02$ for $CD14^+CD16^-$ monocytes in the PhenoGenetic cohort and $p = 0.20$ for unselected PBMCs in the BWH MS cohort; Supplementary Fig 9).

The incidence of death also varies by time of day.[9] We therefore assessed the association between rs7221412 genotype and time of death in the ROS/MAP cohort, excluding subjects who were part of the primary association analysis. Time of death was obtained from autopsy reports. In both additive and recessive models, adjusting for age, sex, presence/absence of dementia, and source cohort, genotype at rs7221412 was associated with time of death ($p = 0.011$ for the additive model and $p = 0.015$ for the recessive model). Under the recessive model, the rs7221412[GG] group (n = 134) had a mean time of death nearly 7 hours later than the rs7221412[AA/AG] group (n = 578; 5:50 PM vs 10:51 AM; Supplementary Fig 10).

Discussion

In this study, a polymorphism near *PER1* was significantly associated with the timing of directly recorded human behavioral rhythms in 2 independent cohorts and with time of death in a third. The associated functional variant was
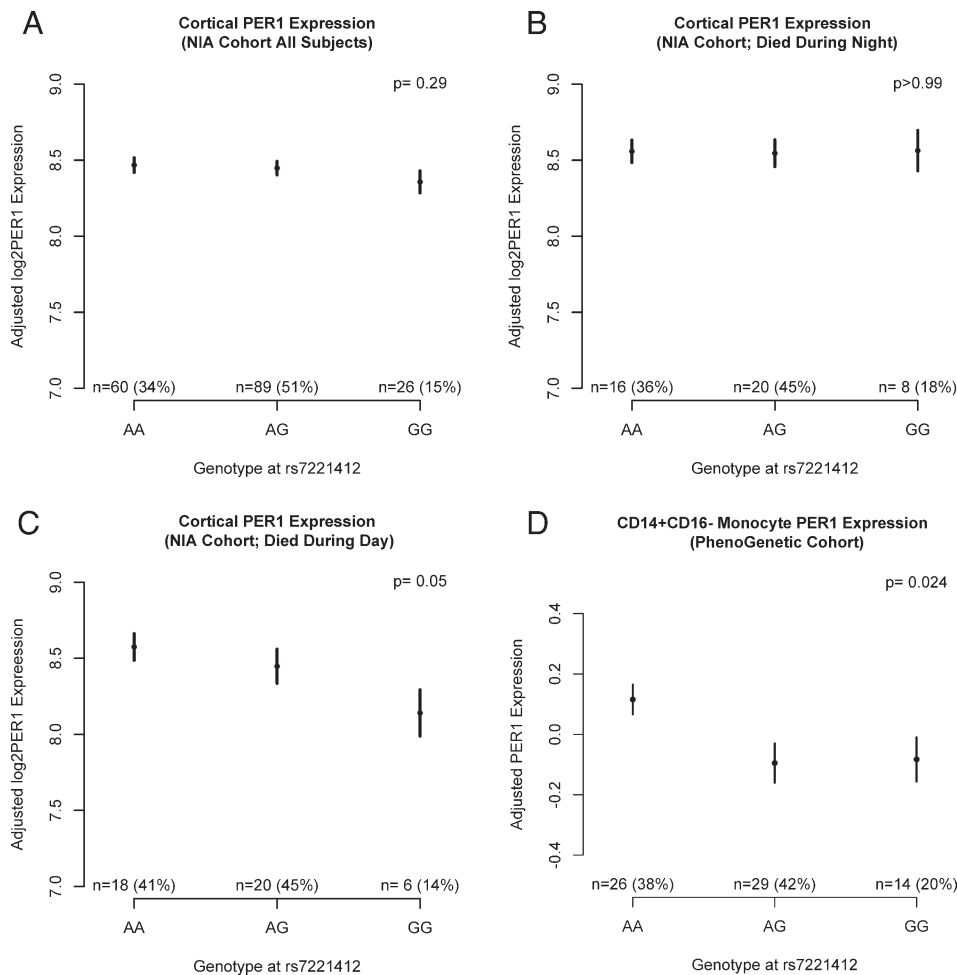
FIGURE 4: Adjusted cortical and CD14$^+$CD16$^-$ monocyte *PER1* transcript expression as a function of genotype at rs7221412. (A) Cerebral cortex, National Institute on Aging (NIA) cohort, all participants. (B) Cerebral cortex, NIA cohort, participants inferred to have died during the night (see Supplementary Methods). (C) Cerebral cortex, NIA cohort, participants inferred to have died during the day (see Supplementary Methods). (D) CD14$^+$CD16$^-$ monocytes, PhenoGenetic cohort, all participants. Dots indicate means, and bars indicate 95% confidence intervals of the mean. Y-axis indicates log$_2$ expression levels adjusted for methodological and clinical covariates as described in Subjects and Methods.

mapped to a 14kb region straddling the 3′ end of the *PER1* gene, and the region downstream of it. Moreover, there was suggestive evidence for a circadian time-dependent association between genotype at this variant and both cortical and PBMC *PER1* expression, which could be a mechanism that mediates its effect. To our knowledge, this is the first genetic polymorphism shown to be associated with the timing of directly observed human behavioral rhythms at the population level, and the first showing evidence of association with time of death.

Methodological Considerations

In this study, biological circadian phase was inferred from actigraphic data. Actigraphy is among the few objective means to gather circadian data from large cohorts in their home environments. The relationship between activity and circadian rhythms can be complex, with social and other factors influencing activity patterns.

However, in the subset of participants who underwent actigraphy followed by monitoring of melatonin and temperature rhythms, 2 standard markers of circadian phase, activity acrophase timing was strongly correlated with the timing of both, suggesting that activity acrophase timing is a reasonable marker of circadian phase under the conditions of this study. Others have found a similarly strong correlation between actigraphic timing and biochemical markers of phase.[34]

We used 3 markers of actigraphic phase in this study: nonparametrically determined acrophase, and parametrically determined nadir and acrophase by the cosinor method. Although the precise effect sizes for rs7221412 were slightly different for these 3 markers, there was a consistent direction of effect for all 3, and the associations were highly statistically significant (joint $p = 2.0 \times 10^{-7}$ for nonparametric acrophase and $p = 7.1 \times 10^{-6}$ for cosinor acrophase and nadir), indicating

that our results are not specific to the precise actigraphic phase marker used, and are not merely due to changes in the shape of the activity profile.

This study was not a comprehensive screen for genetic variants influencing circadian phase. Because of our sample size, we limited the first stage to genes with biological plausibility, examined only common variants, and validated only the most significant variant. With this strategy, several polymorphisms previously associated with subjective diurnal preference[17–20] were not assessed. Moreover, of the 138 single nucleotide polymorphisms (SNPs) assessed, only 1 was found to be statistically significantly associated with the timing of activity rhythms below the Bonferroni-corrected threshold of 3.7×10^{-4}, although a further 8 SNPs had an unadjusted $p < 0.05$. This does not preclude a role for human homologs of animal clock genes other than *PER1* in the generation of human circadian rhythms. Rather, the design and sample size of our study meant that it would have had power to detect only relatively common variants with large effect sizes, and less frequent variants with smaller effect sizes would have been missed. Post hoc power estimations by simulation (see Supplementary Methods) indicate that we would have had 80% power to detect only those variants with a minor allele frequency of 0.45 and a difference in timing between the minor and major allele homozygote genotypes of at least 64 minutes. At a minor allele frequency of 0.10, the required effect size rises to a minimum difference in timing of ≥ 150 minutes. It is likely that with larger sample sizes in future studies, additional variants in other genes will be shown to be associated with human circadian phase.

Genetic Determinants of Circadian Phase

Rare mutations in homologs of conserved circadian genes have been associated with extreme sleep timing in select human pedigrees.[15,16] Although these mutations corroborate the role, in humans, of clock genes identified from animal models, their population-level relevance is unclear. Meanwhile, several common variants have suggestive evidence of association with self-reported day/night preference[17–20] or usual bedtime.[12] However, not all of those results have been replicated, and none of these variants has been associated with the timing of directly observed human behavior or another physiological marker of circadian timing in a real world setting—an important consideration given that self-reported day/night preference is prone to misperception and poor recall, and that its relationship to physiological markers of circadian timing in real world settings can be incomplete.[35]

This previous work established the importance, in humans, of evolutionarily conserved clock genes and provided evidence that variants in these genes can in principle influence human sleep and circadian traits. However, the variant rs7221412 differs from these previously identified variants in a number of important respects. First, unlike the highly penetrant variants identified in rare pedigrees,[15,16] rs7221412 is a much more common variant (minor allele frequency 0.43), meaning that it is likely to play a much more important role at the population level. Second, genotype at rs7221412 was shown to be associated with the timing of directly observed human behavior—an objective physiological marker of circadian timing—as opposed to self-reported subjective day/night preference, as has been the case for many of the previously reported variants.[17–20] This is important because although subjective diurnal preference is likely to be influenced in part by underlying biological circadian phase, these are not identical. For example, a *PER3* variable length tandem repeat (VNTR) polymorphism has been reported to be associated with subjective diurnal preference[17]; however, it was subsequently shown not to be associated with underlying circadian phase as measured using actigraphy or serial serum cortisol, and it appears that this *PER3* VNTR influences subjective diurnal preference by affecting sleep homeostatic function rather than circadian phase.[35] Third, our result was highly statistically significant, with an unadjusted p value approaching genome-wide significance, retained significance following rigorous correction for multiple comparisons, and was replicated in an independent cohort. By contrast, many of the previously reported associations between common variants and subjective diurnal preference had nominal p values with borderline statistical significance $(0.01 < p < 0.05)$[17–19] that would not have been statistically significant after correction for multiple comparisons.[18,19] Moreover, most of these associations have not been replicated, and where replication was attempted, discordant results have been found.[20,36] Fourth, genotype at rs7221412 showed evidence of association not only with the timing of human behavioral rhythms, but also with *PER1* expression in 2 additional cohorts, and with time of death in another, providing several lines of converging evidence of the population-level physiological and clinical significance of this variant. Examination of rs7221412 and other common variants near *PER1* in association with objective measures of circadian timing in additional large cohorts will be needed to further establish the association of this and other *PER1* variants with circadian timing in different human populations.

Remarkably for so common a variant, the effect size was large, with >1 hour separating the daily activity peak in homozygotes for 1 or the other allele. This result

was significant even after correction for multiple comparisons. Several other polymorphisms near rs7221412 are in linkage disequilibrium with it and exhibited a similar level of association, so identification of the precise causal variant within the *PER1* locus will require further studies, likely involving sequencing of the 3′ end of *PER1* as well as the *PER1* downstream region in a large number of people with objective characterization of circadian timing.

Functional Correlates

The *Per* locus was first identified in *Drosophila melanogaster*[37] and shortly thereafter 3 orthologs were identified in the mouse.[10] PER1–3 play a critical role in a negative-feedback transcriptional cycle at the core of the molecular circadian clock[10]. Transcription of *PER1–3* is activated by a complex of CLOCK and BMAL1.[10] Once translated, PER1–3 heterodimerize with CRY1–2 and translocate into the nucleus to inhibit their own transcriptional activation by the CLOCK:BMAL1 complex, forming a 24-hour negative feedback loop.[10]

Another *PER1* polymorphism, rs2735611, 4.5kb upstream of rs7221412 ($r^2 = 0.049$), has been associated with self-reported day/night preference, although phase was not measured directly.[19] In our study, rs2735611 was ascertained in the MAP but not BWH Circadian cohort, and was not associated with activity acrophase timing ($p = 0.24$). As day/night preference was not assessed in the MAP cohort, we cannot comment on whether rs2735611 would have been associated with this phenotype.

Coding mutations in *PER2*[15] and *CKSKNID*,[16] a PER2 kinase, are associated with autosomal dominant familial advanced sleep phase syndrome in rare pedigrees. In these pedigrees, altered phosphorylation of PER2 is hypothesized to lead to a shortened intrinsic period and hence advanced phase. By contrast, rs7221412 was not associated with significant differences in the length of the intrinsic circadian period. Although it remains possible that our study did not have sufficient power to detect a subtle effect, an alternative is that the association of rs7221412 with phase may be mediated by factors independent of period, such as a differential phase response to circadian synchronizing signals. We hypothesize that these changes in turn may be mediated by altered *PER1* expression. In this regard, rs7221412[A] was suggestively associated with higher cortical *PER1* expression during the presumptive day, but not night. This effect was also seen in PBMCs collected during the day, suggesting that rs7221412[A] may be associated with a differential increase of *PER1* expression during the day, leading to an earlier peak in the activity acrophase. This putative association

between genotype at rs7221412 and *PER1* expression will need to be replicated in other human tissues obtained from other cohorts but is consistent with the observation that the association peak containing rs7221412 overlaps not only with several exons and introns at the 3′ end of *PER1* but also with the 3′ untranslated region and a phylogenetically conserved region of open chromatin immediately downstream of *PER1* as revealed by examination of existing maps of DNase hypersensitivity[38] and phylogenetic conservation[39] (Supplementary Fig 11). These conserved downstream regions in particular are suggestive of a potential *cis*-regulatory region. Identification of the precise functional variant mediating the rs7221412 association will allow dissection of its molecular and physiological effects in animal model systems.

Time of Death

It has been shown that the probability of nontraumatic death,[9] as well as stroke,[3] ST-elevation myocardial infarction,[6] and ventricular arrhythmia,[7] varies by time of day, with a peak in the late morning or early afternoon. Consistent with this, in the ROS/MAP cohort, death was most likely to occur in the late morning/early afternoon. However, this varied with rs7221412 genotype, with the rs7221412[GG] group having a mean time of death nearly 7 hours later than the rs7221412[AA/AG] group. There are several possible interpretations for this disparity. First, it may reflect primarily genotype-related group differences in circadian phase in the days leading up to death. Whereas when individuals are relatively well, their biological circadian tendencies are constrained by social commitments, proximate to death, these social constraints are no longer relevant, and the rs7221412[GG] and rs7221412[AA/AG] groups may have drifted apart by 7 hours in the phase of their underlying biological rhythms, accounting for the large difference in mean time of death. A second possibility is that rs7221412 genotype confers differential susceptibility to causes of death that peak at different times of the day. Cause of death in the ROS/MAP cohort was not available for the vast majority of participants. Moreover, circadian phase was not measured by actigraphy near the time of death (and probably would not have been valid if it had been measured given the nature of terminal illness in many people). Therefore, it was not possible to distinguish these 2 possibilities with the available data. Replication of these results in additional cohorts in whom time of death and cause of death are carefully recorded will allow differentiation of the 2 potential interpretations noted above.

Conclusions and Future Directions

This work identifies a 14kb region overlapping with the 3′ end of *PER1* containing a polymorphism associated with a >1 hour difference in the timing of directly observed human behavioral rhythms and a >6 hour difference in average time of death. It suggests that there may exist additional common variants that influence circadian traits. Studies with larger sample sizes will be helpful to replicate the current findings and facilitate identification and validation of additional variants influencing circadian phase. Meanwhile, further work is needed to delineate the effect of rs7221412 on other clinical traits and diseases with circadian modulation, such as cognitive function, stroke, epilepsy, tumor growth, or cardiac arrhythmia. One might hypothesize, for instance, that genotype at rs7221412 might influence the optimal timing of cognitive testing in dementia, or drug therapy in epilepsy, stroke, or cardiac disease. Moreover, recognition and prediction of individual biological rhythms may provide important dividends not just in the clinical arena but also in identifying individuals suited to early or late work, school, or other schedules.

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Potential Conflicts of Interest

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