Effects of aging on circadian patterns of gene expression in the human prefrontal cortex

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With aging, significant changes in circadian rhythms occur, including a shift in phase toward a "morning" chronotype and a loss of rhythmicity in circulating hormones. However, the effects of aging on molecular rhythms in the human brain have remained elusive. Here, we used a previously described time-of-death analysis to identify transcripts throughout the genome that have a significant circadian rhythm in expression in the human prefrontal cortex [Brodmann's area 11 (BA11) and BA47]. Expression levels were determined by microarray analysis in 146 individuals. Rhythmicity in expression was found in ∼10% of detected transcripts ($P < 0.05$). Using a metaanalysis across the two brain areas, we identified a core set of 235 genes ($q < 0.05$) with significant circadian rhythms of expression. These 235 genes showed 92% concordance in the phase of expression between the two areas. In addition to the canonical core circadian genes, a number of other genes were found to exhibit rhythmic expression in the brain. Notably, we identified more than 1,000 genes (1,186 in BA11; 1,591 in BA47) that exhibited age-dependent rhythmicity or alterations in rhythmicity patterns with aging. Interestingly, a set of transcripts gained rhythmicity in older individuals, which may represent a compensatory mechanism due to a loss of canonical clock function. Thus, we confirm that rhythmic gene expression can be reliably measured in human brain and identified for the first time (to our knowledge) significant changes in molecular rhythms with aging that may contribute to altered cognition, sleep, and mood in later life.

aging | postmortem | circadian rhythms | gene expression | prefrontal cortex

Nearly all processes in the brain and body are controlled by a 24-h circadian rhythm. These rhythms are important in regulating the sleep/wake cycle, metabolism, alertness, cognition, and other processes (1). Environmental or genetic disruptions to circadian rhythms are strongly associated with chronic sleep problems, increased rates of cancer, lowered immune function, metabolic disorders, and psychiatric disorders (2, 3). The molecular clock is controlled by a transcriptional/translational feedback loop with the circadian locomotor output cycles kaput (CLOCK) and brain and muscle Arnt-like protein 1 (BMAL1; also known as ARNTL) proteins acting as the major transcriptional activators, and the Period (PER1, PER2, PER3) and Cryptochrome (CRY1, CRY2) proteins acting as the major repressors (4). This core circadian feedback loop regulates the diurnal expression patterns of many different genes as it is estimated that 10–20% of all transcripts have a circadian rhythm (1, 5). Although the master circadian pacemaker in the suprachiasmatic nucleus (SCN) of the hypothalamus synchronizes rhythms throughout the brain and body, the genes that control circadian rhythms are expressed in nearly every cell (6). In recent years, it has become apparent that these genes serve important functions in specific brain regions, including the control of daily rhythms in neuronal activity and the response to environmental stimuli (7–9).

Evidence from preclinical and clinical studies suggests rhythms in the prefrontal cortex (PFC) are particularly important for cognitive performance and executive function. Several studies in humans have reported diurnal differences in cognitive performance and a significant decrease in performance following circadian rhythm disruption (10–12). Interestingly, these measures vary by age with older adults performing better on cognitive tasks in the morning and getting worse throughout the day (12, 13). In older women, there is a direct correlation between weak circadian activity rhythms and poorer executive function (14). In preclinical studies, mice trained in cortical-driven cognitive tasks show pronounced diurnal differences in performance (15–17). Moreover, mice housed under a shortened day (20-h light–dark cycle of 10-h light, 10-h dark) displayed reduced cognitive flexibility and a loss of dendritic length and complexity in the PFC (18).

Physiological and activity rhythms are generally known to deteriorate with aging and show a phase advance toward early morning wakening (19, 20). Daily rhythms in hormones like melatonin and cortisol are decreased as are sleep and body temperature rhythms in older individuals (21). Interestingly, the recognized stimulation of alertness and cognition by blue light seen in young people is diminished in older people, suggesting decreased input to the clock (22). Moreover, the addition of serum from older people to cultured cells that express a circadian reporter construct (*Bmal1*-luciferase) leads to a shortening of molecular rhythms and a phase advance that is not seen with serum from younger people (23). This finding suggests the presence of circulating factors that alter molecular rhythms with age.

Significance

Circadian rhythms are important in nearly all processes in the brain. Changes in rhythms that come with aging are associated with sleep problems, problems with cognition, and nighttime agitation in elderly people. In this manuscript, we identified transcripts genome-wide that have a circadian rhythm in expression in human prefrontal cortex. Moreover, we describe how these rhythms are changed during normal human aging. Interestingly, we also identified a set of previously unidentified transcripts that become rhythmic only in older individuals. This may represent a compensatory clock that becomes active with the loss of canonical clock function. These studies can help us to develop therapies in the future for older people who suffer from cognitive problems associated with a loss of normal rhythmicity.

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In the human brain, the investigation of brain region-specific transcriptional rhythms across the life span has been challenging due to the lack of control of environmental factors, such as time of death, sleep–wake cycles, and exposure to other factors known to influence rhythms. A recent study overcame some of these obstacles by ordering postmortem samples around a 24-h clock based on their time of death, essentially reconstructing a pseudotime series by treating each individual sample as an independently sampled data point over one 24-h cycle (24). In 55 healthy controls and 34 patients with major depressive disorder (MDD), they found robust expression rhythms of hundreds of genes in several brain regions in control subjects, with many of these genes displaying significantly disrupted or altered expression rhythms in subjects with MDD. Here, we used a similar analytical approach to identify rhythmic transcripts in two areas of the PFC [Brodmann's area 11 (BA11) and BA47] in a large sample of 146 subjects from across the life span with no history of psychiatric illness. This large sample allowed us to investigate the effects of normal aging on molecular rhythms in the human PFC and to test the prediction that older individuals display altered or even a loss of molecular rhythms in this brain region.

Materials and Methods

Human Postmortem Brain Samples. Using the resources of the University of Pittsburgh's Brain Tissue Donation Program, 210 subjects were identified. Samples were obtained after consent from next of kin during autopsies conducted at the Allegheny County Medical Examiner's Office (Pittsburgh). The absence of lifetime psychiatric disorders was determined by an independent committee of experienced clinical research scientists using information from clinical records, toxicology results, and a standardized psychological autopsy. Subjects were removed from the study if death was not witnessed because their time of death (TOD) cannot be precisely determined. Subjects that did not meet the criteria of rapid death were also removed from the study. Sixty-four subjects were removed based on these criteria. The final sample consisted of 146 individuals with the following characteristics: mean (range) age of 50.7 (16–96) years, 78% male, 85% Caucasian, mean postmortem interval (PMI) for brain collection of 17.3 h (4.8–28 h), mean pH of 6.7 (5.8–7.6), and RNA integrity number (RIN) of 8.0 (5.9–9.6) (Table S1).

For each subject, the right hemisphere was blocked coronally, frozen, and stored at −80 °C. Blocks containing BA11 or BA47 of the orbital PFC were cut on a cryostat, and cortical gray matter was collected for RNA extraction as previously described (25). All procedures were approved by the University of Pittsburgh's Institutional Review Board for Biomedical Research and Committee for Research Involving the Dead.

Time of Death Analysis in the Zeitgeber Timescale. To analyze rhythmic gene expression, the TOD for each subject was normalized to a zeitgeber time (ZT) scale. For each subject, both place and time of death was collected, where the time zone of the place of death was used to adjust the reported TOD from local time to coordinated universal time. Daylight savings time was also incorporated when appropriate. The sunrise time was calculated according to the date and the longitude and latitude of the place of death. Subject's TOD was set as ZT = t hours after previous sunrise (if $t < 18$) or before next sunrise (if $t \geq -6$).

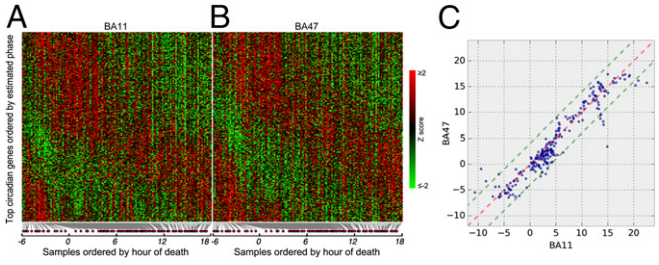

Fig. 1. Heat map of expression levels and comparison of circadian acrophase for the top circadian genes ($n = 235$, $q < 0.05$) in BA11 and BA47. (*A* and *B*) Expression levels were *Z*-transformed for each gene. Red indicates higher expression level; green indicates lower expression levels. (*C*) The circadian phase (peak hours) of 235 circa genes derived from metaanalysis are plotted on TOD axes for BA11 (x axis) and BA47 (y axis). Red dashed line: 1:1 diagonal line. Green dashed lines: ±4-h phase concordance boundaries. Ninety-two percent of circa genes (217 of 235) are located within the concordance interval.

Microarray Data Preprocessing. All samples were analyzed using the Affymetrix Human Gene 1.1 ST microarray platform. Gene expression values were corrected, quantile-normalized, and $\log_2$-transformed via Affymetrix Expression Console software (build 1.2.1.20) using RMA algorithm. A total of 33,297 probe sets were available on each array; only those probe sets with annotated gene symbols were selected (20,237 gene symbols). If a gene was represented by multiple probe sets, the one with the largest intensity interquartile region was selected to represent that gene. Microarray data for each brain region were analyzed separately. The raw and processed microarray data were deposited in the National Center for Biotechnology Information Gene Expression Omnibus database (GSE71620).

Detection of Circadian Gene Expression Patterns. Nonlinear regression was used to detect circadian gene expression patterns. All individual samples were ordered by their TODs. Temporal expression was fitted to a sinusoidal curve using the nonlinear least-squares method. The coefficient of determination (R^2) was used as a proxy of goodness-of-fit. The empirical P value was estimated from a null distribution of R^2 generated from 1,000 TOD-randomized expression datasets for BA11 and BA47 separately. Adaptive weighted Fisher method was adopted to combine multiple P values across brain regions (26). The q value was estimated using R package *qvalue* (27).

Analysis of Aging Effects on Expression Rhythmicity. Subjects were classified as younger (<40 y, $n = 31$) or older (>60 y, $n = 37$) and analysis of rhythmic gene expression was performed on each group independently as described above. Age effect P values were estimated from 1,000 age-shuffled data matrixes for BA11 and BA47 separately (*SI Materials and Methods*).

Results

Gene Expression Rhythms in Human BA11 and BA47. In 146 subjects, we performed a comprehensive gene expression analysis in BA11 and BA47 of the PFC (Fig. S1*A*). In BA11 2,475 genes (~12% of the genome) and in BA47 a total of 1,615 (~8% of the genome) displayed detectable circadian rhythmicity ($P < 0.05$) (Dataset S1). To identify a set of genes with consistent circadian regulation of gene transcripts across BA11 and BA47, we applied a metaanalysis approach across the brain regions and limited the false-discovery rate to 5% (i.e., $q < 0.05$). A total of 235 genes with a significant circadian expression pattern were detected at $q < 0.05$ (Dataset S1 and Table S2). Notably, these genes displayed coordinated temporal expression patterns across BA11 and BA47, as illustrated by the nearly identical patterns observed across the two areas (Fig. 1 *A* and *B*). Indeed, the peak or acrophase of expression of those 235 genes was highly concordant (92%) between BA11 and BA47 (Pearson's $r = 0.95$, $P = 5.8e\text{-}120$) (Fig. 1*C*). The core genes that make up the molecular clock showed robust expression rhythms in the PFC (Fig. 2). Their patterns of expression were strikingly similar across the two brain regions and also to those published previously (24), demonstrating the consistency in the use of these analyses to detect significant rhythms in gene expression in human postmortem tissue. The top 50 genes with significant circadian rhythms in expression are listed in Fig. 3. For example, *C1ORF51* (more recently known as *CIART*) has the most robust expression rhythm (Fig. S2). The mouse ortholog of this gene, *Gm129*, has been recently identified as having a circadian pattern of expression in mice (28, 29). Many of these genes display circadian expression patterns in peripheral tissues of the mouse, such as the liver, heart, and/or skeletal muscle, and in the brain, including the SCN and cerebellum. For example, *DUSP4*, *ANKRD12*, *TRIM24*, and *TMEM119* display expression rhythms in the mouse SCN (30), whereas *DUSP11*, *USP2*, *SESN3*, *BACE2*, and *PEX1* are expressed rhythmically in other areas of the brain (30, 31). Notably, some of the genes we identified here as displaying rhythmicity in the human PFC are not known to be involved in circadian rhythms, or even to be expressed in a circadian pattern, suggesting these genes may have previously unidentified roles within or downstream of the molecular clock. For example, *KCNH4*

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(potassium voltage-gated ion channel) is a top-ranked gene with strong circadian expression patterns across BA11 and BA47 (Fig. 3 and Fig. S2) and there are no previous reports of this transcript being regulated in a circadian manner. Other genes with a similarly strong pattern and no prior knowledge of rhythmicity include *KIAA1370*, *RSP02*, *ZNF43*, *BACE2*, *ZBBX*, and *FGD5*.

Effects of Aging on the *PER* Genes Across BA11 and BA47 of Human PFC. To test the prediction of altered rhythmic patterns of gene expression with aging, we stratified our subjects into younger (<40 y, $n = 31$) and older (≥60 y, $n = 37$) groups (Fig. S1*B* and Fig. S3) and performed an analysis of circadian rhythms of canonical circadian genes of the molecular clock within the two subgroups. Sinusoidal curve analyses were used to investigate differences in total expression levels, amplitude, acrophase, and absolute changes in rhythmicity (loss or gain of rhythm). We found consistent effects of age (younger vs. older) on the circadian pattern of expression for *PER1* and *PER2*, but very minimal changes in *PER3*, across BA11 and BA47 (Fig. 4). Specifically, the acrophases of *PER1* rhythms were shifted from ZT5 (BA11) and ZT7 (BA47) to ZT1–ZT2 with a reduction in amplitude in both regions (Fig. 4 *A* and *B*), suggesting significantly disrupted temporal expression of *PER1* in older individuals. Similar phase shifts in peak expression were observed for *PER2* (Fig. 4 *A* and *B*)—acrophase was shifted from sunset (~ZT12) to the middle of the day (ZT6). However, there were no obvious age effects on expression of *PER3* in either BA11 or BA47 (Fig. 4 *A* and *B*).

Effects of Aging on Rhythms of Gene Expression in BA11. We next conducted separate, whole-genome analyses on BA11 and BA47 to determine whether the age effects on patterns of expression differed between these two regions beyond the canonical circadian genes of the molecular clock. We identified 1,186 genes in BA11 that exhibited age-dependent rhythmicity or alterations in rhythmicity patterns with aging ($P < 0.05$; Table S3), which can be categorized into five characteristics (Fig. 5): (*i*) a base shift (meaning a change in levels of expression but not necessarily a change in rhythm); (*ii*) a decrease (but not complete loss) in amplitude; (*iii*) a phase shift; (*iv*) a significant loss of rhythmicity; and (*v*) a gain of rhythmicity. A total of 201 genes in BA11 do not display any impairment in rhythmicity with aging, including many of the canonical clock genes (Dataset S2). A single gene can display multiple kinds of age-related effects on its rhythmicity pattern. For example, a gene may display both a base shift and a phase shift. Complete lists of genes in each category are in Datasets S2 and S3. When differences between groups were analyzed, we found that, in BA11, there were 30 genes that showed significant changes in level of expression (base shift) while retaining overall rhythmicity from younger to older individuals—for example, several of the most robustly rhythmic genes, *PER1*, *KCNH4*, and *SPRY4* fell into this category (Fig. S4). There were 67 genes that displayed significant phase shifts in older individuals—for example, among the top circadian genes, *PER2*, *BACE2*, and *SPRY4* are all significantly phase-advanced in older individuals (Fig. S4). An impressive 588 genes showed a complete loss of rhythmicity due to age, including *ADRA1b* of the top circadian genes, and a canonical circadian gene, *CRY1*. Only two genes had dampened amplitudes between age groups. These were *C1ORF51* and *LACRT*. Finally, we identified 533 genes in BA11 that were not rhythmic in younger adults but appeared to gain rhythmicity with age, such as *ATM*, *ME2*, and *MEPCE* (Dataset S2).

Effect of Aging on Rhythms of Gene Expression in BA47. Compared with BA11, there were many more alterations in patterns of gene expression in BA47 between older and younger individuals (1,591 genes), suggesting that this area might be more strongly influenced by age (Table S3 and Dataset S3). We identified 35 genes that had a change in overall level of expression including *ARNTL* (*BMAL1*), *BHLHE40*, *DBP*, *KCNH4*, *NR1D1*, and *SPRY4* of the top 50 circadian genes (Fig. S5). Ninety-four genes displayed a significant shift in acrophase due to age, such as *BHLHE40*, *DUSP4*, *PER2*, and *SPRY4* of the top circadian genes (Fig. S5). There were three genes that had a reduction in amplitude without a loss of rhythm, *NOVA2*, *ADAMTS12*, and *HNRNPA3*. A large number of genes (1,063) lost rhythmicity due to age in this rain region, with several of these being in the top circadian genes and also having primary roles in molecular clock function, such as *PER1*, and other genes, including *ADRA1B*, *PDE7B*, and *RARA*. Interestingly, there were 434 genes that gained rhythmicity in older individuals, including two microRNAs, *miR128-1* and *miR15a*. Of note, 30 genes that gained rhythmicity in older individuals in BA47 overlapped with BA11, such as *A2M*, *CDKN1A*,

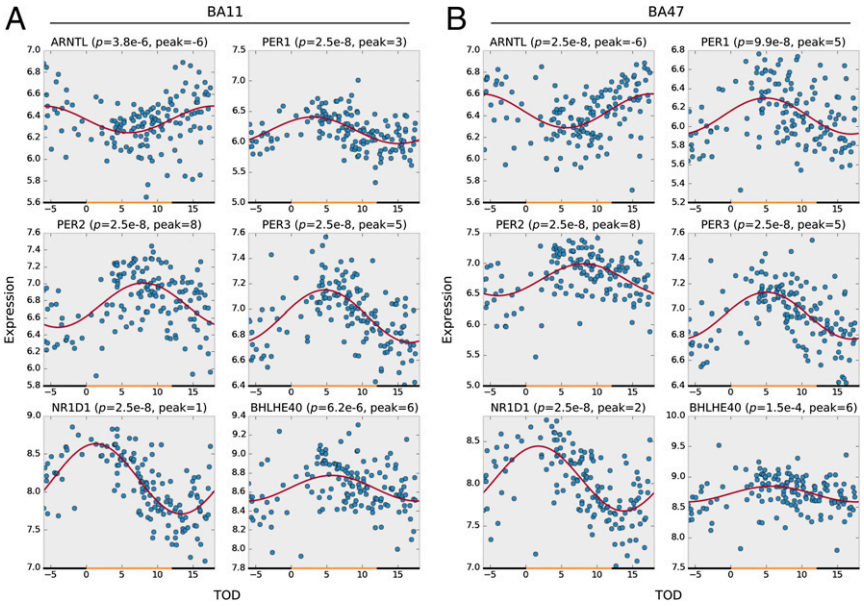

Fig. 2. Circadian gene expression patterns of six canonical circadian genes in BA11 (*A*) and BA47 (*B*). The *x* axis denotes the time of death (TOD) on ZT scale (−6–18 h). Approximate day–night interval within a pseudoday is represented by yellow (day) and black (night) bands. Data points from 146 subjects are plotted in blue. The best-fitted sinusoidal curves are depicted in red. The empirical *P* value and the estimated peak hour are also reported above each panel.

Gene	BA11 R^2	BA47 R^2	q value
C1orf51	0.51	0.50	5.88E-06
NR1D1	0.53	0.41	5.88E-06
PER3	0.41	0.36	5.88E-06
PER2	0.29	0.24	5.88E-06
KCNH4	0.24	0.27	5.88E-06
NR1D2	0.24	0.21	5.88E-06
PER1	0.29	0.21	5.88E-06
ARNTL	0.16	0.23	5.88E-06
OPRL1	0.18	0.18	5.88E-06
BHLHE41	0.18	0.18	5.88E-06
DBP	0.17	0.18	5.88E-06
DUSP11	0.21	0.14	5.88E-06
PIAS1	0.24	0.12	5.88E-06
PDZRN3	0.22	0.08	5.88E-06
ADRA1B	0.12	0.17	5.88E-06
RNF115	0.18	0.11	5.88E-06
ENG	0.14	0.15	5.88E-06
SPRY4	0.14	0.14	1.05E-05
NPAS2	0.10	0.17	1.05E-05
BHLHE40	0.16	0.12	2.50E-05
NFIL3	0.12	0.15	2.86E-05
LRRC39	0.17	0.09	6.36E-05
LDB1	0.16	0.10	6.52E-05
DUSP4	0.11	0.14	9.17E-05
USP2	0.15	0.10	1.04E-04
XRN1	0.13	0.12	1.08E-04
KIAA1370	0.14	0.11	1.33E-04
RARA	0.16	0.07	2.00E-04
RSPO2	0.15	0.08	2.03E-04
IRS2	0.10	0.13	3.32E-04
RC3H1	0.11	0.12	3.32E-04
ZNF43	0.13	0.10	4.38E-04
ANKRD12	0.14	0.08	4.70E-04
SESN3	0.08	0.14	5.00E-04
AKAP1	0.12	0.09	6.89E-04
TRIM24	0.17	0.05	7.25E-04
SLCO2A1	0.08	0.13	7.38E-04
SBK1	0.13	0.09	7.38E-04
TRAF5	0.15	0.06	7.38E-04
ALOX5AP	0.10	0.11	8.18E-04
PDE7B	0.13	0.08	8.54E-04
TMEM119	0.10	0.10	9.81E-04
BACE2	0.11	0.10	1.10E-03
RPS6KA5	0.11	0.10	1.12E-03
ZBBX	0.11	0.10	1.22E-03
FGD5	0.09	0.11	1.33E-03
CCDC91	0.11	0.09	1.35E-03
FHL3	0.06	0.14	1.35E-03
PEX1	0.11	0.09	1.35E-03
DCUN1D4	0.09	0.11	1.39E-03

Fig. 3. Top 50 clock-regulated genes across two brain regions. Gene list is sorted by *q* value after adaptive weighted (AW)-Fisher procedure. Genes in red are known circadian-related genes according to their records in GeneCards database (59, 60).

and *miR30a*. Finally, 193 genes in BA47 do not display any impairment in rhythmicity with aging (Dataset S3).

Several genes are overlapped in BA11 and BA47 in each age effect categories (Fig. S6). Most of the overlapped genes have the same direction of changes (Dataset S4). We also found significant co-occurrence between base shift and phase shift (Fig. S7). Notably, the top circadian genes with detectable age effects tend to have a downward base shift (base dropped) and/or an advanced phase (early peak hour) for the older subject group (Figs. S4 and S5).

Discussion

Our data are the first (to our knowledge) to indicate that age significantly alters the circadian rhythms of gene expression in the human PFC. Our data extend and replicate the findings of Li et al. (24) in larger cohort and further demonstrate that rhythms in gene expression in human postmortem tissue are robust and can be accurately measured. Moreover, the phase and amplitude of the clock-regulated genes analyzed here show remarkable consistency with the rhythms measured in these same genes in the Li et al. study. There are limitations to this type of analysis because we do not have information on these subjects regarding their lifestyle, actigraphy, and sleep time before death, which could contribute to changes in molecular rhythms. However, the independent confirmation and consistency between our cohort and the Li et al. study provides a high level of confidence in the overall results.

We decided to focus this study on two regions of the orbitofrontal cortex, BA11 and BA47. These are areas commonly associated with focused attention, executive functioning, and depression (32, 33). Although the Li et al. paper looked at genes that were rhythmic across six brain regions with more diverse cytoarchitectural structure (cortical, subcortical, cerebellum), we find many of the same genes to be rhythmic in BA11 and BA47 including most of the known circadian genes (i.e., *ARNTL*, *PER1*, *PER2*, *PER3*, *NR1D1*, *NPAS2*, etc.). Neither study identified *CLOCK* as a significantly rhythmic gene, which is consistent with its being constitutively expressed in other brain regions, the SCN in rodents and even in flies (34, 35). Interestingly, some of our most rhythmic genes identified, *C1ORF51*, *KCNH4*, and *OPRL1*, were not found in the Li et al. study, suggesting that perhaps their rhythmicity is specific to the areas of the brain that we investigated. Generally, we find very high concordance between rhythms in BA11 and BA47. This is interesting because studies in rodents have suggested that there can be differences in phase between adjacent brain regions (36, 37).

Several of the genes that have a significant rhythm in expression have not been described previously as core circadian genes or clock-regulated genes. One example is *KCNH4*, which is a voltage-gated potassium channel in the ether-a-go-go family (38). Its expression is largely restricted to the brain, although little is known about its function. Another is *PDZRN3*, a ubiquitin ligase known to be critically involved in cell differentiation via Wnt signaling in a variety of cell types throughout development (39, 40). We also identified certain genes that are known to play a role in rhythm regulation in the SCN or other tissues in rodents. For example, *OPRL1* (also known as nociception receptor) is an opiate receptor that is strongly expressed in the mouse SCN where it binds nociception/orphanin FQ, which suppresses 88% of SCN neurons (41). Moreover, *OPRL1* activation down-regulates *PER2* in the SCN and accelerates reentrainment of rhythms following a shift in the light–dark cycle (42). The importance of the rhythms in this protein in BA11 and BA47 function in human brain is not known. Another example is *C1ORF51* (also known as *Gm129*, *CHRONO*, and *CIART*). Annayev et al. (29) recently found that this protein acts as a novel transcriptional repressor with high-amplitude oscillations in the mouse liver that directly interacts with BMAL1 to repress CLOCK/BMAL1 function in this tissue. Here, we find that this transcript has the highest level of rhythmicity of any gene that we identified in human cortex, suggesting it may have a similar important circadian function in human brain. Interestingly, *RNF115* [also known as breast cancer-associated gene 2 (BCA2)] was strongly rhythmic in our study. RNF115 is another ubiquitin E3 ligase that is strongly associated with increased tumor growth, particularly in response to estrogen (43). Many studies have found significant correlations between disrupted circadian rhythms and increased risk for certain cancers, including breast cancer (44). Ubiquitination regulates the stability of core clock proteins; thus, it is possible that this protein interacts with core circadian proteins to regulate rhythms in cell growth and differentiation (45). Again, the role of this protein in the human PFC is not clear.

Age-stratified analysis enables us to identify age group-specific circadian genes that are undetectable from a cohort of heterogeneous age composition. For example, *FKBP5* shows a reversal of circadian rhythmicity between the younger and the older subjects (Fig. S8*A*). Its rhythmicity thus cannot be detected from a mixed age cohort composed of both younger and older subjects.

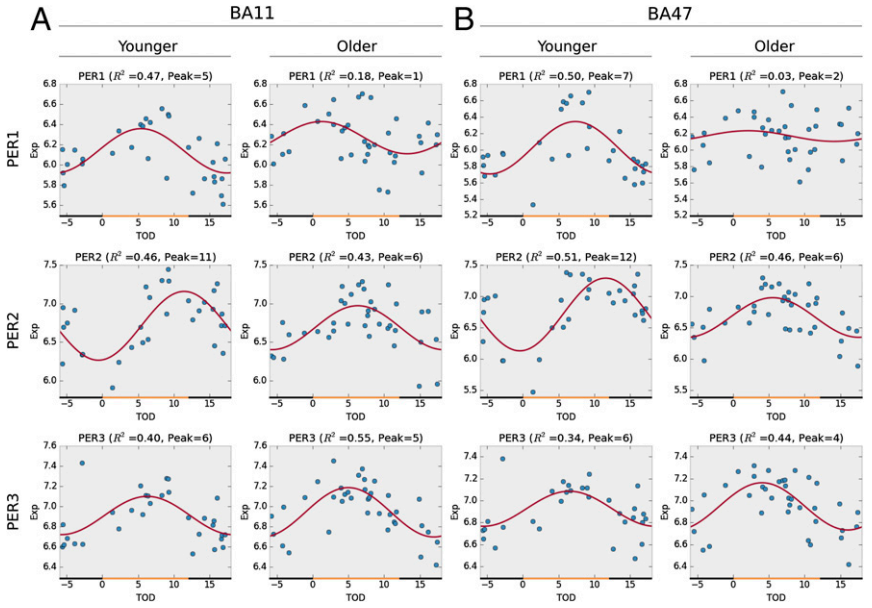

Fig. 4. Aging has unique effects on the circadian gene expression patterns of period genes (PER family) in both BA11 (*A*) and BA47 (*B*). Older people have disrupted PER1 circadian expression patterns ($P = 0.027$ in BA11; $P = 0.0005$ in BA47), together with a phase advance of PER2 expression from sunset to noon ($P = 0.005$ in BA11; $P = 0.004$ in BA47), whereas their PER3 expression remained intact.

Another example is *IGF1*, which also shows distinct rhythmicity between the younger and the older (Fig. S8*B*).

With aging, we found a disruption in several rhythmic transcripts, with the effects somewhat more pronounced in BA47 than in BA11. The reason for this difference is not clear. Samples were processed in parallel from dissection to array hybridization, making technical differences unlikely. Thus, differences may reflect distinct functions and underlying biology of the two regions. The most common deficit that we identified was a complete or partial loss of rhythmicity in transcripts that were rhythmic in younger people. We also identified certain transcripts that had a shift in phase. This is interesting because it is well documented that healthy older individuals tend to experience a shift toward "morningness" where they prefer to wake early in the morning and go to sleep relatively early in the evening (20, 46). There are natural variations in chronotype at all ages with some showing morning preference and some showing evening preference. Extreme chronotypes are associated with multiple psychiatric disorders (47–50), and it would be interesting in future studies to determine how molecular rhythms correlate with chronotype. Interestingly, a condition called "sundowning" or "sundown syndrome" affects some 20–40% of older people with dementia or Alzheimer's disease where they become confused, delirious, anxious, and agitated in the evening when the sun goes down, causing them to wander, become combative, and have difficulties sleeping (51). Sundowning is thought to be directly related to the breakdown in circadian rhythms in these individuals (52). Although our study examined only healthy individuals, it is possible that some of the genes that experience a loss of rhythmicity with aging might be linked to temporal changes in cognition.

Somewhat surprisingly, we identified a number of transcripts that gain rhythmicity with aging. Very interestingly, two of these transcripts were micro-RNAs *miR128* and *miR15a*. *miR128* is involved in the regulation of amyloid-β degradation in Alzheimer's disease and is involved in tumor suppression (53, 54), and *miR15a* is also involved in tumor growth and its expression is correlated with plaque score in Alzheimer's disease (55, 56). A survey of age-related microRNA expression changes in the neuronal stem cell niches of the short-lived annual fish *Nothobranchius furzeri* found that *miR15a* was abundant only in older animals (57). It will be interesting in future studies to examine postmortem samples from subjects with conditions such as Alzheimer's disease to determine whether there are more extreme changes in circadian gene expression. The gain of rhythmicity in these micro-RNAs could impact the cyclic expression of a whole host of proteins, perhaps leading to a novel, compensatory clock that is activated when the canonical clock breaks down. A recent study by Eckel-Mahan et al. (58) had a similar finding in the liver of mice fed a high-fat diet. Mice on regular chow showed normal cycling of canonical circadian genes in the liver and the rhythms in these genes were severely disrupted with a high-fat diet. However, another set of genes became rhythmic with the high-fat diet, suggesting a reprogramming of the liver to allow it to handle this change in diet. It is possible that a similar mechanism occurs in the brain of older individuals, concurrently with a partial breakdown of normal rhythmicity. More studies will need to be done, however, to determine the true significance and potential mechanisms regulating the gain of rhythmicity in these transcripts.

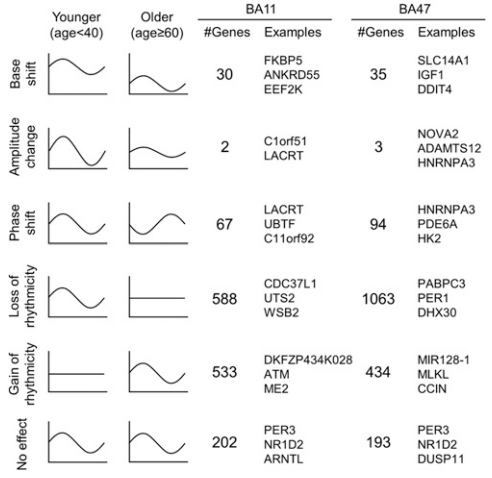

Fig. 5. Illustration of age effect on circadian gene expression patterns. The age effect on circadian gene expression patterns can be classified into five categories, as illustrated (rows 1–5). The number of instances and the top three most significant examples (sorted by *P* values) were also reported. See Datasets S2 and S3 for complete lists.

Conclusions

In conclusion, our study demonstrates the power of TOD transcriptomic analysis to identify transcripts with a circadian rhythm in human postmortem brain. We find many genes to have a significant rhythm in BA11 and BA47 with a high concordance in their phase. Furthermore, we have identified age-related changes in rhythmic transcript expression and somewhat surprisingly have uncovered a previously unidentified set of genes that gain rhythmicity in older individuals. These studies will help us better understand how rhythms change during aging and how targeted therapies that enhance molecular rhythmicity might be developed to prevent conditions like sundowning or enhance cognition. Moreover, in the future, we can use this approach to identify changes in molecular rhythms in a variety of brain regions or specific cell types that associate with psychiatric and neurological diseases.

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