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TRENDS IN MOLECULAR MEDICINE

Circadian genes and bipolar disorder

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Abstract
Bipolar disorder (BD) is a chronic, potentially disabling illness with a lifetime morbid risk of approximately 1%. There is substantial evidence for a significant genetic etiology, but gene-mapping efforts have been hampered by the complex mode of inheritance and the likelihood of multiple genes of small effect. In view of the complexity, it may be instructive to understand the biological bases for pathogenesis. Extensive disruption in circadian function is known to occur among patients in relapse. Therefore, it is plausible that circadian dysfunction underlies pathogenesis. Evidence for such a hypothesis is mounting and is reviewed here. If circadian dysfunction can be established as an 'endophenotype' for BD, this may not only enable identification of more homogenous sub-groups, but may also facilitate genetic analyses. For example, it would be logical to investigate polymorphisms of genes encoding key proteins that mediate circadian rhythms. Association studies that analyzed circadian genes in BD have been initiated and are reviewed. Other avenues for research are also discussed.

Key words: *Association, bipolar disorder, circadian, genetics*

Introduction

Bipolar disorder I (BD1) is common, potentially disabling and has a lifetime morbid risk of approximately 1% (1). The characteristic feature of bipolar disorder 1 (BD1) is the occurrence of one or more manic or mixed (manic/depressive) episodes, interspersed with major depressive episodes. Bipolar II disorder (BD2) is defined by milder manic episodes (hypomania) and major depressive episodes. Because non-genetic etiological factors are obscure and the heritability is relatively high (70%–80%), genetic investigations may hold the key to understanding etiology (2). Unfortunately, it has been difficult to identify genetic susceptibility factors (3,4).

The necessity for finer characterization of phenotypes for disorders such as bipolar disorder (BD) and schizophrenia (SZ) is being increasingly recognized in order to facilitate identification of susceptibility genes (5–7). Of particular interest are quantitative phenotypes genetically correlated with liability to BD1, because it is hoped that the genetic analysis of these traits will be more straightforward, more informative and have greater power than the diagnosis itself. Unfortunately these phenotypes, or endophenotypes, are largely unknown for BD1. Circadian variations appear to be excellent candidates (8,9). In the following sections, we outline the biology of circadian rhythm and the genes underlying circadian function. The evidence incriminating circadian dysfunction in bipolar disorder is then reviewed, along with the possibility that genetic variation underlies such abnormalities. The data are placed in the context of results from other mood disorders. Avenues for further research are suggested.

The mammalian circadian system

The rhythmic variation in the physiological and behavioral processes such as core body temperature, hormonal secretion, food intake, sleep/wakefulness and mood is 'circadian' and takes close to 24 hours from start to finish. This rhythm is endogenous in

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humans and other organisms (10). In the absence of external time cues, the rhythm persists with a period slightly longer than 24 hours (free run), but in normal conditions, it is entrained to daylight stimulus with a period of 24 hours. It has been suggested that the free running period shows marked variation and seems to change with age in humans (10,11), though Czeisler et al. have suggested that there are no age-related changes in the free running period, with a tight distribution consistent with other species (4).

The suprachiasmatic nucleus (SCN), located in the anterior hypothalamus above the optic chiasm is considered the master pacemaker for the circadian rhythm in mammals (12,13). Lesions of the SCN disrupt circadian oscillations leading to arrythmicity in animals. The circadian rhythmicity can be restored by implanting fetal SCN cells into the arrhythmic host (14,15). However, the new circadian oscillation follows the pattern of the donor rather than the recipient (16,14). These experiments suggest that SCN is the pacemaker of the circadian clock.

The SCN is also the site where self-regulatory feedback loops are initiated. These feedback loops induce and maintain circadian rhythms in other tissues. The retino-hypothalamic pathway carries the photic inputs from the retina to the SCN (17) (Figure 1 B). Projections from the SCN to other hypothalamic nuclei and other brain regions control various rhythms such as sleep-wake cycles, body temperatures and hormonal levels (18–20). Although the circadian clock in the small-paired nucleus has the ability to function autonomously without need for external cues (indicating the presence of a pacemaker), environmental cues can reset the clock, especially the light-dark cycles (11,21).

Key messages

- Several lines of evidence suggest circadian dysfunction among patients with mood disorders.
- Extensive research is under way to investigate the possible role of circadian genes in the pathophysiology of bipolar disorder.

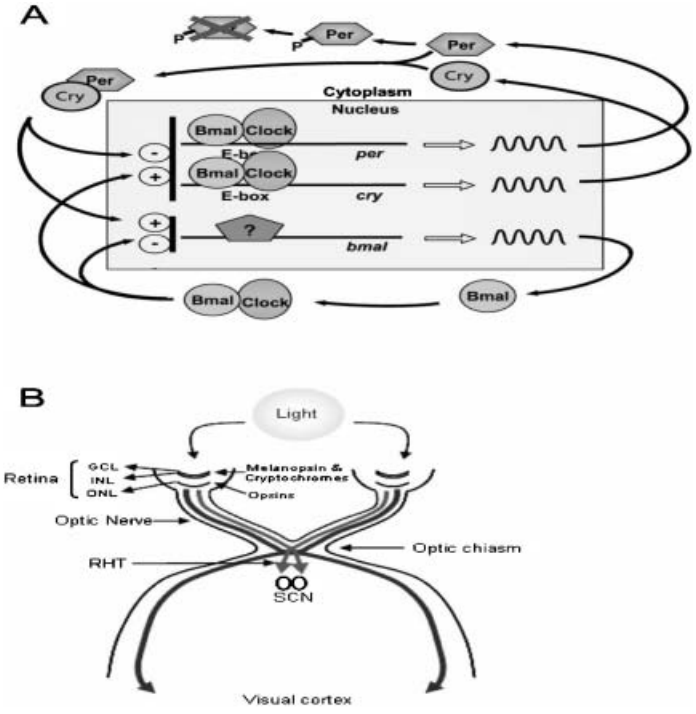

Figure 1. Dark and light function of the mammalian cryptochromes. **A,** dark function. This is a simplified scheme for the mammalian circadian clock. Clock and Bmal1 form a heterodimer that binds to the E-box sequences of target genes and stimulates their transcription. Crys and Pers also make heterodimers in the cytoplasm, translocate into the nucleus, and inhibit Clock-Bmal1-activated transcription (40,125). **B,** light function. The light signal received by the opsins in rods and cones in the outer (back) retina is transmitted to the visual cortex by the optic nerve. The light signal received by both the rods and cones in the outer retina and by the cryptochromes and melanopsin in the inner retina (front) is transmitted to the master circadian clock, the suprachiasmatic nucleus (SCN), by a specialized group of retinal ganglion cells constituting the retinohypothalamic tract (126,127). Adopted from (128), with permission from the American Society for Biochemistry and Molecular Biology.

Individual cells in the SCN can independently regulate their own rhythm and function as individual circadian oscillators that couple to form pacemakers (11,22). The basic theme underlying the mechanism of circadian rhythmicity appears to involve interlinked auto-regulatory transcriptional / translational feedback loops linked in a complex pattern (23,24). In mammals, the transcription factors *CLOCK* and *BmaL1 (ARNTL)* form a heterodimer that drives the expression of several genes such as *Period (Per)* and *Cryptochrome (Cry)* in mammals (25,26) (Figure 1 A). *Cry* and *Per* proteins inhibit the transcription induced by *CLOCK*/*BmaL1* complex, thus regulating their own expression (27,28). It is realized increasingly that there are added complexities in the molecular clock. Thus, Rora, an orphan nuclear receptor was recently identified as an activator of *BmaL1* transcription within SCN (29). Rora and Rev-erb α, which represses *BmaL1* expression, have been suggested to play an important role in maintenance of clock function through their opposing effects on *BmaL1*(29). The precise interactions among different clock genes as well as interactions between clock genes and other elements are still unclear. For example, there may be inherent differences between the SCN and other oscillators. It has been noted that oscillators in the forebrain use NPAS2 (neuronal PAS domain protein 2, alias MOP4). NPAS2 is a paralog of CLOCK that is not utilized in the SCN (30). Indeed, mice lacking NPAS2 have diffuse abnormalities in circadian function (31). Thus, circadian biology continues to be an area of intensive and fruitful research.

Clock genes across species

Clock genes encode proteins that generate the internal circadian rhythm in many physiological and behavioral processes in lower organisms and humans (21). Mutations in the clock genes can lead to disturbance in the circadian clock. Much progress in our understanding of the molecular basis of the circadian system has been achieved recently. It was initiated with the isolation of the *period (per)* locus in the fruit fly *Drosphila* (32,33), though it took several years to isolate the corresponding genes in humans (33–35). Through positional cloning, *CLOCK* was the first gene to be cloned and characterized in mammals (36,37). A few years later, additional circadian genes were discovered, enabling the definition of the circadian clock and its molecular mechanism (38–40).

The basic mechanism of the circadian clock is conserved from fruit fly to human (39). Strikingly, it has been shown that the structure for *PER1* and *CLOCK* genes is very similar in both humans and mice. For example, sequence analysis of human *Period 1 (hPer1)* and mouse *Period 1 (mPer1)* revealed that exon-intron organization is almost completely conserved with six highly conserved segments in the 5′ upstream showing 77%–88% identity (41). The coding sequence of human *CLOCK* is 89% identical to its mouse ortholog with highly conserved intron/exon organization. Further, its deduced amino acid sequence is 96% identical to mouse *CLOCK*(42). Several clock genes have been described in humans such as *ARNTL (BmaL1), PER1, PER2, PER3, CRY1, CRY2, CLOCK, Rora, Rev-erb* α*, GSK3-*β*, NPAS2 and CSNK1E* (29,41–51). As discussed above, intensive efforts are under way to explore more circadian proteins.

Evidence for circadian abnormalities in BD and other mood disorders

During the 1970s and 1980s there was a genuine hope that by understanding the chronobiology of mood disorders, non-pharmacological treatments for depression and bipolar disorder could be developed (52). For unipolar depression, the 'phase advance hypothesis' posited that the natural free-running rhythm of depressed patients was (unlike that of healthy controls) significantly shorter than 24 h, leading to abnormal phase relationships within different circadian rhythms, and to unwanted early morning awakenings, and abnormal diurnal mood swings, in these individuals who were posited to be living in something akin to a permanent 'jet-lag'. The hope was that by rectifying this jet lag, the patient's symptoms could be alleviated. Unfortunately, hard empirical evidence favoring the phase advance hypothesis could not be found, although circadian dysfunction (particularly in the form of reduced circadian rhythm amplitude was indeed there (e.g. (53)). Also, evidence for reduced REM latencies in depression (54) was also supportive of an early phasing pacemaker explanation, since REM sleep appears to be the sleep architecture variable most under the control of the SCN.

Regarding bipolar disorder, the hope was that the cycling of manic and depressed states could in some way be evidence of a 'beating effect' between the (aberrant) period length of the patients circadian pacemaker, and the imposed 24 h routine of society. Again, however, hard empirical evidence favoring this hypothesis was not forthcoming. Having said that, though, it remains the case that several lines of evidence suggest that patients with bipolar disorder have disrupted circadian rhythms. Marked alterations in circadian function such as sleep, activity and

appetite, as well as the diurnal secretion of hormones and other endogenous substances are well known among patients in relapse and even during remission (55–65). Disruption in circadian function can predate relapse and its normalization usually accompanies satisfactory treatment. Therefore, it has been proposed that disrupted circadian rhythms may be involved in the pathogenesis of BD, especially rapid cycling BD (66,67).

Indirect support for the circadian rhythm hypothesis comes from treatment studies. Lithium, a mood stabilizer, is known to modulate circadian rhythms, possibly by lengthening the circadian period (68–72). It has been suggested that such effects may mediate the therapeutic effect of the mood stabilizers (73). Non-pharmacological techniques have been shown to prevent mania and rapid cycling bipolar disorder by fostering sleep and stabilizing its timing (74). Further, it has been suggested that the disruptions in social zietgebers may also predate the circadian rhythm changes and that stabilization of social zeitgebers may reduce the risk of relapse (62,75–77).

Other techniques that modulate circadian rhythms, such as bright light treatment are beneficial for seasonal affective disorders and may even be effective for non-seasonal depression (78–80). Medication used for seasonal affective disorder can be combined with light therapy (80–82). Total sleep deprivation (TSD) is another non-pharmacological technique that may be efficacious in treating depression. It, too can be combined with other medications as well as light therapy (83–87). Diurnal variation of symptom intensity among patients with major depression could be a predictor of clinical response to antidepressant medication (88) and to antidepressant sleep-deprivation therapy (89). Thus, variations in individual chronobiological profiles may be important for determining therapeutic options (79). Analogous studies involving patients with bipolar disorder have not been conducted, to our knowledge.

Other factors can have important effects on circadian function and hence need to be taken into account when evaluating the circadian hypothesis for pathogenesis of mood disorders. For example, age has a significant impact on circadian rhythms and may thereby affect sleep architecture (90). Seasonal variation is another factor, but the interaction between season, circadian function and mood disorders is poorly understood. To evaluate this possibility, investigators measured the duration of melatonin secretion in constant dim light in winter and in summer among patients with major depressive disorder (major depressive episode with seasonal pattern for the current episode, DSM-IV) and matched healthy volunteers. The patients, but not the control individuals had significantly longer nocturnal periods of active melatonin secretion in the winter than in the summer (91). These data suggest that patients with seasonal affective disorder generate a biological signal of change of season similar to the signal used by mammals to regulate seasonal changes in their behavior (91).

Thus, additional evidence implicating circadian dysfunction in BD pathogenesis could be provided by investigation of clinically stable individuals, as well as longitudinal evaluation of such persons. If circadian abnormalities were shown to be 'trait' related, and especially if it predates relapse, a powerful argument for circadian arrythmicity as a trigger for relapse could be made. Studies to evaluate this possibility would be valuable, but are difficult to conduct.

Our recent work supports the circadian hypothesis. We used the Composite Scale (CS) (92), to evaluate 'morningness – eveningness' (M/E), a stable, heritable trait reflecting circadian phase among patients with BD1, unscreened controls and patients with schizophrenia (SZ) or schizoaffective disorder (SZA). This 13-item scale yields a single score, with higher values representing a greater morning-type orientation. The heritability of CS was estimated at 57% among multi-generational pedigrees ascertained on the basis of two first-degree relatives diagnosed with schizophrenia/schizoaffective disorder (SZ/SZA; $n=4$ families, 55 participants, $P<0.01$), following adjustment for age and diagnostic effects. Gender was not a significant correlate. These estimates are consistent with heritability of 44%–47% from a study of unscreened twins (93).

In separate studies, we found that patients with BD1 differ from two sets of comparison groups with respect to the M/E. CS scores are correlated with clinically relevant sub-groups such as the presence of rapid mood variation. Changes in circadian phase are also observed in SZ/SZA, but the pattern and magnitude of the changes are distinct from BD1 (94). These studies suggest distinctive phasic variation in BD, since M/E scales have been shown to be associated with inter-individual differences in the phase (timing) of physiological circadian rhythm in variables such as plasma cortisol and core body temperature (95). However, the impact of medication, mood state and chronicity on CS scores needs to be considered (96).

Genetic studies and the circadian hypothesis

It is plausible that mutations in circadian genes could lead to disruption in the auto regulatory

feedback loops, which could be manifested as bipolar disorder. Indeed, it has recently been reported that expression of the circadian pacemaker protein (*PER1*) is altered in postmortem temporal cortex from patients with schizophrenia (97). Microarray analyses of postmortem samples from BD1 patients would provide further support for the circadian hypothesis.

In the following sections, we describe studies implicating circadian gene mutations in classic circadian diseases, followed by studies of circadian gene variation in psychiatric disorders other than BD. Genetic association studies of BD are then described. Such studies can potentially provide direct evidence implicating circadian dysfunction in their pathogenesis.

Studies of circadian genes and non-psychiatric disorders

A serine to glycine mutation within the casein kinase I epsilon (CKIepsilon) binding region of *hPer2* gene, appears to cause familial advanced sleep phase syndrome (FASPS) in some families. FASPS is an autosomal dominant circadian rhythm disorder (50). A haplotype of the human Period3 gene *(hPer3)*, one of the human homologs of the *Drosophila* clock-gene period (Per), is associated with the delayed sleep phase syndrome (DSPS) suggesting that structural polymorphisms in the *hPer3* gene may be implicated in the pathogenesis of DSPS (98). It has been suggested that a length polymorphism in the same gene *(hPer3)* is linked to (DSPS) and diurnal preference, with the long allele associated with morningness and the short one associated with eveningness (99). A recent study from Japan suggested that a missense variation (S408N) within the casein kinase I epsilon *(CKIepsilon)* gene has a protective mechanism in the development of DSPS non-24-h sleep-wake syndrome through alteration of the enzyme activity (100). Variations in the CLOCK gene are associated with sleep dysregulation in mood disorders (101). It has been reported that the polymorphism 3111 T/C in the 3' flanking region of the human CLOCK gene is associated with diurnal preference, allele 3111C is associated with eveningness (49). However, two subsequent studies could not replicate this finding (102,103). Therefore, it is arguable whether circadian gene polymorphisms confer susceptibility to mood disorders.

Genetic studies of circadian genes and other psychiatric disorders

Despite circumstantial evidence for disruption of the circadian rhythm in patients with psychiatric disorders such as major depression and seasonal affective disorders (SAD), few studies have investigated circadian genetic associations. A study involving 159 European SAD patents and 159 matched controls reported significant differences between patients and controls for NPAS2 471 Leu/Ser polymorphism. No significant differences were found with *CLOCK 3111 C/T*, *PER2 1244 Gly/Glu*, or *PER3 647 Val/Gly* polymorphisms (104). Another study involved 143 European Americans; with history of major depression, 137 screened controls and 58 African-American screened controls did not find an association between *CLOCK* gene alleles at *3111 T/C* locus and major depression (105). It is difficult to say from these limited analyses whether circadian genetic variation imparts liability to these conditions or even to circadian variation within the diagnostic phenotype.

Genetic studies of circadian gene polymorphisms and bipolar disorder

There have been few published association studies of circadian genes and BD. A study from Japan analyzed the CKIepsilon binding region of hper2 gene in a sample of 88 bipolar disorder patients and 127 controls. No significant case-control differences in the frequencies of the S662G allele and genotypes were detected (106).

Intriguing associations have been recently reported with regard to glycogen synthase kinase 3-β (GSK3-β) which is the mammalian ortholog of the *Drosphila* gene SHAGGY. *GSK3*-β maps to chromosome 3q21.1 and plays an important role in the regulation of the molecular clock in the SCN (107,108). Lithium inhibits the phosphorylation activity of GSK3-β, and this effect may be of therapeutic relevance (109–112). Thus, *GSK3*-β is a plausible candidate gene for bipolar disorder liability. Analysis of the -50 T/C SNP in the promoter region of *GSK3*-β among 185 Italian patients with BD1 (DSM-IV) showed no association with BD1. However, homozygotes for the wild variant (T/T) had an earlier age at onset than carriers of the mutant allele (113). These results are intriguing, because early onset increases morbid risk for affective disorders (114,115). Hence, age at onset of illness among probands may be useful for defining homogenous subgroups of mood disorder (113).

To evaluate the association of GSK3-β with response to total sleep deprivation (TSD), the same SNP was analyzed among 60 Italian patients with BD1 (depressive episode) without psychotic features. Homozygotes for the mutant alleles had a later

onset of illness and a better acute effect of TSD treatment, suggesting a protective role for this genotype (GSK3-β C/C) (116).

Case-control comparisons involving candidate gene polymorphisms are prone to artifacts introduced by population sub-structure, though the impact of sub-structure is debated (117,118). Still, it is more convincing to demonstrate associations using family-based controls (119). Another shortcoming of association studies is the low prior probability of detecting association with functional candidate genes (120). Therefore, replicate studies are important before genetic associations can be accepted.

We recently investigated polymorphisms in eight circadian genes assessing 138 patients with BD1 and available parents. Unrelated controls (n=182) were also available. We detected modest significant association with three genes. Our analyses revealed significant case-control differences for the SNPs rs1481892; rs7107287; rs4757142 and rs1982350 at *ARNTL*. Consistent with the case-control differences, our family-based analysis revealed significant transmission distortion at SNP rs895682 at *ARNTL* gene using the transmission disequilibrium test (TDT). Further, we could detect significant case-control differences for the SNP rs2859387 at *PER3*. At *TIMELESS*, significant over-transmission of allele 1 (A) was detected using the TDT at SNP rs2291738, along with a trend for over-transmission of allele 1 (G) at SNP (121). These analyses were uncorrected for genome-wide multiple comparisons. Hence, we sought replication in an independently ascertained sample (96 case-parent trios), recruited through the National Institute of Mental Health (NIMH) Collaborative Genetics Initiative. Significant association could not be detected in this sample (121). This sample had insufficient power to replicate the initial association, so it is unclear if it represents a failure to replicate. We are presently conducting replicate studies using sufficiently powered samples.

It has been suggested that BD and schizophrenia may share some genetic susceptibility factors, and circadian dysfunction was noted among patients with schizophrenia (122). Therefore, we also analyzed polymorphisms of these three genes in our schizophrenia sample (331 cases with available parents). Associations with *TIMELESS* and *PER3* genes were detected using family-based analyses, albeit with different SNPs. TDT analysis revealed significant transmission distortion at the SNP rs2859387 at *PER3*. At *TIMELESS*, significant increased transmission of allele 1 (C) at SNP rs774026 was noted (121).

Conclusion:

Important advances have been accomplished in our understanding of circadian biology and its genetic basis. As there is considerable evidence for circadian dysfunction among patients with mood disorders, it is important to investigate the role of circadian gene variation in their pathogenesis. It may also be feasible to use circadian functions as complementary quantitative phenotypes (endophenotype) as tools for gene mapping studies. Both types of studies are in the nascent phase.

An important first step would be the demonstration of circadian abnormalities predating the onset of illness or predating relapse. As such studies would require analysis of 'first break' and/or drug naïve patients they are difficult to conduct. A practical alternative is to investigate unaffected relatives of BD patients. If persistent circadian abnormalities are demonstrated among such individuals, there would be a convincing argument for co-segregation of circadian abnormalities with susceptibility to BD. The family-based sample could also be used for genome wide evaluation through linkage analysis, followed by association analysis (123).

Several studies have investigated the circadian genes as plausible candidates for the susceptibility to bipolar disorder. In our study, modest associations were observed with three circadian genes, namely *ARNTL* (*BmaLI*), *TIMELESS* and *PERIOD3* genes using case-control and family-based samples. As the associations were not replicated in a smaller NIMH sample, further investigations in larger independent samples are needed. Since these genes are not localized to any of the regions implicated in prior linkage studies (124), it remains possible that as yet misidentified circadian genes confer susceptibility to bipolar disorder.

Based on the extensive body of research in the genetics of circadian rhythm, the involvement of circadian genes in the pathogenesis of bipolar disorder is plausible. Several association studies have provided suggestive leads. Further systematic studies are required in order to address these important questions.

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