Genetic contributions to circadian activity rhythm and sleep pattern phenotypes in pedigrees segregating for severe bipolar disorder

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Abnormalities in sleep and circadian rhythms are central features of bipolar disorder (BP), often persisting between episodes. We report here, to our knowledge, the first systematic analysis of circadian rhythm activity in pedigrees segregating severe BP (BP-I). By analyzing actigraphy data obtained from members of 26 Costa Rican and Colombian pedigrees [136 euthymic (i.e., interepisode) BP-I individuals and 422 non–BP-I relatives], we delineated 73 phenotypes, of which 49 demonstrated significant heritability and 13 showed significant trait-like association with BP-I. All BP-I–associated traits related to activity level, with BP-I individuals consistently demonstrating lower activity levels than their non–BP-I relatives. We analyzed all 49 heritable phenotypes using genetic linkage analysis, with special emphasis on phenotypes judged to have the strongest impact on the biology underlying BP. We identified a locus for interdaily stability of activity, at a threshold exceeding genome-wide significance, on chromosome 12pter, a region that also showed pleiotropic linkage to two additional activity phenotypes.

bipolar disorder | endophenotypes | circadian rhythms | actigraphy | behavior

Quantitative sleep and activity measures are hypothesized to be endophenotypes for bipolar disorder (BP). Disturbance of sleep and circadian activity typically precedes and may precipitate the initial onset of BP (1, 2). Decreased sleep and increased activity occur before and during manic and hypomanic episodes. Conversely, increased sleep and decreased activity characterize BP–depression. Extreme diurnal variation in mood features prominently in both mania and depression, whereas shifts in circadian phase (the time within the daily activity cycle at which periodic phenomena such as bed time or awakening occur) can induce mania and ameliorate symptoms of BP–depression (3).

Twin studies have identified multiple heritable sleep and activity phenotypes, including sleep duration, sleep quality, phase of activity preference and sleep pattern, and sleep architecture variables [e.g., the amount of slow wave and rapid eye movement (REM) sleep (4) and polysomnography profiles during non-REM sleep (5)]. Euthymic BP individuals, compared with healthy controls, display trait-like alterations in several such phenotypes—for example, sleep time and time in bed, sleep onset latency, and periods of being awake after sleep onset (6). However, no prior investigations have assayed the heritability of such phenotypes in BP individuals and their relatives.

We report here the delineation of sleep and activity BP endophenotypes through investigations of 26 pedigrees (n = 558) ascertained for severe BP (BP-I), from the genetically related populations of the Central Valley of Costa Rica (CR) and Antioquia, Colombia (CO) (7–9). Pedigrees ascertained for multiple cases of severe BP (BP-I) should be enriched for extreme values of quantitative traits that are BP endophenotypes, enhancing their utility for genetic mapping studies of such phenotypes. Additionally, such pedigrees derived from recently expanded

Significance

Characterizing the abnormalities in sleep and activity that are associated with bipolar disorder (BP) and identifying their causation are key milestones in unraveling the biological underpinnings of this severe and highly prevalent disorder. We have conducted the first systematic evaluation of sleep and activity phenotypes in pedigrees that include multiple BP-affected members. By delineating specific sleep and activity measures that are significantly heritable in these families, and those whose variation correlated with the BP status of their members, and by determining the chromosomal position of loci contributing to many of these traits, we have taken the first step toward discovery of causative genetic variants. These variants, in turn, could provide clues to new approaches for both preventing and treating BP.

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founder populations are likely to show increased frequencies for many deleterious alleles—another feature that will enhance their utility for mapping these traits (10, 11). We previously described, in these pedigrees, multiple heritable and BP-associated phenotypes from the domains of temperament, neurocognition, and neuroanatomy (10).

Actigraphy (activity measurement using wrist-worn accelerometers) can be conducted over prolonged periods without impinging on an individual's usual activities, enabling assessment of sleep and activity on a scale sufficient for genetic investigations. Actigraphy data on sleep quality and duration correlate strongly with those obtained through polysomnography, the gold standard for sleep research (12). Using actigraphy, one can estimate the main circadian activity parameters, which follow a sinusoidal waveform with a ~24-h period: phase, amplitude (the strength of circadian rhythms, as measured by the difference in the amount of activity between active and inactive moments), and coherence of the rhythm (the degree of consolidation and stability of activity, rest, and sleep). Finally, actigraphy enables quantification of BP-associated features, such as fragmentation of rest and activity within and between days, that do not fit a sinusoidal waveform and cannot be analyzed parametrically (13).

We recorded activity in euthymic BP-I individuals and their non–BP-I relatives from the CR and CO pedigrees for, on average, 14 consecutive days. For each actigraphy phenotype, we evaluated association with BP-I, assessed the heritability of each trait, and performed genome-wide genetic linkage analyses on all significantly heritable traits.

Results

Through actigraphy, we obtained activity recordings (illustrated in Fig. S1) from 638 members of 26 CR and CO pedigrees. After applying quality control (QC) procedures (*SI Methods*) that led to the exclusion of 80 recordings (Fig. S2), we analyzed activity data from 558 individuals, including 136 BP-I individuals and 422 of their non–BP-I relatives (Table 1). A series of algorithms obtained from published sources (14–17) were then applied to the activity data to obtain 116 quantitative sleep and activity phenotypes. These phenotypes can be classified into six broad domains that quantified patterns of activity and sleep during the major rest period of the day (*i*) and during the awake period (*ii*), the fragmentation or consolidation of activity (*iii*), overall activity levels (*iv*), and the fit of daily activity patterns to curves based on sine and cosine functions (using two different approaches) (*v* and *vi*). Details on the construction of phenotypes that fall into each domain are provided in *SI Methods*.

To reduce the multiple testing burden and eliminate completely redundant variables, we calculated pair-wise correlations among all 116 phenotypes (Fig. S3) and performed a hierarchical clustering analysis using 1 – correlation as the distance metric. We then selected one representative phenotype from each cluster, yielding 73 phenotypes (Fig. S3), which we analyzed further as described below. The 43 phenotypes excluded at this stage were very highly correlated with other phenotypes, all with $r > 0.90$ and most with $r > 0.99$.

Heritability of Phenotypes and Their Association to BP-I. Estimating the familial aggregation of the 73 phenotypes (an indicator of heritability) and their relationship to BP-I allowed us to determine which phenotypes have a significant genetic component, to proceed with analyses to identify genes contributing to phenotypes that are potentially important in the etiology of BP-I. We subjected each phenotype to an inverse-normal transformation, and to control for covariates, we regressed [in the software SOLAR (Sequential Oligogenic Linkage Analysis Routines) (18)] the transformed phenotypes on age, gender, and country. The residuals from this

Table 1. Sample characteristics and recording days by country and family

Family	n (BP-I cases)	Female	Mean age (SD), range	Mean recorded days (SD), range
CO All	269 (66)	58%	46.3 (16.6), 18–83	15.1 (2.1), 7–24
CR All	290 (70)	53%	49.2 (16.0), 17–88	15.8 (2.9), 6–27
CO4	33 (7)	61%	42.0 (17.2), 18–76	15.1 (2.3), 13–24
CO7	96 (23)	53%	44.8 (17.0), 18–80	14.8 (1.6), 7–21
CO8	5 (2)	60%	40.8 (12.8), 24–54	14 (4.9), 7–21
CO10	22 (5)	73%	53.4 (15.4), 32–77	15.4 (2), 14–21
CO13	14 (4)	57%	42.1 (14.7), 18–66	17.1 (3.1), 14–21
CO14	13 (4)	46%	43.8 (15.4), 20–74	14.1 (2.4), 10–21
CO15	19 (4)	58%	41.7 (15.3), 19–73	14.5 (1.6), 12–20
CO18	20 (6)	55%	59.3 (14.2), 34–77	15.2 (1.9), 14–20
CO23	21 (6)	67%	45.0 (15.2), 18–83	15.3 (1.9), 14–20
CO25	8 (2)	63%	56.5 (13.5), 45–82	15.6 (2.6), 14–21
CO27	18 (3)	67%	46.7 (16.8), 18–74	15.4 (1.8), 14–21
CR001	5 (1)	60%	56.0 (11.3), 44–68	19.6 (2.6), 15–21
CR004	37 (7)	51%	55.6 (13.5), 30–84	15 (1.9), 12–21
CR006	7 (2)	29%	52.7 (11.8), 38–66	13.9 (4.1), 6–20
CR007	4 (2)	50%	47.0 (6.6), 39–55	13.8 (0.5), 13–14
CR008	9 (3)	44%	43.7 (16.1), 21–68	15.7 (2.5), 14–20
CR009	25 (6)	60%	40.6 (14.4), 20–74	15.6 (2.8), 12–22
CR010	12 (3)	58%	43.9 (15.9), 21–74	14.2 (2.2), 11–19
CR011	9 (2)	56%	48.8 (23.2), 21–86	18.2 (3), 14–21
CR012	19 (5)	68%	40.8 (15.3), 20–68	17 (3.2), 14–21
CR013	5 (2)	80%	52.2 (19.3), 35–74	15.4 (2.2), 14–19
CR014	2 (1)	50%	45.0 (2.8), 43–47	14.5 (0.7), 14–15
CR015	8 (1)	63%	51.6 (14.2), 38–71	18.4 (2.2), 16–21
CR016	13 (4)	38%	50.1 (14.5), 19–66	16.8 (2.6), 13–21
CR201	125 (28)	51%	50.6 (16.2), 17–88	15.8 (3.1), 7–27
CR277	9 (3)	56%	49.3 (11.6), 37–71	14.1 (0.3), 14–15
Grand total	558 (136)	56%	47.8 (16.3), 17–88	15.4 (2.6), 6–27

regression were assessed for heritability and for a mean difference between BP-I individuals and their non–BP-I relatives.

Of the 73 phenotypes, 49 (67%) demonstrated significant heritability. Heritable phenotypes included measures related to sleep and activity duration, timing, fragmentation, and consolidation; activity levels and variability; and the timing and periodicity of mean daily activity (Fig. 1).

Thirteen phenotypes (18%) were significantly associated with BP-I, of which 12 (92%) were also heritable (Fig. 1 and Fig. S4). BP-I subjects awoke later and slept longer than non–BP-I subjects (phenotypes, mean of sleep offset time and mean sleep duration). Outside of the rest period, BP-I individuals were, on average, awake fewer minutes than non–BP-I individuals (phenotypes, mean of awake duration and mean of total minutes scores as awake) and had more variability in the time during the awake period scored as sleeping (phenotype, SD of the total minutes scored as sleep). Similar to previous studies (16, 19), we found that euthymic BP-I individuals display lower activity levels

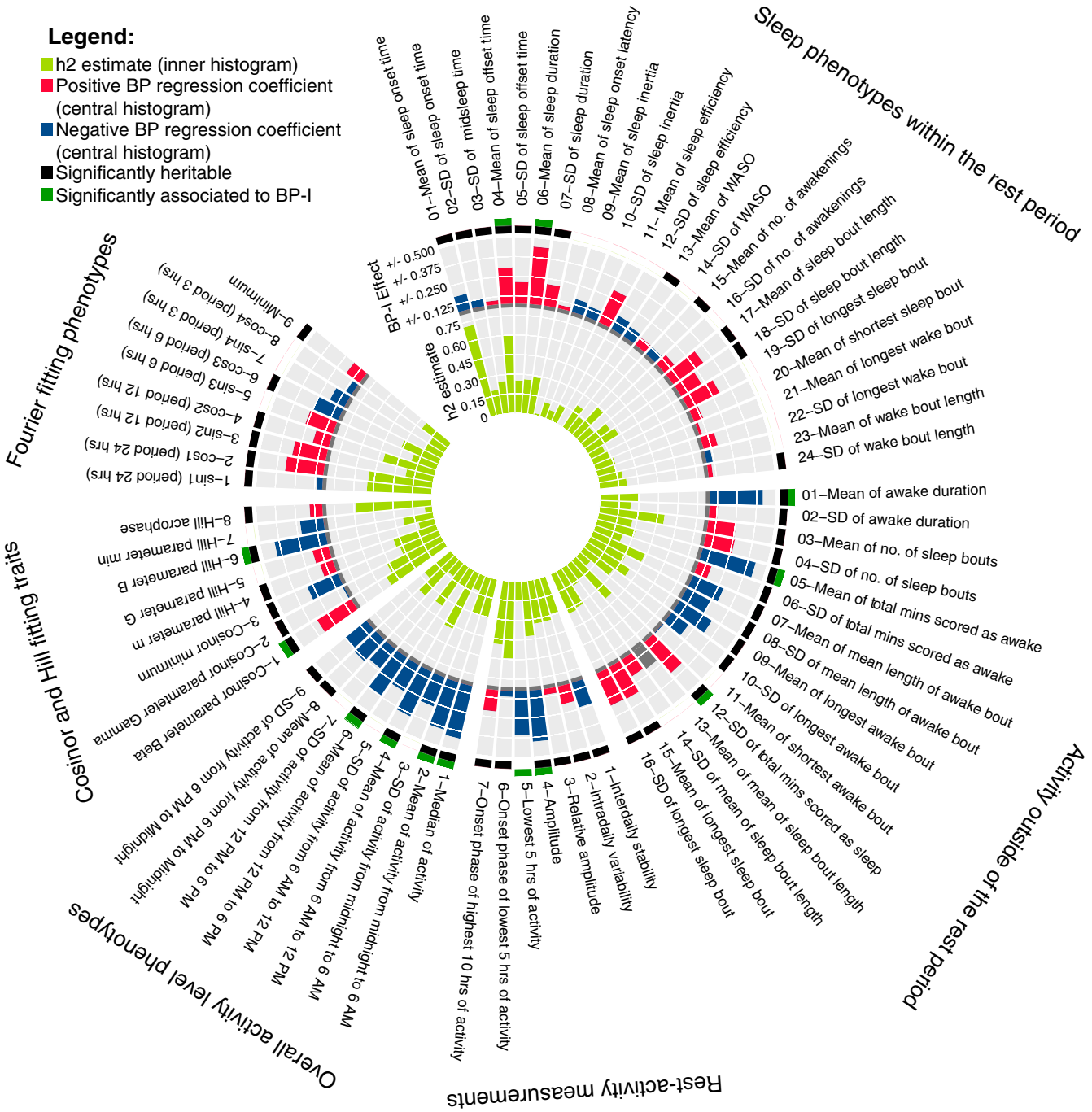

Fig. 1. Polar histogram of heritability traits and association to BP-I. The inner histogram represents the heritability estimate (h2) in yellow–green. The middle histogram represents the association to BP-I; positive associations with BP-I are presented in red (i.e., the trait has a higher value in those with BP-I), whereas negative associations are presented in blue (indicating those with BP-I have lower values on the trait). The outer histogram summarizes the heritable traits (black) and the phenotypes associated with BP-I (green). hrs, hours; mins, minutes; no., number.

than non–BP-I individuals, by multiple measures: amplitude; L5, 5-h period of minimal activity; median activity; mean activity in 6-h windows; and two parameters related to the amplitude and phase of curve fits to mean daily activity (parameter B from the fit of the Hill transformation to the daily activity profile and Cosinor parameter Beta).

Linkage Analysis of Selected Heritable Traits. To identify genetic loci with the largest impact on sleep and activity phenotypes in the BP-I pedigrees, we conducted genome-wide linkage analysis using a dense set of SNPs. Our primary analyses focused on 13 phenotypes that we considered most relevant to BP. These phenotypes included traits with prior evidence of BP association, traits that showed strong BP-I association in our pedigrees, and traits biologically relevant to fragmentation or consolidation of circadian activity or with biological relevance to phase (Table 2).

Using SOLAR, we performed multipoint linkage analysis on these 13 phenotypes. The strongest linkage was for the rest–activity phenotype Interdaily Stability (IS), that represents the degree of variability of activity level on an hourly basis from day-to-day (Fig. 2), for which we observed a maximum LOD [logarithm (base 10) of odds] score (4.73, 4 Mb from chromosome 12pter), exceeding the traditional genome-wide significance threshold ($P < 10^{-4}$). Interdaily stability linkage remains genome-wide significant at the 0.05 alpha level, after correcting for the 13 phenotypes (empirical P values associated to the highest LOD score and to the smallest Simes' P value are 0.03 and 0.02, respectively). The linkage evidence for interdaily stability diminishes only slightly when including BP-I status as a covariate (LOD = 4.65; Table S1).

Fig. 3 presents a summary of the significance of linkage results considering all 49 heritable phenotypes (20). Two linkage peaks stand out. The largest peak includes the interdaily stability linkage on chromosome 12pter, having a Simes P value < 0.0001. In the same region of linkage to interdaily stability, we observed suggestive linkage for two additional phenotypes (Fig. 4): the mean number of sleep bouts in the awake period, and amplitude (the difference in activity between the 5 most active hours and the 10 least active hours), with peak LOD scores, respectively, of 2.53 (8 Mb from pter) and 2.13 (1 Mb from pter). Interdaily stability and mean number of sleep bouts show moderately negative phenotypic correlation ($rho_p = -0.51$) but strongly negative genetic correlation ($rho_g = -0.93$); joint multipoint linkage analysis suggests complete pleiotropy, indicating a common genetic component to both phenotypes, centered 4 Mb from 12pter (LOD = 3.35); lower interdaily stability indicates a weaker rhythm, while a lower value of mean number of sleep bouts is associated to a more consolidated rhythm, leading to a negative correlation between these variables. Amplitude and interdaily stability display phenotypic and genotypic correlation of a similar magnitude, albeit in a positive direction ($rho_p = 0.51$ and $rho_g = 0.92$); their joint linkage analysis (LOD = 3.41) suggests near-complete pleiotropy between these phenotypes, at the same location.

A second (nonsignificant) linkage peak on chromosome 1q displayed LOD > 3.0 for four correlated phenotypes related to the SD of sleep onset and activity (SD of the time of sleep onset, LOD 3.9 at 183 Mb; SD of the active phase, LOD 3.6 at 185 Mb; SD of the time of midsleep, LOD 4.3 at 186 Mb; SD of the total minutes awake, LOD 3.4 at 186 Mb). These phenotypes all have a pair-wise genetic correlation >0.85, as is reflected in joint linkage results suggesting complete pleiotropy among them (joint LOD scores range from 3.1 to 4.2 at 185 Mb; Table S2).

Discussion

We report here, to our knowledge, the first large-scale delineation of sleep and activity phenotypes in BP-affected individuals and their relatives. More generally, it is the first genetic investigation of such a comprehensive set of sleep and circadian measures in any human study.

Phenotypes significantly associated to BP-I paint a consistent picture; activity is lower in euthymic BP-I individuals than in their non–BP-I relatives, reflecting a longer sleep duration, with a later time of sleep offset and rest offset, resulting in a shorter duration of the active phase. Furthermore, during the active phase, BP-I individuals have fewer total minutes scored as awake and more variability in the total minutes scored as asleep. Such individuals also display lower amplitude, mainly due to their lower activity level during the least active hours of the day.

As the BP-I individuals who participated in the study were all euthymic at the time of recording, the phenotypes that we observed could be considered representative of the remission phase of the disorder. Previous studies have suggested that disturbances in sleep and circadian activity are early signs of manic episodes, particularly in individuals affected with rapid-cycling forms of BP (1, 2). We did not observe a high rate of subsequent mania among those BP-I individuals in whom we detected the most extreme sleep and activity phenotypes; however, it is possible that we may have missed such a relationship given that we

Table 2. Thirteen phenotypes chosen for the primary linkage analysis

Phenotype	Trait	Importance
Amplitude	Relative amplitude	BP-I associated (19)
	Amplitude	Association with BP-I in the present study
	Median of activity level	Association with BP-I in the present study
Phase	Time of sleep onset	BP-I associated (17)
	Time of sleep offset	BP-I associated (17)
	Hill acrophase	Biologically relevant for phase
Fragmentation/ consolidation	IV, Intradaily Variability in activity	BP-I associated (17)
	IS, Interdaily Stability in activity	BP-I associated (17)
	Mean of the number of sleep bouts during the awake period	Biologically relevant to fragmentation/consolidation
	Mean of the length of sleep bouts during the sleep period	Biologically relevant to fragmentation/consolidation
Efficiency of sleep	WASO, total minutes in awake bouts after sleep onset	BP-I associated (24)
	Mean of awake duration	Association with BP-I in the present study
	Mean total minutes scored as awake during the awake period	Association with BP-I in the present study

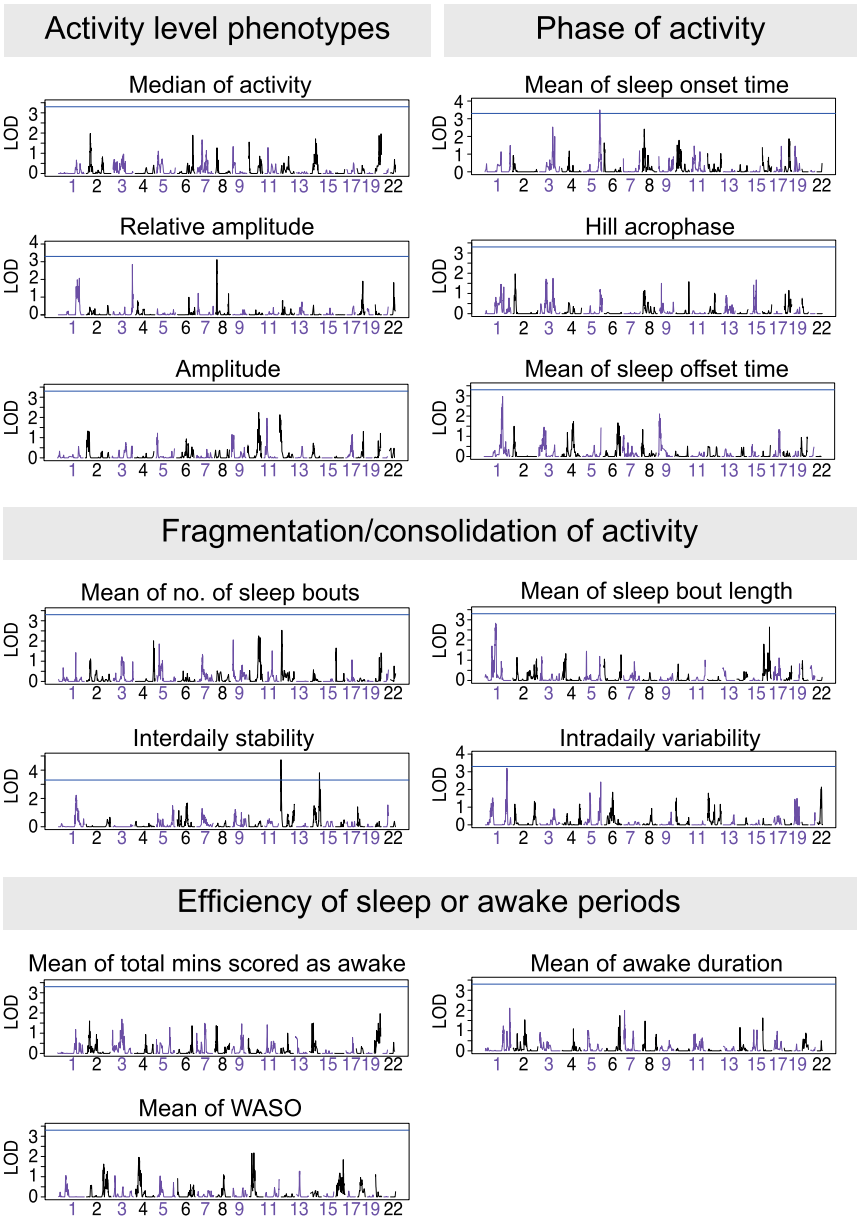

Fig. 2. Representation of the multipoint LOD scores on different chromosomes (which are depicted in alternating violet and black) for 13 traits that we considered of highest biological significance. Blue line indicates an LOD score of 3.3, corresponding to a P value of 5×10^{-5}. Mins, minutes; no., number.

did not systematically monitor clinical state after the 2-wk recording period.

Our observation of longer sleep duration in BP-I individuals accords with the results of a meta-analysis conducted in euthymic BP cases compared with controls (6). However, although the meta-analysis observed a difference between cases and controls in sleep onset latency, we did not observe such a difference between BP-I individuals and their non–BP-I relatives. Our study differs from the previous investigation in two ways: First, whereas it compared BP cases (defined broadly) to normal controls, we compared BP cases (defined narrowly) with participants defined only by the absence of BP-I. Second, our comparisons involved close relatives rather than independent participants.

We evaluated the possible effects of medication use on the association of activity phenotypes to BP-I. Twelve variables, including several measuring mean activity levels, were lower for BP-I subjects on neuroleptics than for those not prescribed these agents. When subjects receiving neuroleptics were removed from the analysis, however, activity levels remained significantly lower in BP-I subjects for the majority of variables (Table S1). Antidepressant medication and lithium treatment appeared to have no significant effect on the group differences except for one variable, mean activity from midnight to 6:00 AM, which was lower in subjects taking lithium. The finding of decreased activity in BP-I subjects also remained significant, when lithium-treated patients were removed from the analysis.

The size and composition of the pedigree set enabled us to identify significant heritability for most measures that we evaluated. This finding encouraged us to conduct, to our knowledge, the first genome-wide mapping study of quantitative traits representing the most important features of human circadian behavior: phase, amplitude, and rhythm coherence or robustness.

Previous studies of rare, autosomal dominant circadian rhythm disorders have implicated genes [including *PER2* (*PERIOD2*),

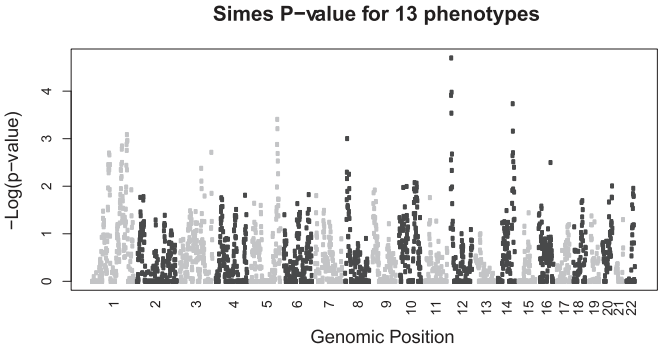

Fig. 3. Multipoint linkage analysis across all 49 heritable phenotypes. Chromosomes are represented in alternating colors (light gray–dark gray).

CK1δ (Casein kinase 1 delta), and *DEC2* (BHLHE41, basic helix-loop-helix family member e41)] already known to function in the regulation of the circadian clock (21–23). In contrast, the linkage regions identified here for quantitative activity traits do not include any known clock genes. Similarly, prior work has found little evidence that such genes play a role in quantitative circadian activity phenotypes in mice (24).

Our most striking genetic finding was the genome-wide significant linkage for interdaily stability, a measure of day-to-day variability of the waveform of activity, near chromosome 12pter. The suggestive linkage peaks in this region for two additional phenotypes, amplitude and mean number of sleep bouts, which show pleiotropy with interdaily stability in bivariate linkage analysis, underline its importance for the regulation of circadian activity. Several genes in this region could plausibly influence activity-related behaviors, including the histone lysine demethylase *JARID1a* (*KMD5A*) and calcium channel subunit 1C (*CACNA1C*). *JARID1a* forms a complex with the core clock proteins CLOCK and BMAL1, thereby recruiting them to the *Per2* promoter. On this promoter, *JARID1a* enhances *Per2* transcription through a demethylase-independent mechanism. Depletion of JARID1a, in mammalian cells in culture, shortens the circadian period (25).

CACNA1C demonstrates a circadian expression pattern. Its loss affects the ability to phase advance wheel running behavior in mouse after a light pulse and impairs the induction of *Per2* and *Per1* expression (26). In multiple GWASs (genome-wide association studies), *CACNA1C* has shown genome-wide significant associations to BP (27–29) as well as to other psychiatric disorders (27). Studies in two cohorts have suggested a role of variants in *CACNA1C* in sleep habits and insomnia (30, 31), and *CACNA1C* knockout mice display lower EEG spectral power and impaired REM sleep recovery (32). These diverse GWAS findings, together with the pleiotropic effects that we observed, and the moderate association in our dataset between interdaily stability and BP-I, suggest that variants in this region could have a complex phenotypic impact beyond BP; however, SNPs in *CACNA1C* known to be associated to BP (30, 31) are not associated to interdaily stability and when included as covariates in our linkage analysis do not decrease our evidence for linkage to chromosome 12. Whole-genome sequencing underway in these pedigrees will enable us to evaluate, in relation to 12pter-linked sleep and activity phenotypes, variants in the genes noted above as well as other genes in this region.

Although the study demonstrates the feasibility of large-scale genetic investigation of human circadian activity, its limitations reflect the imprecision of actigraphy as a representation of circadian rhythm. By conducting the recordings while individuals were carrying out their usual activities, we were unable to exclude the possibility that various masking and confounding factors, such as social and natural/artificial light entrainment, could have influenced our results. An alternative study design might have been to analyze sleep and circadian parameters in traditional laboratory conditions, such as constant routine or forced desynchrony (33). We did not, however, consider such a design feasible. Not only were we concerned that exposure of BP-I–affected individuals to such conditions might trigger an acute episode, but such laboratory studies are extremely expensive and labor-intensive, making the recording and analysis of circadian activity and sleep patterns in such a big cohort virtually impossible.

To counter the limitations noted above, future investigations of circadian rhythms in these pedigrees will use molecular assays that record circadian rhythms in skin fibroblasts, from affected and unaffected individuals, in real time for several days. From this analysis, we will obtain information on the period length of the cells as well as parameters such as amplitude, phase, and entrainment. Compared with behavioral phenotypes, circadian phenotypes resulting from this analysis will more directly reflect the underlying genetic properties of the clock (34, 35).

In summary, this is the first large-scale analysis of activity phenotypes in pedigrees ascertained for BP. We demonstrate lower activity in euthymic BP-I individuals compared with their non–BP-I relatives and heritability for phenotypes assaying multiple facets of sleep and activity. The genome-wide significant linkage to interdaily stability, a phenotype associated with BP in case-control studies (17), provides an opportunity to identify sequence variants contributing to the biological underpinnings of this disorder.

Methods

Activity Recording Procedures. We used the Actiwatch Spectrum (Philips Respironics) to record activity count (in 1-min epochs) and ambient light level. At the time of purchase and after annual servicing and battery replacement, we performed two independent procedures, to calibrate devices and minimize interdevice variability (*SI Methods*). Project staff in CR and CO provided calibrated Actiwatches to all participants, whom we ascertained as reported previously (10) and who provided informed consent, as approved by US and local Institutional Review Boards (University of California–Los Angeles Medical Institutional Review Board, the Ethics Committees of the University of Costa Rica, the Ethics Committees of the University of Antioquia, and University of Texas Southwestern Medical Center Institutional Review Board). As close as possible to the time of other phenotypic assessments, we placed the Actiwatch on the nondominant wrist and instructed participants to not remove it for 14 d, press the marker button when they lay down to

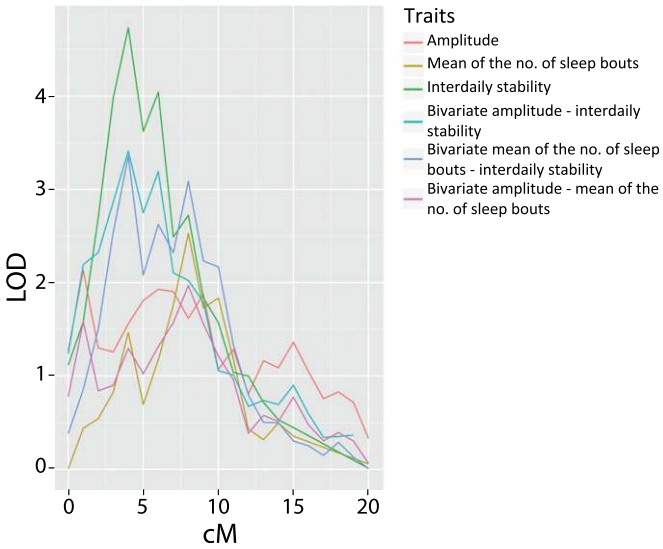

Fig. 4. Multipoint linkage analysis of the most biological significant phenotypes; depiction of the region of pleiotropic linkage on chromosome 12p. no., number.

sleep and when they got out of bed, and keep a sleep log, registering bed times and nap times.

Activity Data Analysis. Two research assistants visually inspected activity recordings (Fig. S1) for gross abnormalities or deficiencies in data collection that would exclude them from analyses (*SI Methods*). For acceptable recordings, we first delineated the rest period for each 24 h (the interval from the time an individual gets into bed until he/she gets out of bed), combining actigraphy data with information from sleep logs using an algorithm written in R (36) (*SI Methods* and Figs. S5 and S6). We analyzed sleep parameters using a script written in R, based on published Respironics definitions and algorithms (14–17). R scripts are available upon request.

Analysis of Heritability and Association to BP-I. As in the previous endophenotype analyses of these pedigrees (10), we analyzed heritability and association to BP-I in SOLAR (18), which implements a variance component method to estimate the proportion of phenotypic variance due to additive genetic factors (narrow sense heritability). Under the null hypothesis that the value of the additive genetic variance is zero, testing significance of the estimate of genetic variance compared with the null value is a one-sided test.

Variance components analysis is sensitive to outliers and nonnormal trait distributions. To guard against statistical artifacts induced by skewed distributions, before analyses we used, in SOLAR, a standard rank-based procedure (37) to inverse-normal transform all phenotypes and thereby avoid correlations between relatives or inflated heritability estimates (38).

We regressed all phenotypes on three covariates [sex, age, and country (CR vs. CO), also used in all analyses described below]; of six potential covariates that we evaluated, only these three significantly affected phenotype values (*SI Methods*). We initially considered household effects as a potential source of phenotypic variation; however, we found that "household" was not a significant component of variation for any phenotype and therefore did not include it as a variable in further models. We implemented regressions in SOLAR, using pedigree structures, using residuals from these models in all further analyses. We tested for BP-I association (difference in trait means between individuals with and without this diagnosis), using SOLAR to account for dependencies among relatives in a two-sided test of the null hypothesis of no association. As the non–BP-I category includes individuals diagnosed with other psychiatric disorders, this is not a case-control comparison (and likely underestimates the degree of BP-I association of each measure). We controlled family-wise error rate at the 0.05 level, using a Bonferroni-corrected threshold for each test (heritability and BP-I association; $P < 6.7 \times 10^{-4}$).

Genome-Wide Genotyping and Quantitative Trait Linkage Analysis. Genotyping of 856 individuals, performed in three batches, used the Illumina Omni 2.5 chip (for QC details, see *SI Methods*). We implemented genome-wide multipoint linkage analysis in SOLAR, which uses a variance component approach to partition the genetic covariance between relatives for each trait into locus-specific heritability (h2q) and residual genetic heritability (h2r). The software package Loki (39, 40), which implements Markov Chain Monte Carlo, provided estimated multipoint identical by decent (MIBD) allele-sharing among family members from genotype data, using a linkage disequilibrium (LD)-pruned subset of markers that passed QC procedures (*SI Methods*). We performed linkage analysis at 1-cM intervals focusing primarily on phenotypes that we considered most relevant to BP; for these phenotypes, we also performed a secondary genome-wide linkage analysis including BP status as a covariate. There were 558 individuals with genotype and phenotype data for all analyses (136 BP-I and 422 non–BP-I). Power analysis in SOLAR, using simulated data, indicated that this sample size provided >80% power to detect a LOD score of 3, provided the estimate of locus-specific heritability was ≥35%.

To evaluate the significance of the strongest linkage finding while appropriately accounting for multiple comparisons among phenotypes most relevant to BP, we used simulations. Specifically, we used gene dropping to generate genotypes consistent with the relationships between the pedigree members, independent from the recorded phenotypic values. This approach allowed us to simulate null datasets that maintain the specific dependency structure existing across these 13 phenotypes. We used gene-drop simulations to construct a null distribution of LOD scores, rather than permuting phenotypic values, because individuals in pedigrees are not exchangeable; phenotypic permutation would likely render most of our phenotypes nonheritable, therefore biasing the null distribution toward zero LOD scores. For each of 100 datasets so generated, we carried out linkage analysis for each phenotype, recording both the highest LOD score obtained and the most significant *P* value for the hypothesis of no linkage to any phenotype (Simes *P* value).

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