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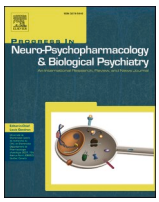

Gene expression of circadian genes and CIART in bipolar disorder: A preliminary case-control study

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ABSTRACT

Based on the observed circadian rhythms disruptions and sleep abnormalities in bipolar disorders (BD), a chronobiological model has been proposed suggesting that core clock genes play a central role in the vulnerability to the disorder. In this context, the analysis of circadian genes expression levels is particularly relevant, however studies focused on the whole set of core clock genes are scarce. We compared the levels of expression of 19 circadian genes (including the recently described circadian repressor (*CIART*)) in 37 euthymic individuals with BD and 20 healthy controls (HC), using data obtained by RNA sequencing of lymphoblastoid cell lines and validated the results using RT-qPCR. RNA sequencing data showed that *CIART* gene expression was correlated with those of *ARNTL*, *ARNTL2*, *DBP*, *PER2* and *TIMELESS*. Data from RNA sequencing showed that the level of expression of four circadian genes (*ARNTL*, *ARNTL2, BHLHE41* and *CIART)* discriminated individuals with BD from HC. We replicated this result using RT-qPCR for *ARNTL* and *CIART*. This study suggests that an imbalance between activation/repression of the transcription within the circadian system in individuals with BD as compared to HC and as such opens avenues for further research in larger independent samples combining both expression and epigenetic analyses.

1. Introduction

Bipolar Disorders (BD) affect 1–2% of the adult population and are characterized by recurrent episodes of major depression and (hypo)-mania interspaced by periods of remission (i.e. euthymia). Sleep abnormalities and circadian rhythms (CR) disruptions have been identified as predisposing factors to BD (Murray et al., 2020). First, meta-analyses suggested that these altered patterns pre-exist to the onset of BD (Scott et al., 2021; Scott et al., 2022). Second, meta-analyses of studies using self- or clinician-based questionnaires demonstrated that, as compared to healthy controls (HC), euthymic individuals with BD have an evening chronotype (preference for activities in the evening) and lower amplitude, lower flexibility and less synchronization of CR (Meyrel et al., 2021). Studies using actigraphy recordings also suggested abnormalities in timing, amplitude and stability of CR (Krane-Gartiser et al., 2019). Altogether, these observations have served as bases for a chronobiological model of BD. According to this model, underlying abnormalities of the biological clock lead to greater CR irregularities and sleep disturbances, then to alterations in mood regulation and further to mood episodes, especially when individuals are exposed to life events that can desynchronize CR or disrupt sleep (Harvey, 2008).

This chronobiological model mainly relies on the assumption that some abnormalities of the circadian genes may predispose individuals to altered circadian phenotypes. Core-clock circadian genes (i.e. those expressed in the suprachiasmatic nuclei) interact through complex positive and negative loops to control CR. Genetic variants of these genes have been studied in association with BD, however with inconsistent or un-replicated results (Milhiet et al., 2011; Etain et al., 2014; McCarthy et al., 2021). In this context, the study of the level of expression of circadian genes may offer a complementary approach to

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explore the contribution of this particular biological pathway to the susceptibility of BD. Some studies have investigated such an issue, mainly focusing on only one circadian gene (Bengesser et al., 2021), but extremely few have offered a comprehensive study of the whole set of genes (Yang et al., 2009; Zhang et al., 2022). For instance, differential gene expressions have been reported in individuals with BD as compared to HC for *BMAL1*, *NR1D1*, *DBP*, *ARNTL2*, *CRY1*, *CRY2*, *PER2*, or *PER3*, however among non-euthymic participants (mostly during depression). Moreover, the expression levels of certain genes such as *CIART* (Circadian Associated Repressor Of Transcription), recently identified as a component of the circadian clock (Goriki et al., 2014) have not been studied yet in BD.

Gene expression levels are generally analyzed in peripheral blood samples of individuals with BD. However, in the specific case of circadian genes, this requires a very rigorous sample collection conditions and schedules to take into account the circadian aspect of the expression of these genes at the peripheral level. Lymphoblastoid cell lines (LCLs), derived from lymphocytes of patients, are much easier to obtain and to grow in standardized and controlled conditions thus reducing variability (Viswanath et al., 2015). This model has been successfully used for the investigation of core clock genes expression in BD (McCarthy et al., 2011; Etain et al., 2012; Kittel-Schneider et al., 2015; Geoffroy et al., 2018) and therefore represent a useful model to explore the contribution of this set of genes in association with BD.

Therefore, the aim of this study was to compare the levels of expression of 19 circadian genes between euthymic individuals with BD and HC, using data obtained by RNA sequencing of LCLs and validated using RT-qPCR.

2. Methods

2.1. Sample

The present study is an ancillary analysis of the research protocol (Clinical Trials Number NCT02627404) that investigated the genetic and environmental factors of vulnerability to BD. The sample consisted of Caucasian euthymic individuals with a diagnosis of BD type 1 according to DSM-IV criteria. Euthymia at inclusion was confirmed by scores below 8 for both the Montgomery Asberg Depression rating Scale (MADRS) and the Young mania rating Scale (Young et al., 1978; Montgomery and Asberg, 1979). Caucasian HC were recruited from the general population and had no personal history of mood disorders, schizophrenia, substance use (except nicotine use) and suicidal attempts. The appropriate ethical committee approved the study. All participants provided written informed consent prior to inclusion. Details on inclusion and exclusion criteria have been described previously (Etain et al., 2012).

2.2. Cell culture and RNA isolation

A blood sample was drawn from all participants. LCLs (Lymphoblastoid Cell Lines) were established from fresh blood by transforming lymphocytes using standard with Epstein-Barr virus transformation, as previously described (Neitzel, 1986). LCLs were cultured in RPMI-1640 glutaMAX medium containing 10% fetal bovine serum and 1% gentamycin (Life Technologies, France) in a 5% CO2 humidified incubator at 37 °C. All procedures were standardized and LCLs from individuals with BD and HC were seeded and collected at the same time of day in parallel. LCLs were seeded at 2×10^5 cells/mL. After 4 days, cells were harvested and washed twice with PBS before RNA extraction. Total RNA was extracted, for individuals with BD and HC, from 2×10^6 cells pellets using the miRNeasy Mini Kit according to the manufacturer's protocol (QIAGEN, France) and quantified with a NanoDrop One ND-1000 spectrophotometer (ThermoFisher Scientific,Ozyme, France). Total RNAs were stored at −80 °C until processing.

2.3. RNA sequencing

RNA sequencing was performed at the Institut du Cerveau (IGenSeq platform - Paris). Samples were randomized and libraries were generated from total RNA using TruSeq Stranded mRNA Kit (Illumina, USA). Paired-end sequencing (2×75 bp) was performed on a Nextseq500 instrument (Illumina, USA). Quality of raw reads was assessed with FastQC49, trimming was done with Trimmomatic39 to cut adapters and exclude reads <40 bp. The processed reads were aligned on human reference genome hg19 with Star v 2.5.3a (Dobin et al., 2013). RSEM (Li and Dewey, 2011) was used to obtain expected read counts and FPKM (Fragments Per Kilobase of transcript per Million mapped reads), which eliminated the influence of different gene length and discrepancy of the library size. Samples types, RNA isolation and sequencing methods were identical for samples from individuals with BD and HC, therefore these data were used for the following analyses (Zhao et al., 2020).

2.4. Genes selection

FPKM data for the following circadian genes were selected from the dataset: *ARNTL1* and *ARNTL2* (Aryl hydrocarbon receptor nuclear translocator-like protein), *BHLHE40* and *BHLHE 41* (Basic helix-loop-helix family, member E), *CLOCK* (Circadian Locomoter Output Cycles Protein Kaput), *CRY1*, *CRY2* (Cryptochrome Circadian Regulator), *CSNK1D*, *CSNK1E* (Casein Kinase 1 Delta and Epsilon), *DBP* (D-Box Binding PAR BZIP Transcription Factor), *GSK3B* (Glycogen Synthase Kinase 3 Beta), *NR1D1* (Nuclear Receptor Subfamily 1 Group D Member 1), *PER1*, *PER2*, *PER3* (Period Circadian Regulator), *RORA* (RAR Related Orphan Receptor A), *NPAS1* (Neuronal PAS Domain Protein 1) and *TIMELESS* (Timeless Circadian Regulator) (Kavakli et al., 2022). We added to this list *CIART* (Circadian-Associated Transcriptional Repressor) because of its recent characterization as a potential contributor to CR alterations (Yang et al., 2020).

RORB (RAR Related Orphan Receptor B), *NPAS2* (Neuronal PAS Domain Protein 2) and *PPARGC1A* (Peroxisome Proliferator Activated Receptor Gamma Coactivator 1 Alpha) were excluded from the analyses because of low expression levels.

2.5. Validation using PCR

In order to validate the differential expression of genes identified in the RNA-seq analyses, RT-qPCR were performed on the same samples. One μg of total RNA was reverse transcribed using the iScript™ Reverse Transcription Supermix following the manufacturer's protocol (Bio-Rad laboratories, France). One patient and one HC with no RNA left could not be included in the PCR experiments. Commercially available Prime PCR primers targeting identified genes from RNAseq data analyses were purchased from BioRad laboratories (France). Based on previous results, prime PCR primers targeting *GUSB* and *TBP* were used as reference genes (Curis et al., 2019), as described in the GeNorm method the geometric mean of the two reference genes was used as a normalization factor. SsoAvanced Universal SYBR Green Supermix (Bio-Rad laboratories) was used for amplification following the manufacturer's instructions. The specificity of PCR products was verified using a melting curve analysis step. Amplifications were performed, in triplicate, on a CFX384 instrument and gene expression levels (ΔΔCq) were calculated using the built-in gene expression mode of the CFX Maestro software (Bio-Rad laboratories).

2.6. Statistical analysis

Some of the studied genes had observed distributions in the whole sample (cases and controls) that did not fit a normal distribution. This was the case for *ARNTL*, *BHLHE40*, *BHLHE41*, *CSNK1E*, *DBP*, *GSK3B*, *NR1D1*, *PER1*, *PER3*, and *RORA*. Therefore, we mainly used non-parametric tests for the analyses. Qualitative data were described by

numbers and percentages and quantitative data by medians and interquartiles (IQR). Correlations between variables were investigated using Spearman correlation tests. Comparisons between groups for quantitative data were performed using Mann-Whitney tests. For this main analysis we used a False Discovery rate (FDR) correction for multiple testing. Spearman correlations were performed to analyze covariations between circadian genes and results were visualized as a network. The association between clinical status and gene expression levels was tested using logistic regression, and further adjusted for age, sex, BMI (Body mass Index) and tobacco use. ROC analyses and AUC were generated to investigate the accuracy of the model.

3. Results

3.1. Comparison between BD and HC

The sample consisted in 57 individuals (37 with BD type 1 and 20 HC). The groups were similar for sex, age at inclusion, BMI, and current tobacco use (see Table 1). The mean age at onset of BD was around 27 years old. The mean number of lifetime mood episodes was 7.6 (+/− 4.6). At inclusion, most individuals with BD were treated with lithium ($n = 28$, 75%), around one third were on anticonvulsants ($n = 12$, 32%), and around one quarter were on atypical antipsychotics ($n = 10$, 27%). None of the individuals with BD was drug naive.

3.2. Correlation between genes expression

We constructed a network plot in the whole sample ($N = 57$) to visualize the correlations of expression of the 19 genes (threshold for edge = 0.3). As seen in Fig. 1, the network is dominated by two main groups of genes linked with a large number of edges: *DBP*, *ARNTL2*, *GSK3b*, *CLOCK*, *CRY2*, *PER1*, *NR1D1*, *BHLHE40*, *CSNK1E* and *CSNK1D* on the one part and *ARNTL*, *BHLHE41*, *PER2*, *NPAS1*, *CRY1* and *TIMELESS* on the other. The expression level of *CIART* is correlated with both groups. The main correlations between the level of expression of *CIART* and those of other circadian genes were found with *ARNTL* (rho = −0.39 $p = 0.003$) and *DBP* (rho = 0.33 $p = 0.01$). *PER3* and *RORA* were not connected to the two main groups of genes with correlated expression levels.

3.3. Comparison between groups for gene expression

As compared to HC, individuals with BD had significantly lower levels of expression of *ARNTL* ($p = 0.00006$) and of *ARNTL2* ($p = 0.0003$) and higher levels of expression of *CIART* ($p = 0.00009$) and of *BHLHE41* (p = 0.003). These differences resisted to the FDR correction. Other associations (not significant after the correction) were found with *BHLHE40* ($p = 0.015$), *RORA* ($p = 0.038$) and *TIMELESS* ($p = 0.023$) (see Table 2).

We used a logistic regression with clinical status as the dependent variable, and the following independent variables: *ARNTL*, *ARNTL2*, *CIART* and *BHLHE41* (model 1 with a stepwise option), then adjusted for covariates (model 2 with a forced entry). Clinical status was associated with *ARNTL*, *ARNTL2*, *CIART* but not with *BHLHE41* (see Table 3), which remained significant after adjustment. We used ROC analyses to explore whether each single gene, and then the combination of the three significantly differentially expressed genes, better discriminated BD from HC (see Fig. 2). The AUC was 0.79 for ARNTL2 ($p = 3.10^{-4}$), 0.82 for CIART ($p = 9.10^{-5}$), 0.82 for ARNTL ($p = 6.10^{-5}$) and was the highest when combining the three genes (AUC = 0.95 p = 3.10^{-8}). The increment in the AUC obtained when combining the three genes was significant as compared to the AUC obtained with each single gene (*p* values between 0.03 and 0.01 for the increments) (see Fig. 2).

Table 1
group comparison for age, sex, BMI and tobacco use.

Variables	BD group	HC group	p values
Females N (%)	21 (57%)	9 (45%)	0.39
Age at inclusion (median IQR)	47 (42–54)	44 (42–45)	0.37
BMI (median IQR)	26 (23–28)	25 (23–26)	0.45
Tobacco current use N (%)	17 (46%)	5 (28%)*	0.19

* Two missing variables. BD: Bipolar disorder, HC: healthy control, N: number, IQR: interquartile, BMI: body mass index (kg/m2).

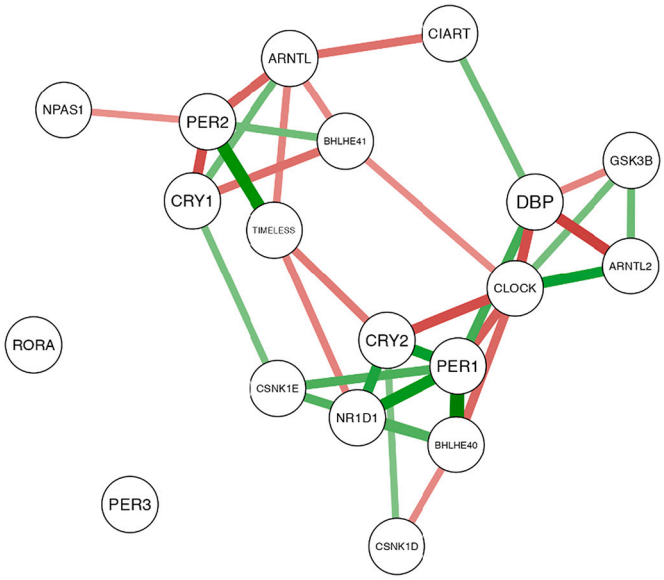

Fig. 1. Correlation network of levels of expression of circadian genes. The edges of the network refer to Spearman correlation coefficients between gene expression. Only edges with a threshold of 0.30 are drawn.

3.4. Validation using PCR

RT-qPCR experiments were performed in the same samples to validate the results observed with RNA-seq. Based on the FDR corrected *p*-values obtained with the RNA-seq data, only *ARNTL*, *ARNTL2*, *CIART* and *BHLHE41* were tested. (Table 4). In individuals with BD as compared to HC, we confirmed lower levels of expression of *ARNTL* ($p = 0.002$) and higher levels of expression of *CIART* ($p < 0.001$) and of *BHLHE41* (p < 0.001). As seen in Table 4, the log2 fold change were similar to the ones observed with the RNA Seq data. However, expression levels of *ARNTL2* were similar in patients with BD and HC (0.95 fold change, $p = 0.55$).

We used the logistic regression model adjusted for covariates (Model 2) using the RT-qPCR data. Both *ARNTL* and *CIART* levels of expression were found associated with diagnosis (Table 5). The AUC for this model was 0.97 (0.93–0.99).

4. Discussion

This is the first study that explored the level of expression of a comprehensive set of 19 circadian genes, also including the recently described circadian repressor (*CIART*), in individuals with BD as compared to controls. First, we suggest for the first time that *CIART* is co-expressed with other circadian genes in humans. Second, using RNAseq data, we show that the level of expression of four circadian genes (*ARNTL*, *ARNTL2, BHLHE41* and *CIART)* discriminated individuals with BD from healthy controls. Third, we replicated the results using RT-qPCR for two of these genes: *ARNTL* and *CIART*.

We constructed a correlation network of 19 circadian genes based on their expression levels in LCLs from 57 individuals. Indeed, the complex interplay between circadian genes at the transcriptional and functional

Table 2
group comparison for the level of expression of 19 circadian genes.

Status	HC		BD		log2 fold change	p values
	Median	IQR	Median	IQR		
ARNTL	10.14	9.04–12.59	7.67	7.16–8.74	−0.40	**0.00006**
CIART	0.99	0.72–1.47	1.64	1.34–1.92	0.73	**0.00009**
ARNTL2	36.01	30.77–42.68	30.06	23.97–33.22	−0.26	**0.0003**
BHLHE41	2.31	1.42–4.71	4.54	2.46–7.18	0.97	**0.003**
BHLHE40	46.49	29.80–66.81	62.55	51.62–72.26	0.43	0.015
TIMELESS	14.77	12.45–17.65	16.79	14.95–17.75	0.18	0.023
RORA	4.30	2.67–6.58	5.76	4.32–7.13	0.42	0.038
PER2	4.80	4.26–5.39	5.21	4.86–5.54	0.12	0.070
CSNK1E	28.94	25.63–35	32.04	29.91–34.71	0.15	0.074
DBP	5.41	4.72–6.81	6.18	5.61–7.13	0.19	0.075
CSNK1D	30.97	29.6–33.31	30.03	29.07–31.33	−0.04	0.084
PER1	11.08	8.5–15.73	12.98	10.68–15.66	0.23	0.095
CRY1	3.35	2.22–4.76	2.75	1.67–3.47	−0.28	0.098
PER3	0.74	0.55–2.58	1.51	0.68–2.34	1.03	0.173
NR1D1	4.53	3.57–6.17	4.16	3.74–5.04	−0.12	0.259
CLOCK	4.39	4.14–4.62	4.14	3.91–4.76	−0.08	0.266
NPAS1	0.29	0.22–0.44	0.29	0.20–0.39		0.598
GSK3B	21.42	20.88–23.31	21.74	20.66–22.24	0.02	0.738
CRY2	7.63	7.04–8.43	7.77	7.05–8.41	0.03	0.874

In bold, p values that remained significant after a FDR correction.
Genes are ordered by *p* values.

Table 3
Comparisons between BD and HC for ARNTL, ARNTL2, CIART without and with adjustment.

	MODEL 1 *					MODEL 2 *				
Variables	B	S.E.	Wald	df	p	B	S.E.	Wald	df	p
ARNTL	−0.72	0.25	8.32	1	**0.004**	−1.29	0.52	6.29	1	**0.01**
ARNTL2	−0.29	0.11	6.88	1	**0.009**	−0.38	0.15	6.45	1	**0.01**
CIART	3.51	1.38	6.46	1	**0.01**	6.32	2.45	6.65	1	**0.01**
Age	–	–	–	–	–	0.21	0.12	3.33	1	0.07
Sex	–	–	–	–	–	−0.27	1.33	0.04	1	0.84
Tobacco	–	–	–	–	–	−2.67	1.80	2.18	1	0.14
BMI	–	–	–	–	–	0.17	0.22	0.59	1	0.44

* Intercept is not shown. B: beta, SE: standard error, df: degree of freedom.

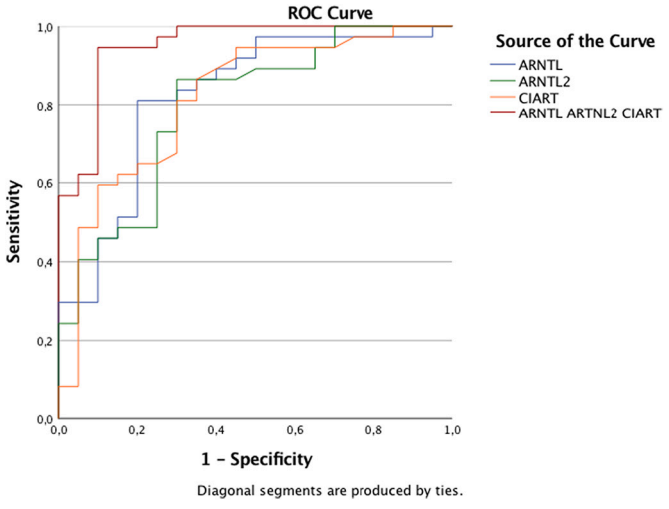

Fig. 2. Prediction of BD status for ARNTL, ARNTL2 and CIART alone, then combined.

levels depends on numerous factors and varies along the nycthemere (Rijo-Ferreira and Takahashi, 2019; Sun et al., 2020). However, we were able to identify two main groups of inter-correlated genes and show that the expression level of *CIART*, a recently described circadian repressor, is correlated to these two groups in this cellular pathway. The study of circadian gene expression over 24 h under controlled conditions should allow to describe more precisely the genes correlated with *CIART* and better understand its role.

Our study suggests that *ARNTL* and *CIART* may be relevant candidate circadian genes whose levels of expression may discriminate BD from HC. ARNTL is an Aryl Hydrocarbon Receptor Nuclear Translocator-Like Proteins (also known as Bmal1 for Brain and Muscle ARNT-Like 1) that dimerizes with CLOCK. This heterodimer is able to bind DNA and activate transcription from an *E*-box element, thus participating in the activation of the transcription of core clock genes such as *PER1–3* and *CRY1–2*. The loss of Bmal1 in mice results in immediate and complete loss of circadian rhythmicity in constant darkness and *Bmal1* knockout is the only single gene deletion that fully eliminates circadian clock function in the suprachiasmatic nucleus and in peripheral tissues (Bunger et al., 2000; Tamaru and Takamatsu, 2018; Haque et al., 2019). CIART (Circadian-Associated Transcriptional Repressor) also known as CHRONO (computationally highlighted repressor of the network oscillator) or Gm129 (Gene Model 129) encodes a nuclear-localized protein that directly interacts with BMAL1 and represses CLOCK-BMAL1 activity (Annayev et al., 2014). Overexpression of *CHRONO* leads to suppression of BMAL1-CLOCK activity (Goriki et al., 2014) and *Chrono* knockout mice display a prolonged free-running circadian period (Anafi et al., 2014). Given the physiological functions of these two genes, we suggest that, as compared to controls, individuals with BD might display a lower expression of one circadian transcription driver (ARTNL) and a higher expression of one circadian repressor of transcription (CIART). If replicated, these results lead to the hypothesis that BD might be characterized by an imbalance between activation/repression of the transcription in the biological clock.

Table 4
group comparison for the level of expression of ARNTL, CIART, ARNTL2 and BHLHE41 using RT-qPCR.

Status	HC		BD		log2 fold change	p values
	Median	IQR	Median	IQR		
CIART	0,50	0.33–0.58	0.82	0.68–1.04	0.71	**<0.001**
BHLHE41	0.24	0.16–0.48	0.7	0.37–1.05	1.54	**<0.001**
ARNTL	0.79	0.66–1.07	0.62	0.49–0.78	−0.35	**0.001**
ARNTL2	0.92	0.79–1.05	0.89	0.76–0.97	−0.05	0.557

In bold, p values <0.05.
Genes are ordered by *p* values.

Table 5
Comparisons between BD and HC for ARNTL, BHLHE41 and CIART with adjustment.

	MODEL 2 *				
Variables	B	SE	Wald	df	p
ARNTL qPCR	−7.01	3.35	4.38	1	**0.04**
BHEHL41 qPCR	3.04	2.51	1.47	1	0.23
CIART qPCR	11.29	5.47	4.25	1	**0.04**
Age	0.25	0.14	3.22	1	0.07
Sex	0.42	1.11	0.14	1	0.71
Tobacco	−3.26	2.04	2.55	1	0.11
Body Mass Index	0.08	0.19	0.18	1	0.67

* Intercept is not shown. B: beta, SE: standard error, df: degree of freedom.

Several studies have explored genetic variants of circadian genes as vulnerability factors to BD. Even though some studies provided replication in independent samples (Etain et al., 2014), for example with *TIMELESS* and *RORA*, it cannot be concluded that one particular circadian gene may represent a risk factor to BD based on genetic approaches. Of note, while most genome-wide association studies (GWAS) failed to demonstrate any robust associations between genetic variants in circadian genes and BD, a recent one nevertheless suggested an association with *ARNTL* ($p = 1.8\ 10^{-6}$) using gene-based tests (Mullins et al., 2021). In this context, a gene expression study may represent a complementary approach. Since we found associations between BD and levels of expression for some circadian genes, this may suggest that the abnormalities leading to alterations in gene expression might be linked to epigenetic modifications rather than to abnormalities at the level of genetic variants. Further studies should then explore epigenetic marks of these circadian genes (DNA methylation levels, histone modifications and/or targeted miRNAs expression) that may differ between individuals with BD and controls in order to disentangle the mechanisms at stake. Interestingly, methylation of *ARNTL* has been suggested to differ significantly between BD and controls (Bengesser et al., 2018), which may be one possible explanation of the lower *ARNTL* gene expression in BD observed in our study.

Several limitations of this study should be discussed. First, the sample size is relatively limited, although standard for a gene expression study. This does not preclude some false negative findings, especially regarding genes that we did not include in the multivariable model after the correction using FDR. Replications in samples of larger size are thus deserved. Second, this study was performed on RNA from LCLs. Although this model has been shown to retain characteristics of patients with BD and to express most of the circadian genes (McCarthy et al., 2011; Viswanath et al., 2015), we cannot exclude that potential artefacts might have been caused by the transformation of the lymphocytes. In addition, LCLs have repeatedly been shown to capture differences in circadian gene expressions in individuals with BD (Kato et al., 2011; Geoffroy et al., 2014; Kittel-Schneider et al., 2019; Grillault Laroche et al., 2021). These results need to be replicated using RNA samples directly extracted from whole blood. Third, we focused on genes expressed in the suprachiasmatic nuclei. We did not explore melatonergic genes that may also display some abnormalities in terms of gene expression between cases and controls (Etain et al., 2012). This analysis may also be extended to upstream clock modulator genes (genes that modulate circadian rhythms) and downstream clock-controlled genes (McCarthy et al., 2012), however this would massively increase the number of genes to be investigated. We did not provide any methylation analysis to further explore potential underlying epigenetic mechanisms. Finally, all individuals with BD were currently treated by mood stabilizers and a complementary study should be proposed ideally in drug-naive participants (which may be impossible in BD).

In conclusion, this study suggested abnormalities in two circadian genes (*ARNTL*, and *CIART*) in individuals with BD as compared to controls. We reported for the first time that a recently characterized circadian repressor (*CIART*) may contribute to the vulnerability to BD. Our results suggest that BD might be characterized by an imbalance between activation/repression of the transcription within the circadian system. Replications in larger independent samples that would combine both expression and epigenetic analyses are required.

Statement of ethics

This research protocol (Clinical Trials Number NCT02627404) was approved by the French medical ethics committee (Comité de Protection des Personnes (CPP) – IDRCB2008_AO1465_50 VI – Pitié-Salpêtrière 118–08) and carried out according to the approved guidelines. Written informed consent was obtained from all participants.

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Author contribution

BE is the principal investigator of this study and obtained the funding. EM performed the RNA-seq experiments. JG performed the QC and extraction of the RNA-seq data. CC performed the cellular experiments, RNA isolation and qPCR validation. BE and CMC performed the first exploratory statistical analyses and wrote the first draft of the manuscript. BE, CMC and GG reviewed the statistical analyses and wrote the final draft of the manuscript. BE, GG, VH and MM contributed to the recruitment of participants. FB critically reviewed the final version of the manuscript. All authors approved the submitted version of the manuscript.

Declaration of Competing Interest

None in relation to this article.

Data availability

Data will be made available on request. Due to ethical and legal restrictions, data involving clinical participants cannot be made publicly available. All relevant data are available upon request to the authors for researchers who meet the criteria for access to confidential data.

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