Circadian clock gene expression: a key player in inflammation underlying chronic lung disease?

Heidi Makrinioti and Sung Chun

Division of Pulmonary Medicine, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA.

Corresponding author: Heidi Makrinioti (Heidi.Makrynioti@childrens.harvard.edu)

Shareable abstract (@ERSpublications)
The circadian clock genes might play a key role in regulating the pathophysiological processes underlying COPD. Their role in regulating pathophysiological processes in other chronic lung diseases is still unclear, but important to elucidate. https://bit.ly/4f3KXQk

Cite this article as: Makrinioti H, Chun S. Circadian clock gene expression: a key player in inflammation underlying chronic lung disease? *ERJ Open Res* 2025; 11: 01066-2024 [DOI: 10.1183/23120541.01066-2024].

Received: 17 Oct 2024
Accepted: 19 Oct 2024

The lung circadian clock lung is an internal time-keeping system that regulates daily rhythms in various pathophysiological processes within lung tissues [1, 2]. Principally, the circadian clock controls the timing of inflammatory responses [3, 4]. Therefore, disruptions in the clock have been associated with aberrant inflammatory responses in the context of chronic respiratory disease (*e.g.*, asthma [5], COPD [6] and pulmonary fibrosis [7]) pathogenesis (figure 1). With regard to triggers, disruption in the circadian clock has been considered to occur in response to environmental stimulants (*e.g.*, air pollutants [8], viral [9] and bacterial infections [10], smoking [11], shift working [12] and stress [13]).

The genetic regulation of inflammatory responses is modulated by core clock gene expression (*e.g.*, the *Brain and Muscle ARNT-Like 1* (*BMAL1*) activator complex gene) [14]. *BMAL1* drives the expression of the period (*PER1* to *PER3*) [15] and cryptochrome (*CRY1* to *CRY2*) circadian regulator genes [16], and the *PER* and *CRY* proteins form phosphorylated heterodimers that translocate back to the nucleus, where they repress their own transcription by inhibiting the *BMAL1* complex [17]. In addition, the nuclear receptors *Reverse Erb Alpha* (*REV-ERBα*) and *Retinoic Acid Receptor-Related Orphan Receptor Alpha* (*RORα*) help stabilise the clock by regulating the timing and amplitude of *BMAL1* expression [18]. Disruptions in the timing or amplitude of this molecular clock can lead to dysfunction, affecting downstream processes. Epidemiological studies have supported an association between disturbed circadian rhythm and asthma, COPD or pulmonary fibrosis exacerbations [19, 20]. However, the molecular pathways underlying these associations are still unclear. Therefore, studies shedding light on these pathways are of utmost importance.

The study by Li *et al.* [21], published in this issue of *ERJ Open Research*, aimed to address the aforementioned research gap by examining associations between the core clock genes (CCGs) and proinflammatory gene expression in patients with COPD using RNA sequencing data from bronchial brushes [21]. The team calculated a composite score (CCG-score) based on the expression of five core clock genes (*PER1*, *PER2*, *PER3*, *CRY1* and *CRY2*) that inhibit or suppress the activity of the activating arm in the circadian rhythm cycle (*i.e.*, the *BMAL1*/*CLOCK* gene complex) [21]. Patients with high CCG scores tended to have high expression of both positive and negative regulators (genes), whilst the patients with low CCG scores had low expression of both regulators [21]. Interestingly, high CCG scores were associated with low proinflammatory gene expression, meaning that higher clock gene expression was associated with lower levels of inflammation-related genes, such as *CXCL8*, *IL1A*, *IL1B* and *TGFB1* [21].

Pathway enrichment analysis highlighted that genes positively correlated with the CCG score were mainly involved in cilium assembly and cell maintenance, while negatively correlated genes were associated with cytokine and inflammatory processes. To further investigate the inflammatory factors, they analysed 1796 genes using the Molecular Signatures Database (MSigDB) of predefined gene sets based on biological

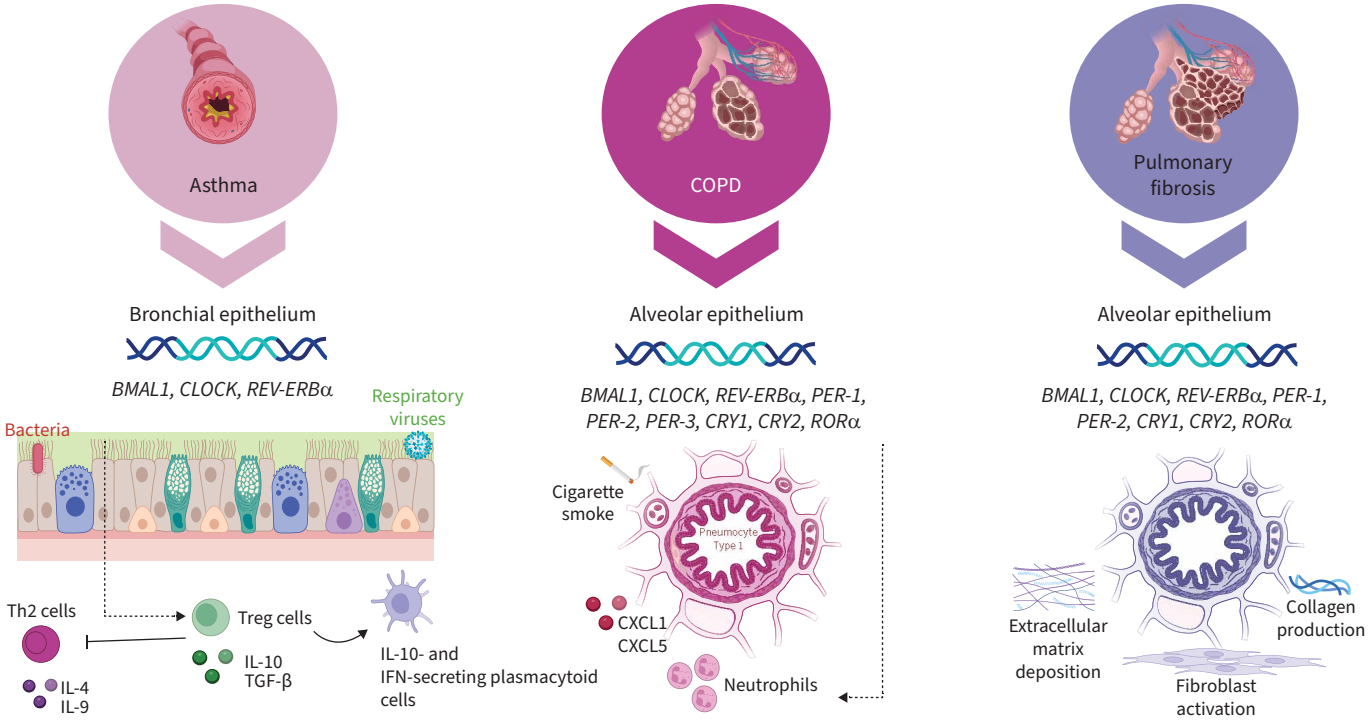

FIGURE 1 An illustration of the impact of circadian clock gene dysregulation on cellular functions within the bronchial and alveolar epithelium across three chronic lung diseases: asthma, COPD and pulmonary fibrosis. In response to viral and bacterial infection, circadian gene (*i.e.*, *BMAL1, CLOCK, REV-ERBα* genes) dysregulation leads to impaired T regulatory cell induction and subsequent Th2 and Th9 cell expansion. The latter leads to increased type 2 cytokine secretion and heightened bronchial hyperresponsiveness. In the alveolar epithelium, in response to pollutants (*e.g.*, cigarette smoke) and circadian clock gene dysregulation, including *PER* and *CRY*, the function of pneumocytes type I and type II is impaired; this leads to abnormal surfactant production, fibrosis and defective gas exchange. In addition, the increased neutrophil recruitment is associated with chronic inflammation and with thickening and narrowing of the bronchial wall. In pulmonary fibrosis, circadian clock gene dysregulation (*i.e.*, *BMAL1, CLOCK, REV-ERBα, PER-1, PER-2, CRY1, CRY2, RORα*) is associated with enhanced fibroblast activation and collagen production, contributing to fibrotic lung remodelling. *BMAL1*: *Brain and Muscle ARNT-Like 1*; *CLOCK*: *Circadian Locomotor Output Cycles Kaput*; *CRY*: *Cryptochrome Circadian Regulator*; IL: interleukin; *PER*: *Period Circadian Regulator*; *REV-ERBα*: *Reverse Erb Alpha*; *RORα*: *Retinoic Acid Receptor-Related Orphan Receptor Alpha*; TGF: transforming growth factor; Treg: T regulatory.

knowledge [21]. They then identified 39 inflammation-related genes, including *CXCL8*, *IL1A*, *IL1B*, *IL7* and *TGFB1*. To determine potential interactions between these genes and CCGs, they constructed a protein–protein interaction network [21]. The analysis identified key interactions, with the top five inflammatory genes interacting with clock genes, including the *CXCL8*, *IL1B*, *IL1A*, *CSF1* and *TGFB1* [21]. To validate whether cytokine genes identified in the bronchial samples were also differentially expressed in lung tissues, the team performed a differential gene expression analysis using a lung tissue microarray dataset [21]. This analysis revealed significant expression changes in 1107 genes between healthy smokers and COPD patients, with 185 genes upregulated and 922 genes downregulated [21]. The pathway enrichment analysis also confirmed that proinflammatory pathways were prominent among differentially expressed genes, mirroring the findings from the CCG-score-associated genes. Specifically, COPD patients had differentially expressed eight inflammatory cytokine-related genes and four circadian clock genes, as explained considering the disease heterogeneity [21]. Finally, a weighted gene co-expression network analysis (WGCNA) analysis was performed to identify similar expression patterns across further samples [21]. This clustering analysis identified two gene modules associated with the CCG score, with one of the two modules containing many inflammation-related genes [21].

This study is important to understanding molecular mechanisms underpinning chronic lung disease development. The study utilised bronchial brushing samples, thus providing a direct insight into lung pathophysiology [22]. In addition, the findings were validated using an independent lung tissue microarray dataset. The latter added robustness and credibility to the results. Finally, by establishing a clear inverse relationship between circadian clock disruption and inflammation, the study added to the current evidence

suggesting that chronotherapy or interventions aiming to restore normal circadian rhythms need to be carefully considered for the management of chronic lung disease-related inflammation [23].

However, as with all studies no matter how excellent, this study is not without some weaknesses too. A principal weakness was the small sample size (26 patients with COPD). This limits the statistical power of the study and creates concerns about the generalisability of the findings. Additionally, the use of healthy smokers as a control group makes it difficult to separate the effects of COPD on gene expression from those of smoking. This is important to address in a future study with a higher number of patients. Importantly, whilst the study identifies significant gene expression changes, it lacks mechanistic validation to confirm the relevance of these findings to COPD pathophysiology. Furthermore, the cross-sectional design limits the ability to infer causality or observe dynamic changes over time in the relationship between circadian clock disruption and inflammation. Therefore, these observations might be attributed to reverse causality, where the severe prolonged inflammation might cause the disruption of the sleep cycle and circadian rhythm. In addition, the transcriptomic analyses are not cell-type specific and thus may contain multiple distinct cell populations. Specifically, brushing samples might contain a differential fraction of other pro-inflammatory cell types other than epithelial cells.

From a bioinformatics perspective, this study showed that WGCNA is ideally suited for clustering molecular data in studies with small sample sizes [24]. It reduces the complexity of molecular data by grouping highly correlated features (*e.g.*, genes) into modules and alleviates the burden of multiple testing. This is especially important in small sample-size studies where a large number of features are analysed. In addition, WGCNA uses inherent gene co-expression patterns to define modules [25]. This approach helps identify biologically meaningful groupings in smaller datasets where traditional clustering might fail due to limited statistical power. Lastly, the way soft-thresholding power is used helps ensure that the network construction is robust and maintains scale-free topology, capturing meaningful correlations between features in small sample sizes.

To summarise, the study by Li *et al.* [21] showed that circadian clock genes may play a key role in regulating the pathophysiological processes underlying COPD. Previous research has shown that circadian clock genes are implicated in chronic lung diseases other than COPD, such as asthma and pulmonary fibrosis. However, the precise molecular mechanisms remain to be fully elucidated. In asthma, *CLOCK and BMAL1* genes are implicated in controlling inflammation and airway hyperreactivity (figure 1) [25, 26]. In COPD, *PER2* and *CRY1* have been associated with heightened inflammatory responses, in particular in response to adverse environmental stimulants [2, 6]. In pulmonary fibrosis, *BMAL1* and *REV-ERBα* contribute to the dysregulation of fibrosis-related pathways [7]. The authors of this editorial believe that investigating common circadian pathways in chronic lung diseases might offer new therapeutic targets in the future.

It is important to understand how we can translate these findings into prediction of treatment responses or chronotherapy modalities in chronic lung diseases. Will we be able to precisely predict treatment responses in individuals who are differentially exposed to diverse environmental triggers? Will we be able to adjust the timing and doses of medications used on each individual based on circadian clock differences? There are teenagers living with chronic lung diseases who are differentially exposed to screen time or have poor sleeping habits [27, 28]. Understanding whether this exposure can be measured and translated into a precision approach to treating their condition will be of utmost importance. Similarly, shift workers exposed to various pollutants would also benefit from a precision medicine approach. Understanding the role of circadian clock genes in chronic lung disease inception and exacerbation will provide new avenues for improved patient care [29]. We can allow the article from Li *et al.* [21] to pave the way for future research in this field, and we eagerly anticipate breakthroughs in precision medicine that will provide substantial clinical benefits.

Provenance: Commissioned article, peer reviewed.

Conflicts of interest: H. Makrinioti is an associate editor of this journal. S. Chun has nothing to disclose.

References

1 Sundar IK, Yao H, Sellix MT, *et al.* Circadian molecular clock in lung pathophysiology. *Am J Physiol Lung Cell Mol Physiol* 2015; 309: L1056–L1075.

2 Sundar IK, Yao H, Sellix MT, *et al.* Circadian clock-coupled lung cellular and molecular functions in chronic airway diseases. *Am J Respir Cell Mol Biol* 2015; 53: 285–290.

3 Srinivasan A, Giri A, Duraisamy SK, *et al.* Acute HDM exposure shows time-of-day and sex-based differences in the severity of lung inflammation and circadian clock disruption. *J Allergy Clin Immunol Glob* 2023; 2: 100155.
4 Wang XL, Li L. Circadian clock regulates inflammation and the development of neurodegeneration. *Front Cell Infect Microbiol* 2021; 11: 696554.
5 Sundar IK, Srinivasan A. Lung miRNA profiles show a time-of-day response in house dust mite-induced allergic asthma in mice. *Clin Transl Allergy* 2021; 11: e12057.
6 Li L, Zhang M, Zhao C, *et al.* Circadian clock gene Clock-Bmal1 regulates cellular senescence in chronic obstructive pulmonary disease. *BMC Pulm Med* 2022; 22: 435.
7 Wang Q, Sundar IK, Lucas JH, *et al.* Circadian clock molecule REV-ERBα regulates lung fibrotic progression through collagen stabilization. *Nat Commun* 2023; 14: 1295.
8 Hwang JW, Sundar IK, Yao H, *et al.* Circadian clock function is disrupted by environmental tobacco/cigarette smoke, leading to lung inflammation and injury via a SIRT1-BMAL1 pathway. *FASEB J* 2014; 28: 176–194.
9 Mazzoccoli G, Vinciguerra M, Carbone A, *et al.* The circadian clock, the immune system, and viral infections: the intricate relationship between biological time and host-virus interaction. *Pathogens* 2020; 9: 83.
10 Li Z, Bonaldi K, Uribe F, *et al.* A localized *Pseudomonas syringae* infection triggers systemic clock responses in *Arabidopsis*. *Curr Biol* 2018; 28: 630–639.e4.
11 Khan NA, Yogeswaran S, Wang Q, *et al.* Waterpipe smoke and e-cigarette vapor differentially affect circadian molecular clock gene expression in mouse lungs. *PLoS One* 2019; 14: e0211645.
12 Wan X, Wang L, Khan MA, *et al.* Shift work promotes adipogenesis *via* cortisol-dependent downregulation of EGR3-HDAC6 pathway. *Cell Death Discov* 2024; 10: 129.
13 Ota SM, Kong X, Hut R, *et al.* The impact of stress and stress hormones on endogenous clocks and circadian rhythms. *Front Neuroendocrinol* 2021; 63: 100931.
14 Chatterjee S, Nam D, Guo B, *et al.* Brain and muscle Arnt-like 1 is a key regulator of myogenesis. *J Cell Sci* 2013; 126: 2213–2224.
15 Cox KH, Takahashi JS. Circadian clock genes and the transcriptional architecture of the clock mechanism. *J Mol Endocrinol* 2019; 63: R93–R102.
16 Liu T, Wang Z, Ye L, *et al.* Nucleus-exported CLOCK acetylates PRPS to promote *de novo* nucleotide synthesis and liver tumour growth. *Nat Cell Biol* 2023; 25: 273–284.
17 Cao X, Yang Y, Selby CP, *et al.* Molecular mechanism of the repressive phase of the mammalian circadian clock. *Proc Natl Acad Sci USA* 2021; 118: e2021174118.
18 Cho H, Zhao X, Hatori M, *et al.* Regulation of circadian behaviour and metabolism by REV-ERB-α and REV-ERB-β. *Nature* 2012; 485: 123–127.
19 Maidstone RJ, Turner J, Vetter C, *et al.* Night shift work is associated with an increased risk of asthma. *Thorax* 2021; 76: 53–60.
20 Joshi A, Sundar IK. Circadian disruption in night shift work and its association with chronic pulmonary diseases. *Adv Biol (Weinh)* 2023; 7: e2200292.
21 Li X, Srikanthan K, Rahmawati SF, *et al.* A network of pro-inflammatory genes repressed by clock signalling in bronchial epithelium. *ERJ Open Res* 2025; 11: 00605-2024.
22 Looi K, Sutanto EN, Banerjee B, *et al.* Bronchial brushings for investigating airway inflammation and remodelling. *Respirology* 2011; 16: 725–737.
23 Giri A, Rahman I, Sundar IK. Circadian clock-based therapeutics in chronic pulmonary diseases. *Trends Pharmacol Sci* 2022; 43: 1014–1029.
24 Zhao W, Langfelder P, Fuller T, *et al.* Weighted gene coexpression network analysis: state of the art. *J Biopharm Stat* 2010; 20: 281–300.
25 Morrow JD, Qiu W, Chhabra D, *et al.* Identifying a gene expression signature of frequent COPD exacerbations in peripheral blood using network methods. *BMC Med Genomics* 2015; 8: 1.
26 Tran NQV, Le MK, Nguyen TA, *et al.* Association of circadian clock gene expression with pediatric/adolescent asthma and its comorbidities. *Int J Mol Sci* 2023; 24: 7477.
27 Krietsch KN, Lawless C, Fedele DA, *et al.* Influence of asthma status on sleep variability in overweight/obese youth. *J Asthma* 2017; 54: 383–391.
28 Schmidt-Persson J, Rasmussen MGB, Sørensen SO, *et al.* Screen media use and mental health of children and adolescents: a secondary analysis of a randomized clinical trial. *JAMA Netw Open* 2024; 7: e2419881.
29 Baccarelli A, Dolinoy DC, Walker CL. A precision environmental health approach to prevention of human disease. *Nat Commun* 2023; 14: 2449.