Clock genes for joint health: if we could turn back time

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This editorial refers to '**The circadian clock: a central mediator of cartilage maintenance and osteoarthritis development?**', by Raewyn C. Poulsen et al.

In their latest review,[1] Poulsen *et al* have done a great job presenting an overview of recent studies and current knowledge on the contribution of clock genes (e.g. *BMAL1*) in maintenance of cartilage health. In their key messages, authors state that, for treatment, specifically targeting the chondrocyte clock to control differentiation and homeostasis is likely too complicated. Therefore, they advocate that lifestyle-interventions, such as diet and exercise, may provide an opportunity to prevent or slowdown progression of diseases such as osteoarthritis.

'*Circadian*' origins from the Latin words *circa*: 'about', and *dies*: 'day'. It refers to this internal cyclical rhythm, present in most living organisms including humans, that takes 'about a day' and continues even in the absence of external time cues.[2] Importance of the biological clock for life sciences was corroborated when Jeffrey Hall, Michael Rosbash and Michael Young were awarded the 2017 Nobel Prize in Physiology/Medicine. Although the circadian rhythm was already known for long, Hall, Rosbash and Young with their team were able to prove it is generated and maintained at a molecular level. Founders of current chronobiology, were Seymour Benzer and Ron Konopka. Back in the early seventies they discovered the first family of clock genes in flies: Period (*PER*).[3] And, as pointed out in the Nobel Lecture from Rosbash, at the very basis of circadian rhythm is the transcriptional negative feedback loop of clock genes such as *PER* that inhibit their own expression.[2]

The review from Poulsen and colleagues addresses the fact that inconsistencies have been observed for gene expression differences of clock genes in osteoarthritis. Although between-lab and study-cohort differences are a well-known phenomenon in research, highly likely such rhythmic changes even further enhance this problem. Of interest is that the review includes a section with recommendations for research in the field of chronobiology. Three concrete technical considerations are made that may help overcome this challenge for circadian clock studies. All come down to taking into account the time of the day in each study: with patients, with cells, when analysing effects of treatments, and while collecting material. Recent data underscore the importance of such 'timed research'. It was demonstrated that not only at the transcriptional level,[4,5] but also at the protein level,[6] articular cartilage is much more dynamic across 24-hour daytime than previously believed. Notably, with respect to the proteome Dudek *et al* found that 12% of all extractable proteins from mouse femoral head cartilage was synthesized and/or turned over on a daily basis.[6] Of identified rhythmic proteins, approximately 20% appeared to be dysregulated with aging or osteoarthritis. Definitely, among all proteins expressed in cartilage the absolute number of rhythmic proteins associated with loss of homeostasis is relatively small (N=30 proteins in the study mentioned). However small in number, majority of these proteins are part of the extracellular matrix or are associated to it, such as matrilin 1(Matn1), Serpine1, and

connective tissue growth factor (Ctgf) and will therefore have high impact on the overall cartilage quality.

A section of the review less well highlighted is on the circadian clock as a therapeutic target in osteoarthritis. Authors conclude by recommending a healthy lifestyle. Certainly, the importance of lifestyle interventions should not be underestimated, however, to specifically aim at improving joint health via the biological clock this section of the review could have discussed such possibility in more depth. Rather than targeting clock genes, chronopharmacology implies taking the rhythmic physiological changes into account in drug development. This is very well reviewed in 'Medicine in the Fourth Dimension'.[7] Efficiency of treatments depend on drug pharmacokinetics but may also depend on the time of administration since circadian rhythm can affect sensitivity of target cells and pathways to availability of drugs at specific times of the day. This would imply that medication can be optimized by better timing of intake (**Figure 1**). It also offers the possibility of combined medication by alternating drugs for the morning and the evening. Already in 1985 it was found that effectivity of indomethacin treatment for hip or knee osteoarthritis was patient depend. Individuals with night and/or morning pain were helped more from evening intake, while patients with afternoon or evening pain benefitted most from indomethacin in morning or afternoon.[8] Although such results should be interpreted with caution since patients logically seek to ease their pain when it hurts most, most importantly, the timing of an identical indomethacin dose in the same subject was shown to be responsible for a 200% difference in peak plasma concentrations, indeed indicating that some drugs are more sensitive for timing then others.

Previously, Tang and colleagues have shown that conditional deletion of Ctgf in mice results in thicker cartilage and was protective for osteoarthritis.[9] Multiple antibodies targeting different domains of CTGF have been studied, being FG-3019 (pamrevlumab) the most studied which has also been granted so-called 'Orphan Drug Designation' by the Food and Drug Administration (FDA) and is enrolled in different clinical trials.[10] As such, osteoarthritis patients may benefit from anti-CTGF treatment to slow down progression of their disease. Knowing that the expression of Ctgf is cyclic (being: increased during daytime and decreased at night), it is tempting to speculate that targeting Ctgf particularly at night could restore the rhythmic expression thereby facilitating cartilage recovery while one is sleeping. Certainly, for treatments targeting cyclic proteins, apart from time-dependent administration of medication also possibilities for local administration should be considered. This, to avoid interference with the local clocks of distinct organs throughout the body. For that matter, one may think of the application of therapeutic gels or strips for topical and controlled drug release.[11] Alternatively, given that topical application of antibodies such as FG-3019 are not likely to efficiently transverse the skin, development of 'chrono-engineering therapies' for delivery of therapeutic agents in an on-demand and

timely manner as an modification of recently proposed immunoengineering approaches,[12] may be an interesting option to explore.

Taken together, a lot is known about the regulation of clock genes in cartilage. Now, the time has come to take this to the next level and benefit from this in development of treatments aiming at restoring circadian rhythm to maintain cartilage homeostasis with aging and disease. The route to achieve this, is by providing lifestyle recommendations which, unfortunately, have notoriously low compliance. A secondary approach, less well investigated in the context of joint disease, is to follow-up on chronopharmacological studies such as those published in 1985,[8] to improve outcome for patients in a near-effortless way. That is, just by considering the time of drug intake.

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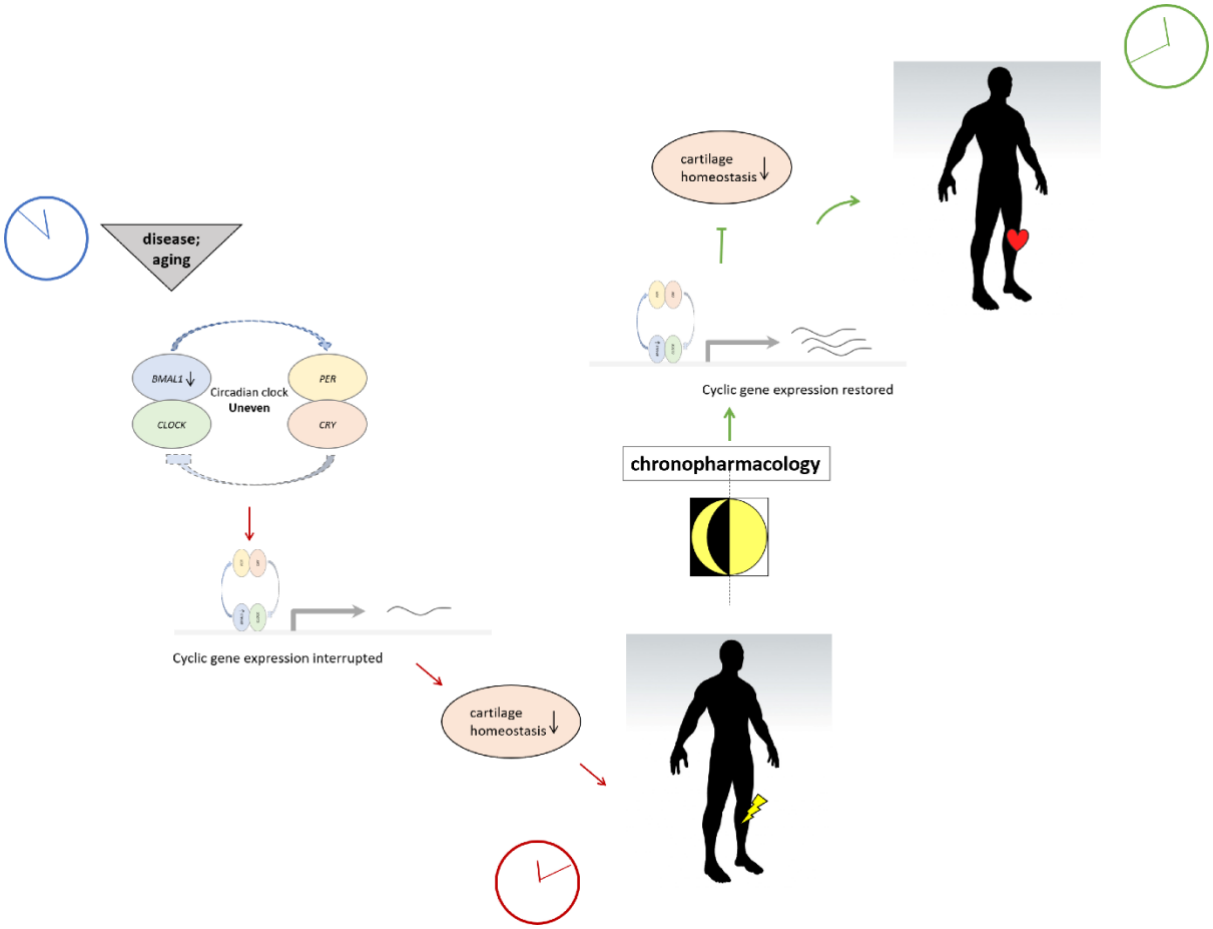

Figure 1. Application of chronopharmacological approaches in osteoarthritis contributes to restore aberrant cyclic gene expression towards the restoration of cartilage homeostasis.