Chronotherapy-informed Mendelian Randomization of PDCD1 Expression and Lung Cancer Survival

Joseph Gilbody[1], Gibran Hemani[1], George Davey Smith[1], Emma Vincent[1,2], Philip Haycock[1], Daniel Rosoff[3], David Ray[3], Rebecca Richmond[1,2]

1. MRC Integrative Epidemiology Unit, University of Bristol, Bristol, UK, 2. Translational Health Sciences, Bristol Medical School, University of Bristol, Bristol, UK

3. OCDEM, Radcliffe Department of Medicine, University of Oxford, Oxford, UK,

Circadian Rhythm of Proteins

Many physiological processes, including protein expression, follow a circadian rhythm[1]. It's unclear whether genetic variants influence plasma protein levels in a time-of-day-dependent manner in humans.
Widespread genetic regulation of circadian gene expression in humans have recently been identified[2].
Whether genetic loci (circadian quantitative trait loci, **circQTLs**) exist which influence variation in circulating protein levels across the 24-hour day is unknown, although a recent study used a sinusoidal genome-wide association study (GWAS) model to identify key circadian regulators of 24-hour blood glucose[3].
The identification of circQTLs offers particular promise in providing genetic evidence for chronotherapy i.e. time-specific dosing of drugs.

Chronotherapy of PDCD1

Chronotherapy of immune checkpoint inhibitors (ICIs) have found significantly improved outcomes if an anti-PD-1 agent is taken in the before or after 3pm in a phase 3 trial of late stage lung cancer (LungTIME-C01)[4]. The Hazard ratio (HR) of an earlier progression was 0.40 [0.29-0.55] ($P < 0.0001$) in early vs late time-of-day group.

Methods

Data:
Proteins:
UK Biobank proteomic data of the PD-1 protein (PDCD1) from 33,219 individuals measured using Olink Explore.
GWAS Model
Interaction terms: cos(2π·sample time), sin(2π·sample time).
Covariates: age, sex, genetic panel, assessment centre, fasting time, first 10 principal components of ancestry.

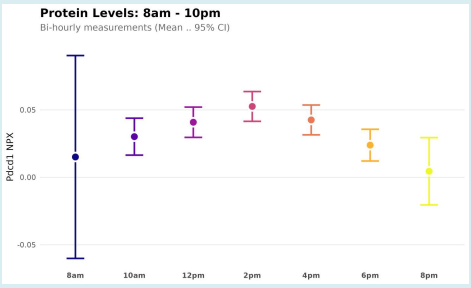
$$y = \beta 0 + \beta_{SNP}G + \beta_{Time}sin(2\pi t) + \beta_{Int}(Gxsin(2\pi t) + \gamma C + \epsilon$$
$$y = \beta 0 + \beta_{SNP}G + \beta_{Time}cos(2\pi t) + \beta_{Int}(Gxcos(2\pi t) + \gamma C + \epsilon$$

Mendelian randomization (MR)
Derivation of time-specific genetic instruments
To estimate the maximal (peak) and minimal (trough) genetic effects of PDCD1 over the 24-hour cycle, we utilized a cosinor interaction framework, calculating the amplitude of effect and adding/subtracting from the main effect.

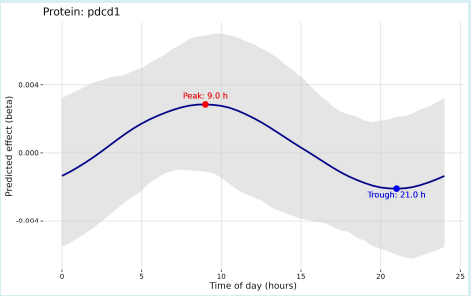
$$\beta Peak = \beta Main + A$$
$$\beta Trough = \beta Main - A$$

Outcomes
Using a GWAS of lung cancer survival (LCS) in the International Lung Cancer Consortium (3428 cases with 1405 deaths) we ran IVW two-sample MR. We compared effects generated from standard MR (time-agnostic effect of PDCD1 on LCS) to early vs. late time-of-day effects, generated using the genetically predicted peak and trough of PDCD1 expression.

Observational Protein Expression Rhythm



Genetically Predicted Protein Expression Rhythm



Using genome wide significant QTLs for the Cos and Sine effects, we estimated genetically predicted effects on protein expression across the day

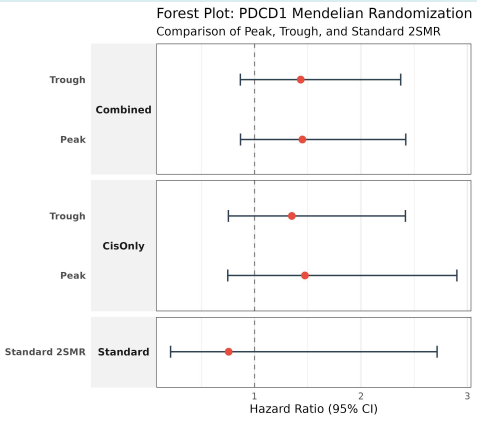
Results

GWAS results:
For PDCD1, we identified one cis and one trans QTL variant independent of the main effect model. Using these variants, we identified a genetically predicted peak expression at 9am and a trough at 9pm.
MR results
Directional Divergence: Standard cis-MR suggests a protective effect of PDCD1 on LCS (HR < 1), but circadian-informed models (Peak/Trough) reveal a flip toward hazard (HR > 1)
Temporal Masking: "Average" expression models appear to dilute the risk signal captured by rhythmic extrema.
Power Constraint: Wide 95% CIs reflect the current bottleneck in outcome GWAS power; however, the consistent hazard trend across all circadian models suggests a biological signal that static models miss.

References

1. S. Jóhönnuson, Elvar M., et al. "Diurnal rhythm of the human plasma proteome." Clinical Proteomics 22.1 (2025): 29.
2. Chen, Ying, et al. "Human genetic variation determines 24-hour rhythmic gene expression and disease risk." Nature Communications 16.1 (2025): 4270.
3. Sinnott-Armstrong, Nasa, et al. "Genetic variants affect diurnal glucose levels throughout the day." *bioRxiv* (2024).
4. Huang, Z., Zeng, L., Ruan, Z. *et al.* Time-of-day immunochemotherapy in non-small cell lung cancer: a randomized phase 3 trial. *Nat Med* (2026). https://doi.org/10.1038/s41591-025-04181-w

Mendelian Randomisation Results



Discussion

Identification of circQTLs:
We comprehensively mapped circQTLs identifying specific genetic variants that drive sinusoidal fluctuations in protein abundance over the 24-hour cycle.

Distinct Genetic Architecture:
We demonstrated that these circQTLs may be independent of standard "main effect" GWAS signals, revealing a distinct layer of genetic regulation, particularly in trans-acting variants, that is missed by time-agnostic analyses.

Translational Application: We propose a framework for Time-of-Day Mendelian Randomization (MR) to emulate chronotherapy trials. This approach allows for the high-throughput prioritization of drug targets that would benefit most from time-specific dosing[4].

Mendelian Randomization:
We found directional shifts from a standard cis-pQTL MR using our time of day approach which is more consistent with the results expected of a drug targeted with an inhibitor.

Limitations:
While we corrected for fasting time, we cannot fully disentangle the effects of the endogenous circadian clock from behavioral factors (sleep/feeding), and data is only available for 8am-8pm. Olink
The outcome GWAS used in the MR analyses contained only 3428 cases, meaning we had little power to detect an effect.
Additionally the proteomic data is from plasma expression of PDCD1 as opposed to T-cell expression.
Next steps :

We plan to replicate with PDL1 analyses, and to use an intermediate outcome, levels of circling T cells.