Universal and robust assessment of circadian time?

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In PNAS, Braun et al. (1) describe the algorithm TimeSignature. We make the following observations.

First, circadian time refers to the phase of internal biological clocks. Any algorithm assessing this should be validated against gold-standard markers of internal circadian phase. For example, the phase of the melatonin rhythm is considered a gold standard for the phase of the suprachiasmatic nucleus, the master circadian pacemaker in mammals. Braun et al. (1) validate TimeSignature against the clock time at which the sample was taken, which is not a marker of internal phase. It is unfortunate the authors do not discuss this major limitation and claim to assess circadian time. Others (e.g., refs. 2 and 3) have developed algorithms for assessing circadian phase from blood samples and have validated against gold standards.

Second, Braun et al. (1) claim superiority of their two-sample method by comparing its performance with one-sample–based methods of others (2, 4, 5). A fair comparison is to compare two apples to two apples. Using two samples, TimeSignature predicts external clock time with an error of less than 2 h in <70% of samples in each of the four tests. Partial least squares regression (PLSR) using the difference of two samples will predict internal circadian time (melatonin phase) with an error of less than 2 h in 82% of samples (2).

Third, Braun et al. (1) state that TimeSignature is independent of any knowledge of time separation. However, the two-sample, within-subject normalization applied produces two samples that are always 180° apart (same magnitude but opposite sign); that is, equivalent to two samples taken 12 h apart. Hence, sampling strategies that best fit with the intrinsic 12-h separation of TimeSignature normalization (i.e., 10- to 14-h intervals) will have the highest prediction accuracy. In short, any two-sample differential method can be applied to two samples with any time separation. It is the 24-h sinusoidal assumption of the modeling approach that dictates that 12-h separation provides the maximum predictive power.

Fourth, the authors claim that TimeSignature is more efficient and more reliable because it does not require cross-study renormalization. This is true, but certainly not novel to the prediction of circadian time. The directly comparable two-sample differential PLSR method also requires only the difference between two samples to be taken. The normalization applied to the data in the original study in ref. 2 was retained only so that a systematic and direct comparison between approaches could be made.

Finally, Braun et al. (1) make inaccurate statements about previous work. For instance, they cited our work (2) in their statement that "in all cases. . . [data] were first combined and conormalized." This is incorrect. We (2) did not conormalize; normalization factors were independently calculated for the training set and subsequently applied to the validation set. Furthermore, we have described our method in detail and provided code for applying our method to new samples (2).

The application of machine learning to high-dimensional data such as transcriptomics offers new and exciting opportunities for assessing circadian parameters in humans and may aid personalized and precision sleep and circadian medicine. Progress in this field will require that any new method be carefully validated and compared with other methods.

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