doi 10.18699/bgrs2024-13-29

Epistemic uncertainty challenges reliable prediction of rejuvenation events with aging clocks

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Key words: rejuvenation; cell reprogramming; epistemic uncertainty; epigenetic aging clocks; dataset shift; DNA methylation

Motivation and Aim: Epigenetic aging clocks have been widely used to validate rejuvenation events during cellular reprogramming [1] and early stages of embryogenesis [2]. However, the predictions of aging clocks can not be directly verified due to the unavailability of true biological ages for reprogramming or embryonic methylation samples. Moreover, the extreme nature of reprogramming and embryogenesis data, compared to the data used for aging clock training, raises questions about the reliability of aging clock predictions (Fig. 1a, b).
Methods and Algorithms: We turned to established knowledge in machine learning and constructed a multifaceted analytical framework to consider rejuvenation predictions from the uncertainty perspective [3] (Fig. 1c–g).

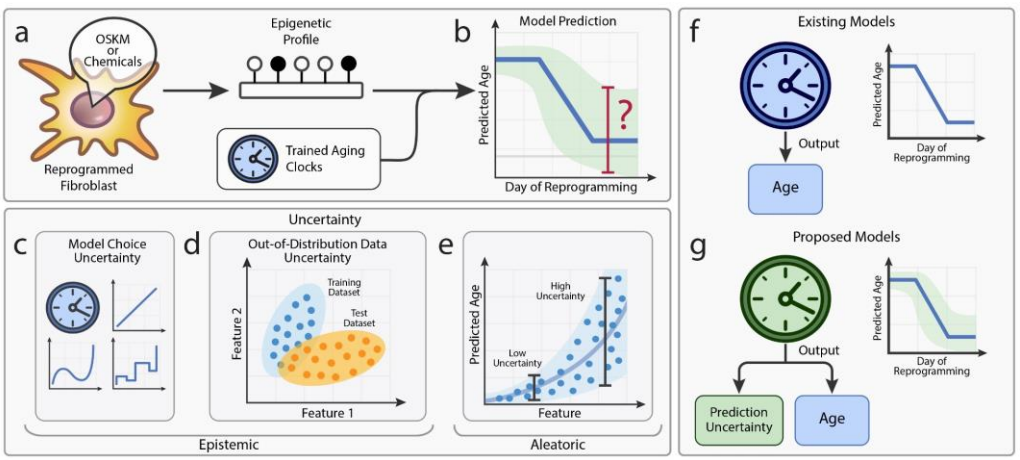

Fig. 1. Prediction uncertainty is an essential component for clinically relevant aging clocks. a, A common pipeline for testing rejuvenation effect using epigenetic aging clocks. b, Current aging clocks estimate biological age during the reprogramming process; however, they lack epistemic uncertainty quantification. c, By selecting a model to make predictions from data, a researcher implicitly introduces model uncertainty. d, Out-of-distribution uncertainty arises when the testing data samples are not represented in the training distribution. e, Aleatoric uncertainty comes from the intrinsic variability in data, e. g. when the same level of a feature corresponds to different ages. f, None of the existing aging clocks estimates epistemic uncertainty. g, We propose to use aging clocks capable of predicting uncertainty, which could mitigate the potentially erroneous effects of clock predictions on clinical decision-making

The framework includes: testing two datasets (training and testing) on the distribution shift [4], testing consistency of rejuvenation prediction by training multiple machine learning models from different model families, direct uncertainty estimation with Gaussian Process Regression model [5]. This framework addresses four key questions: (i) Is there a substantial shift in DNAm values between aging and rejuvenation datasets? (ii) Do different established aging clocks provide consistent predictions for rejuvenation? (iii) Can the rejuvenation datasets be used reciprocally to predict normal aging? (iv) Can an aging clock, capable of estimating its own uncertainty, demonstrate age reversal in putative rejuvenation experiments?

Results: Our analysis shows that DNA methylation profiles of reprogramming are not represented in the aging data used for clock training – a phenomenon also known as dataset shift – which introduces high epistemic uncertainty in aging predictions. Furthermore, predictions of different published aging clocks exhibit poor agreement, with some even suggesting zero or negative rejuvenation. Our developed Inverse Train-Test Procedure further reveals that in vitro reprogramming datasets can not robustly predict normal aging reciprocally, thus challenging the assumption that normal aging can precisely predict reprogramming. We demonstrate that the high prediction uncertainty challenges the reliability of aging clocks in predicting rejuvenation effects observed during in vitro reprogramming prior to pluripotency and throughout embryogenesis. Conversely, our method also reveals a significant age increase after in vivo reprogramming.

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