CASE STUDY

SAPIENT

# Turning back the metabolic aging clock

The human aging process is as diverse as humans themselves. Individuals of the same *chronological age* can experience vastly different health trajectories, with some remaining mentally and physically vibrant well into late life, and others experiencing sharp health or quality of life declines decades earlier.

This heterogeneity can be read out through *biological age,* which uses biomarkers to estimate how genetic factors and environmental exposures influence cellular aging. Various biological disruptions, from oxidative stress to immune dysregulation, can accelerate biological aging faster than chronological age, and such measures can capture individual differences in aging rates based on how the body is actually functioning.

Much effort has been made to develop aging clock models that can accurately predict biological age. To date most have relied on epigenetic markers, specifically measuring patterns of DNA methylation to estimate aging of cells and tissues. Epigenetic clocks have provided strong evidence that aging can be measured in molecular terms, but they do still have shortcomings. Specifically, it is unclear if such measures reflect causal factors of aging or are simply correlated with aging and disease, making it difficult to assess whether interventions can actually slow or reverse biological aging.

### The Question

Can dynamic plasma metabolite measures be used to **predict individual biological aging rates?**

### The Findings

Our metabolic aging clock model **accurately predicted accelerated biological aging** for individuals with chronic disorders, with **dynamic 'reversal' of accelerated aging** following intervention.

## The Opportunity

Small molecule biomarkers, comprising metabolites and lipids, read out both genetic *and* non-genetic factors of health, disease, and drug response, including diet, lifestyle, environmental exposures, toxicants, and microbes – all factors that can influence biological aging. Additionally, given their diverse exogenous and cellular origins, rapid translocation into central circulation from multiple body tissues, and dynamic nature, metabolite and lipid biomarkers can serve as powerful diagnostic and prognostic predictors of human disease.

Clearly, **metabolomics data has high potential utility to predict complex physiological traits like aging,** and metabolites may better capture dynamic changes in aging due to disorders and/or interventions than epigenetic markers that currently define biological age.

## The Challenge

Historically, mass spectrometry methods have been constrained by several bottlenecks that limit overall throughput and have largely precluded the ability to perform non-targeted metabolomics

on population-scale datasets. Large-scale analyses are needed to provide the statistical power for robust biomarker discovery and cross validation – particularly for applications like metabolic markers predicting aging rate differences across individuals.

To address the throughput challenge, Sapient has developed a fully automated rapid liquid chromatography-mass spectrometry (rLC-MS) system that **captures thousands of polar, amphipathic, and nonpolar (lipid) metabolites in human plasma, in 53 seconds of analytical time per sample.** As a result, our rLC-MS pipeline enables high throughput, reproducible, non-targeted metabolite measurements across tens of thousands of samples, enabling large-scale interrogation of exogenous and endogenous small molecule mediators that influence human health and disease.

In this case study, we sought to assess whether our population-scale rLC-MS metabolomics data could be used to predict biological age.

## The Analysis

Sapient first **trained a machine learning model on rLC-MS data generated in 1,640 samples from 887 healthy individuals,** ranging in chronological age from 23 to 82 years old with no diagnosed chronic diseases and healthy BMI. The samples were selected from Sapient's proprietary DynamiQ™ biorepository – comprised of 62,039 total plasma samples collected longitudinally from 11,045 individuals – to represent those whose biological age should closely align with their chronological age.

Through fine tuning of the machine learning model, **a set of 30 metabolites were selected to produce an accurate multivariate model, or metabolic aging clock** (Figure 1). Metabolites negatively associated with age included several sex hormones known to decline with age. Positively associated metabolites included glucuronate, which is involved in detoxification of xenobiotics, and phenylacetylglutamine, a marker recently implicated in senescence during the aging process.

A fully independent validation was run on rLC-MS data from an additional 758 samples from 300 individuals in Sapient's DynamiQ biorepository, which **confirmed that the metabolic aging clock predicted individuals' age with a median absolute error of 8.0 years** (Figure 2).

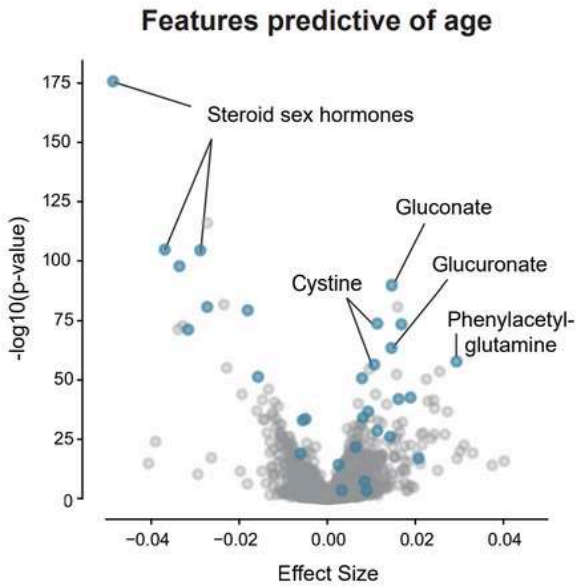



*Figure 1. Features from the training/testing set selected for inclusion in the metabolic aging clock model.*

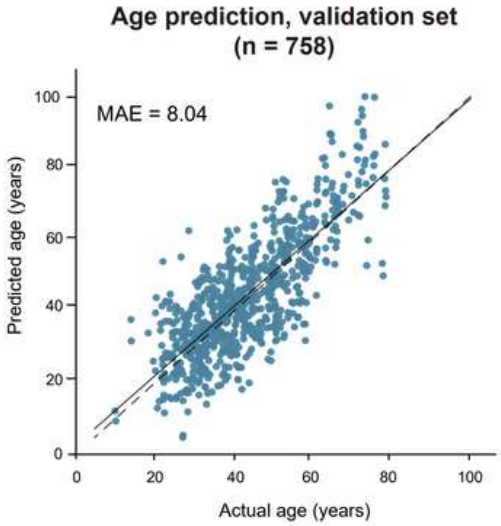



*Figure 2. Performance of the metabolic aging clock model on the validation set of 758 samples from 300 healthy individuals.*

## The Insights

Using the metabolic aging clock model, we were able to interrogate several aspects impacting human aging. Because common human diseases tend to shorten lifespan, we first asked if age predictions by our model would be affected by disease. **In a set of 4,000 individuals not seen by the model during training, the clock predicted accelerated biological aging for individuals with common chronic disorders** (Figure 3). Specifically, obesity was associated with a median age acceleration of 3.7 years, and diabetes with a median of 7.4 years – consistent with reported lifespan reductions for those disease states.

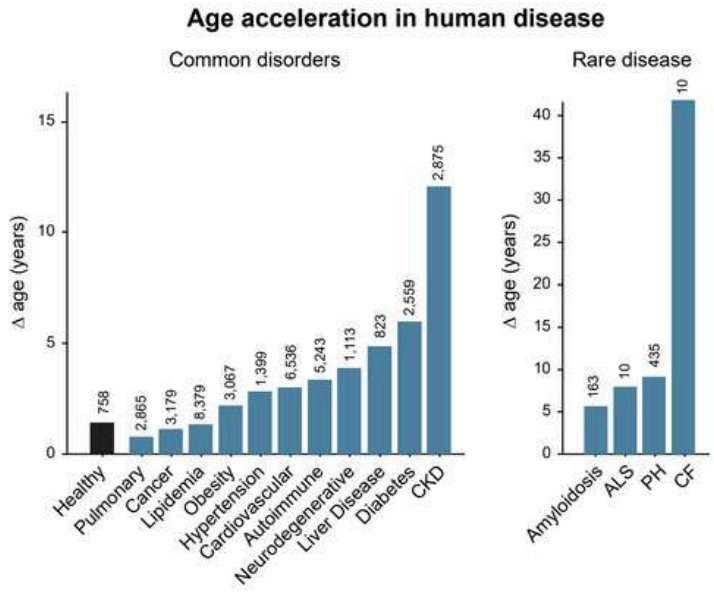



*Figure 3. Age acceleration (Δage), defined as difference between predicted and actual age, of individuals with indicated chronic disorders. Median values per group are graphed.*

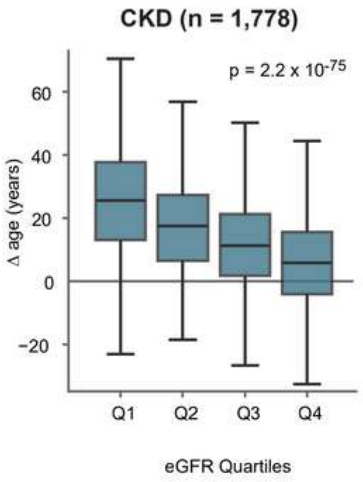



*Figure 4. Age acceleration vs. quartiles of eGFR for individuals diagnosed with CKD.*

Chronic kidney disease (CKD) was estimated to accelerate biological age by as much as 13.0 years, and this age delta was inversely correlated with estimated glomerular filtration rate (eGFR), indicating that within CKD, **greater disease severity was related to more accelerated aging** (Figure 4). This suggests that biological age predicted by our metabolic aging clock reflects disease severity in a more fine-grained manner than traditional diagnosis.

Interestingly, **diseases generally localized to a single organ exhibited a more minimal effect on accelerated aging compared to those impacting systemic physiology.** For instance, non-metastatic cancer increased median biological age acceleration by only 2.4 years. On the other hand, rare debilitating diseases such as amyotrophic lateral sclerosis (ALS) and cystic fibrosis resulted in substantial accelerated aging, suggesting that while those diseases initially manifest in a single organ, they have widespread effects on whole body function that further progress biological aging.

The second analysis asked whether the model would reflect a 'reversal' of accelerated biological aging following therapeutic treatment of disease. The metabolic aging clock was applied to a set of individuals with end-stage renal disease who underwent kidney transplantation, and **remarkably, it predicted a marked decrease in biological age within 3 months following the surgery – essentially 'turning back the metabolic clock' by a median of 9.4 years** (Figure 5). This suggests that definitive treatment normalized the pro-aging factors affecting systemic aging in these patients.

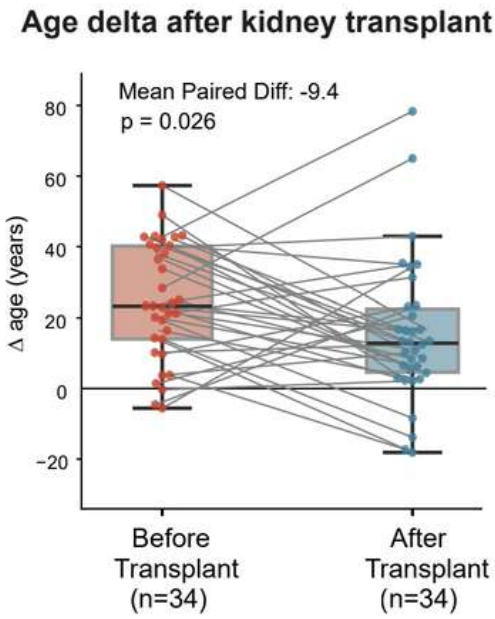
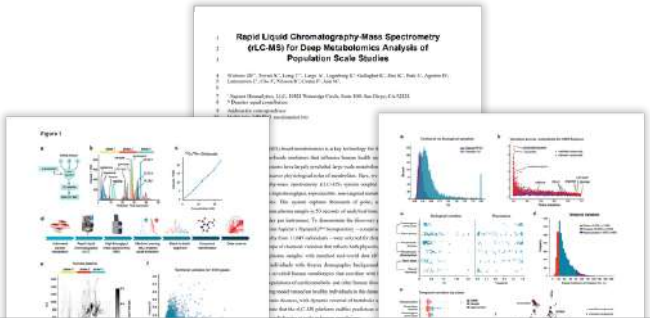



*Figure 5. Biological age acceleration in 34 individuals before and after kidney transplant. Gray lines indicate samples from the same individual.*

## The Impact

The saying "age is just a number" belies the true complexity of human aging. Biomedical data generally cannot perfectly predict chronological age, as individuals of the same age may differ widely in their physiology and biological age. Metabolites and lipids, as exogenous and endogenous small molecule mediators that influence health and disease, can **offer unique insight into biological aging mechanisms and inform on treatment response.**

This case study demonstrates that it is indeed feasible to train predictive models of complex physiological states such as aging from large-scale, non-targeted metabolomics datasets, and that this data may better capture dynamic changes in aging than biomedical data or epigenetic markers alone. It also showcases the exciting potential of **pairing metabolomics data and machine learning models to predict other key outcomes** such as disease onset and response to therapeutic and lifestyle interventions.




*To explore more about the rLC-MS method and metabolic aging clock, you can read our new paper,* ***Rapid Liquid Chromatography-Mass Spectrometry (rLC-MS) for Deep Metabolomics Analysis of Population Scale Studies.***

Interested in integrating dynamic biomarker data into your drug development programs? Schedule a time to speak with our scientists at **sapient.bio/metabolomics.**

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