DNA methylation age mediates effect of metabolic profile on cardiovascular and general aging

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Abstract

Background Alterations in lipid metabolism and DNA methylation (DNAm) are two hallmarks of aging. Connecting metabolomic, epigenomic, and aging outcomes helps unravel the complex mechanisms underlying aging. We aimed to assess whether DNAm clocks mediate the association of circulating metabolites with incident atherosclerotic cardiovascular disease (ASCVD) and frailty.

Methods The China Kadoorie Biobank is a prospective cohort study with a baseline survey from 2004 to 2008 and a follow-up period until December 31, 2018. We used the Infinium Methylation EPIC BeadChip to measure the methylation levels of 988 participants' baseline blood leukocyte DNA. Metabolite profiles, including lipoprotein particles, lipid constituents, and various circulating metabolites, were measured using quantitative nuclear magnetic resonance. The pace of DNAm age acceleration (AA) was calculated using five widely used epigenetic clocks (the first generation: Horvath, Hannum, and Li; the second generation: Grim and Pheno). Incident ASCVD was ascertained through linkage with local death and disease registries and national health insurance databases, supplemented by active follow-up. The frailty index was constructed using medical conditions, symptoms, signs, and physical measurements collected at baseline.

Results A total of 508 incident cases of ASCVD were documented during a median follow-up of 9.5 years. The first generation of epigenetic clocks were associated with the risk of ASCVD ($P<0.05$). For each standard deviation (SD) increment in LiAA, HorvathAA, and HannumAA, the corresponding hazard ratios (HRs) for ASCVD risk were 1.16 (1.05, 1.28), 1.10 (1.00, 1.22), and 1.17 (1.04, 1.31), respectively. Only LiAA mediated the association of various metabolites (lipids, fatty acids, histidine, and inflammatory biomarkers) with ASCVD, with the mediating proportion reaching up to 15% for the diameter of low-density lipoprotein (P=1.2E-02). Regarding general aging, a 1-SD increase in GrimAA was associated with an average increase of 0.10 in the frailty index (P=2.0E-03), and a 33% and 63% increased risk of prefrailty and frailty at baseline (P=1.5E-02 and 5.8E-02), respectively; this association was not observed with other clocks. GrimAA mediated the effect of various lipids, fatty acids, glucose, lactate, and inflammatory biomarkers on the frailty index, with the

mediating proportion reaching up to 22% for triglycerides in very small-sized very low-density lipoprotein (P=6.0E-03).

Conclusions These findings suggest that epigenomic mechanisms may play a role in the associations between circulating metabolites and the aging process. Different mechanisms underlie the first and second generations of DNAm age in cardiovascular and general aging.

Non-standard Abbreviations and Acronyms

AA age acceleration

ACME average causal mediation effect

Apo apolipoprotein

ASCVD atherosclerotic cardiovascular disease

AUC area under the receiver operating curve

BMI body mass index

CA chronological age

CKB China Kadoorie Biobank

-C cholesterol

DNAm DNA methylation

EWAS epigenome-wide association analysis

GlycA glycoprotein acetyls

GOF goodness of fit

HDL high density lipoprotein

CRP C-reactive protein

IDL intermediate-density lipoprotein

IDI integrated discrimination improvement

LDL low density lipoprotein

MET metabolic equivalent task

MUFA monounsaturated fatty acid

NMR nuclear magnetic resonance

NRI net reclassification index

PC principle component

TFA total fatty acid

TG triglycerides

VLDL very low density lipoprotein

Introduction

With the rapid aging of the global population, novel strategies for healthy aging are urgently needed. Novel biological age estimators could depict accelerated or decelerated aging in populations and aid in unravelling the complex molecular mechanisms underlying aging.[1]

DNA methylation (DNAm) level varies dynamically in response to both genetic and environmental factors.[2] DNAm clocks have emerged as promising tools for estimating biological age.[3] The first generation of DNAm clocks were trained to predict chronological age (CA), and the deviation from CA is further defined as biological aging pace[4–6]; whereas the second generation of DNAm clocks were predictors of lifespan and several established biomarkers of aging[7,8]. While these clocks have similarities and differences, we still do not fully understand the mechanisms they capture. We hypothesized that there are multiple distinct epigenetic aging phenomena for different organs and systems, which the clocks capture to varying degrees. For example, studies regarding different DNAm ages and risk of atherosclerotic cardiovascular disease (ASCVD) got mixed results: null[9,10], positively associated with fatal[11] or incident ASCVD[8]. Evidence also demonstrated the associations of DNAm age with mortality and other age-related phenotypes varies across different DNAm age.[3] However, few studies compare the effects of different epigenetic clocks on various health outcomes in the same population.

Aging is characterized by genomic instability, telomere shortening, epigenetic changes and metabolic dysfunction.[12] Previous Mendelian randomization studies found that lipid dysfunction affects DNA methylation levels at age-related genes in a causal way.[13,14] It is plausible that lipids metabolites can alter the risk of age-related disease at least partly through

epigenetic modification. Previous studies have generally used conventional lipid measures, without considering the distinctions in lipoprotein size, density, and composition, all of which are important factors related to biological aging.[15-17] Quantitative nuclear magnetic resonance (NMR) metabolomics offers more comprehensive measurements of lipoprotein particles and other metabolic markers.[18] Studies have found clear opposite effects of different compositions carried in lipoprotein on ASCVD.[19,20] Epigenome-wide association analysis (EWAS) revealed NMR-metabolites had contrasting effect on DNAm change.[21] However, no research has thoroughly examined the connections between NMR-metabolites and various DNAm ages within the same study population. Most importantly, how DNAm age mediates the contrasting effect of metabolites on the aging outcome deserves to be studied.

The present study aimed to compare the potential association of five widely used epigenetic age indicators with cardiovascular (incidence of atherosclerotic cardiovascular disease, ASCVD) and general (frailty and total mortality) aging in 980 participants from the China Kadoorie Biobank (CKB) study. Mediation analyses were used to shed light on the underlying pathway from NMR-metabolomics to general and cardiovascular aging through accelerated epigenetic aging. Finally, we assessed the predictive utility of these metabolic measures and epigenetic age acceleration (AA) for ASCVD risk.

Methods

Because of the sensitive nature of the data collected for this study, requests to access the dataset from qualified researchers trained in human subject confidentiality protocols may be sent to China Kadoorie Biobank at https://www.ckbiobank.org/data-access.

Please see the detailed methods and the major resources table in the **Supplemental Materials**.

Study population

A total of 512,715 participants from ten geographically diverse areas across China, including five urban and five rural areas, were recruited during 2004-2008. Each participant accepted laptop-based questionnaire survey and physical measurements by trained staff at baseline. Random blood samples (10 ml) were collected for all participants at baseline for an immediate on-site test of random plasma glucose and long-term storage (with the time since the last meal recorded). After the baseline survey, all participants were followed up for mortality, morbidity, and hospitalization events by linkage with local death and disease registries and the national health insurance databases, supplemented by active follow-up (i.e., visiting local communities or directly contacting participants). Also, about 5% of surviving participants were cluster sampled for periodic resurveys every 4-5 years. Further details of the CKB have been described elsewhere.[22]

The study was approved by the Ethics Review Committee of the Chinese Center for Disease Control and Prevention (005/2004, Beijing, China) and the Oxford Tropical Research Ethics Committee, University of Oxford (025-04, UK). All participants provided written informed consent.

Study design

The current study involved four interrelated components: (1) the association of 225 NMR-measured metabolites with five DNAm AA; (2) the association of DNAm AA with cardiovascular aging (incident ASCVD during follow-up) and general aging outcomes (frailty

at baseline, frailty change from baseline to resurvey, and total mortality during follow-up); (3) mediation effect through DNAm AA from metabolites to aging outcomes; and (4) evaluation of the incremental predictive ability of metabolites and DNAm age for incident ASCVD.

We selected 988 participants who had previously been measured for NMR-metabolomics to measure the epigenome-wide methylation level of baseline blood leukocyte DNA. Details of the inclusion and exclusion criteria were provided previously.[23] All involved participants were free of heart disease, stroke, transient ischemic attack, and cancer, and were not using statin therapy at baseline.

Measurement of epigenome-wide methylation

Infinium Methylation EPIC BeadChip (Illumina, USA) was used to measure epigenome-wide methylation levels. This latest beadchip interrogates more than 850,000 CpG sites across the genome. The laboratory staff were blinded to case/control status. β-value (ranging from 0 to 1.0) for each CpG site was reported, representing the DNAm percentage of each CpG site. We used minfi[24] package (RRID: SCR_012830) to process methylation data. Details of study design, quality control, filtering, and normalization of the methylation data have been described previously.[23] These filtering processes resulted in 980 samples with 747,726 CpG sites retained.

Measurement of NMR-metabolomics

All 980 participants were measured for 225 lipid, lipoprotein, and other metabolites using baseline blood samples by a high-throughput targeted NMR metabolomics platform at the Brainshake Laboratory (Kuopio, Finland). Three components separated by hyphens are used

to name the lipid subfraction traits. The first component indicates the size (XS, S, M, L, XL, and XXL); the second indicates the fraction according to the lipoprotein density (VLDL, LDL, IDL, and HDL); and the third indicates the measurement (C for total cholesterol, and TG for triglycerides). Details of the sampling process, measurements, and quality control of the metabolomic data have been given elsewhere.[20] C-reactive protein (CRP) and lipids concentrations (TC, LDL-C, HDL-C, and TG) were also quantified using standard clinical chemistry assays (Wolfson Laboratory at University of Oxford, UK) for all participants.

Assessment of aging outcomes and covariates

We ascertained outcome status (incident ASCVD and total mortality) from the baseline to 31 December 2018. Incident ASCVD was defined as the first acute myocardial infarction (ICD-10 I21), fatal ischemic heart disease (IHD) (I20-I25), or ischemic stroke (I63 or I69.3), whichever came first. By reviewing hospital medical records, we have finished diagnosis adjudication for 134 reported cases and confirmed 90% of the ASCVD diagnoses.

Of the 980 participants, 268 were also enrolled in the 2008 resurvey, with the mean time interval from baseline to resurvey being (2.96 ± 0.74) years. We calculated the frailty index at baseline (N=980) and resurvey (N=268) for available participants. The frailty index was constructed using 25 variables, including medical conditions, symptoms, signs, and physical measurements (**Table S1**).[25]

Statistical analysis

DNA methylation age and age acceleration

We calculated five methylation age indicators based on existing prediction models, including the first generation (HorvathAge[4], HannumAge[5], and LiAge[6]) and the second generation

(GrimAge[8] and PhenoAge[7]) of DNAm age (**Table S2**). Pearson correlation test was used to evaluate the correlation of the five DNAm ages with CA. DNAm AA was defined as the residual of regressing DNAm age on the CA. LiAA, GrimAA, and PhenoAA presented significantly skewed distribution, with P value for skewness < 0.05 (**Figure S1**). Rank-based inverse normal transformation (SD=1) was used for AA measurements.

Metabolites and DNAm age acceleration

The associations between metabolites (both clinical chemistry and NMR metabolomics) and DNAm AA were assessed using multiple linear regression in all participants, women, and men separately, with adjustment for CA (year), sex (male or female), ten study areas, fasting time (0- < 6, 6- < 8, 8- < 10, or ≥10 hours), five DNAm measurement batches, education level (middle school and above, or others), marital status (currently married or not), smoking (average cigarettes or equivalents consumed per day), alcohol consumption (average pure alcohol volume consumed per day), total physical activity level (MET-h/d), average days consuming fresh vegetables, fruits, and red meat per week, and BMI (kg/m^2). Categorical variables were included as dummy variables in the model. The models were further adjusted for the estimated cell type proportion (CD4+ T cell, CD8+ T cell, B cell, natural killer, monocytes, and granulocyte). The proportion of leucocyte cells was estimated from DNAm signature by R package minfi (SCR_012830) [24].

DNAm age acceleration and aging outcome

For the associations of DNAm AA with incident ASCVD and total mortality during follow-up, we fitted multivariable Cox proportional hazards models to estimate the hazard ratio (HR) and 95% confidence interval (CI), with age as the underlying time scale and stratified by 5-

year age groups and ten survey areas. Participants contributed person-time from baseline until the date of the outcome, death, loss to follow-up, or December 31, 2018, whichever came first. All models were adjusted for CA, sex, fasting time, education level, marital status, batch, smoking, alcohol consumption, physical activity, average days consuming fresh vegetables, fruits, and red meat per week, BMI, and the estimated cell type proportion.

Multiple linear regression was applied to investigate the association of DNAm AA with baseline frailty index and frailty index change, with adjustment for same set of covariates as above. We further fitted multinomial logistic regression adjusting for the same set of covariates to estimate the odds ratio (OR) and 95% CI for prefrailty and frailty.

Sensitivity analyses with further adjustment for the first 17 principle components (PCs) explaining >95% of variance in 225 NMR-measured metabolites, or further adjustment for prevalent diabetes and blood pressure were also performed. The association of DNAm AA with aging outcomes was also analyzed in women and men separately.

Mediation analyses

We built a directed acyclic graph to illustrate the hypothesis of the mediation framework (**Figure S2**). Subsequent mediation analyses were restricted to metabolites potentially associated with any of the five DNAm AA (raw $P < 0.05$). We performed causal mediation analysis using parametric regression models, achieved by R package mediation.[26] We aimed to calculate how much of the aging outcome (ASCVD risk or frailty) associated with metabolite (average total effect) was attributable to the mediating effect of DNAm AA (average causal mediation effect, ACME).

Evaluation of the incremental predictive ability of metabolites and DNAm age for

incident ASCVD

We first constructed a reference prediction model that included traditional ASCVD risk factors using multiple logistic regression model: CA (years), sex (male or female), ten study areas, smoking status (average cigarettes or equivalents consumed per day), alcohol consumption (average pure alcohol volume consumed per day), BMI (kg/m^2), prevalent diabetes (yes or no), systolic and diastolic blood pressure (mmHg), TC, HDL-C, and TG (mmol/L). We then added five DNAm AA, metabolites one by one, or the first 17 PCs of metabolites, to the reference model separately and jointly. The area under the receiver operating curve (AUC), integrated discrimination improvement (IDI), continuous net reclassification index (NRI), and categorical NRI with a 10% cut-off point for logistic regression models were calculated. The C index for survival data was also calculated to assess the robustness of the findings. The Hosmer-Lemeshow tests were performed to test the goodness of fit (GOF) for logistic regression.

All analyses were performed with Stata version 14.2 (SCR_012763) and R software version 3.5.2 (SCR_001905). Statistical significance was set at two-tailed $P<0.05$. We used the false discovery rate (FDR) <0.05 across each DNAm AA measurement to test multiple metabolites (N=225).

Results

The mean CA for 980 participants was 50.5 ± 7.5 years. The mean correlation coefficients between CA and DNAm GrimAge, LiAge, HorvathAge, HannumAge, and PhenoAge were 0.79, 0.90, 0.80, 0.84, and 0.79, respectively (**Figure S3**). Participants with higher GrimAA

were more likely to be men, have a higher proportion of daily smokers and alcohol drinkers, and have a higher prevalence of diabetes at baseline (**Table 1**). The distributions of the other four DNAm AA across participants with different baseline characteristics were generally similar (**Tables S3-S6**).

Clinical chemistry-measured lipids and DNAm age acceleration

After adjustments for covariates including cell type proportion, HDL-C was negatively associated with GrimAA. Participants with per SD higher HDL-C levels had a 0.035 SD lower GrimAA on average (P=9.0E-03). TC was positively associated with HannumAA (adjusted SD difference =0.076, P=3.5E-02) (**Table S7**). Other association analyses revealed no statistically significant associations.

NMR-measured metabolites and DNAm age acceleration

A total of 20 components of the NMR-measured metabolites were associated with the GrimAA after adjustment for multiple risk factors of DNAm aging and cell type proportion (FDR < 0.05). Cholesterol within various-sized VLDL particles was positively associated with the GrimAA, with a maximum SD difference (95%CI) to be 0.068 (0.022, 0.114) for M-VLDL-C (FDR=2.9E-02) (**Figure 1**). However, cholesterol within IDL, LDL, and HDL particles was not associated with the GrimAA (all FDR > 0.05). TG within IDL, LDL, smaller-sized VLDL and smaller-sized HDL was positively associated with GrimAA. The maximum adjusted SD difference was observed for IDL-TG (0.096, 95%CI: 0.050, 0.141; FDR=3.0E-03). The associations of lipoprotein particle concentration with GrimAA were similar to the associations of cholesterol within lipoprotein subfractions (**Figure 2**). Participants with per SD increase in the LDL diameter showed an average of 0.064 (95% CI:

0.014, 0.114) SD higher GrimAA (FDR=4.8E-02). ApoB and ApoB/ApoA1 were positively associated with the GrimAA. The corresponding SD difference (95% CI) was 0.067 (0.021, 0.112) and 0.072 (0.026, 0.118).

The glycoprotein acetyls (GlycA), an inflammation marker, was positively associated with GrimAA, with an adjusted SD difference of 0.108 (95%CI: 0.060, 0.155) (FDR=1.0E-03). GlycA and high sensitivity CRP were both independently associated with GrimAA (**Table S8**). There was no association of GrimAA with fatty acids, amino acids, and ketone bodies (all FDR>0.05).

The associations between metabolites and GrimAA were more pronounced in men than women, although the general association patterns were similar (**Figures S4-S7**). After FDR correction, statistically significant associations between metabolites and GrimAA were only observed in men.

The association patterns of NMR-measured metabolites with DNAm AA were generally concordant for LiAA, HannumAA, and PhenoAA. In other words, metabolic measures associated with GrimAA also tended to be associated with LiAA, HannumAA, and PhenoAA, with regression slopes of 0.81, 0.72, and 0.91, respectively (**Figure S8 A, C, and D**). However, such associations were no longer statistically significant after multiple comparison corrections (all FDR > 0.05, **Figure 1** and **Figure 2**). In contrast, there was less concordance of the associations on metabolites with GrimAA or HorvathAA, with metabolites generally showing much weaker associations with HorvathAA (regression slope = 0.21) (**Figure S8 B**).

Mediation effects of metabolites on general aging through DNAm age acceleration

A total of 357 deaths were documented during a median of 12.3 years follow-up (25–75th percentile range: 5.9-13.5 years). In the multivariable model adjusted for risk factors of mortality and cell type proportion, per SD increase in HorvathAA and PhenoAA was associated with 14% (P=2.4E-02) and 17% (P=9.0E-03) increased risk of death, respectively (**Table S9**). Per SD higher GrimAA was associated with an average of 0.10 higher frailty index (P=2.0E-03), 33% and 63% increased risk of prefrailty and frailty at baseline (P=1.5E-02 and 5.8E-02). GrimAA also showed marginal positive association with the change of frailty index from baseline to the resurvey (P=9.6E-02) (**Table S10**). Additional adjustment for the first 17 PCs explaining >95% of variance in NMR-metabolites did not change the association between GrimAA and frailty index at baseline materially (**Table S11**). The associations of DNAm AA measurements with total mortality and frailty index at baseline were similar among men and women (P for sex interaction > 0.05, **Tables S9 and S12**). Therefore, we did not perform mediation analysis in men or women separately.

Of the total 357 deaths, the primary cause of death was ASCVD for 327 participants. To better capture the characteristics of general aging, we used frailty as the outcome in the subsequent mediation analyses. Further mediation analysis revealed that GrimAA mediated the effect from various-sized VLDL and IDL particle concentrations and its cholesterol and TG to frailty index (**Table 2**). The mediating proportion ranged from 11.21% for IDL-C (P_{ACME} = 5.0E-02) to 21.98% for XS-VLDL-TG (P_{ACME} = 6.0E-03). GrimAA also mediated the effect from TG in various-sized LDL and HDL to frailty index. The maximum mediating proportion was 20.88% for M-HDL-TG (P_{ACME} = 4.0E-03). For apolipoproteins, 20.00% and 20.37% of the ApoB and ApoB/A1 associated frailty index increment was mediated through

GrimAA. For other metabolites, the effect of glucose, lactate, MUFA/TFA, and GlycA on frailty index was mediated through GrimAA (all P_{ACME} <0.05). The mediating effect pattern was generally similar in the sensitivity analysis without adjustment for cell type proportion (**Table S13**). For the other four DNAm aging indicators, we didn't observe robust statistically significant mediation effects (**Table S14-S21**).

Mediation effects of metabolites on incident ASCVD through DNAm age acceleration

A total of 508 incident cases of ASCVD were documented during a median of 9.5 years follow-up (25–75th percentile range: 4.2-13.1 years). After adjustment for multiple risk factors of ASCVD and cell type proportion, LiAA, HorvathAA, and HannumAA were associated with risk of ASCVD (P<0.05) (**Table S22**). For each SD increment in the LiAA, Horvath, and HannumAA, the corresponding HRs for risk of ASCVD were 1.16 (1.05, 1.28), 1.10 (1.00, 1.22) and 1.17 (1.04, 1.31). Further adjustment for the 17 PCs of metabolites, or further adjustment for prevalent diabetes and blood pressure did not change the association between LiAA and incident ASCVD materially (**Table S23**). The statistically significant association between GrimAA and incident risk of ASCVD was only observed in men after adjustment for potential confounders (P for sex interaction =3.2E-02, **Table S22**). The corresponding HRs for risk of ASCVD with each SD increment in GrimAA were 1.31 (1.08, 1.60) and 0.78 (0.57, 1.08) among men and women respectively. The associations were generally similar among men and women for other DNAm AA measurements (P for sex interaction > 0.05).

LiAA mediated the association of M- to XXL-sized VLDL particles and their cholesterol and TG concentration with incident risk of ASCVD (**Table 3**). The mediating proportion

ranged from 7.89% for M-VLDL-C (P_{ACME} =4.8E-02) to 10.77% for L-VLDL-TG (P_{ACME} =3.4E-02). XL-HDL-C associated ASCVD risk reduction was mediated through LiAA (7.20%, P_{ACME} =4.0E-02); M/S-HDL-TG associated ASCVD risk increase was mediated through LiAA (13.42% and 9.34%, P_{ACME} = 1.6E-02 and 2.2E-02). Other statistically significant mediating pathways through LiAA to ASCVD risk included MUFA/TFA (15.20%), histidine (10.22%), and GlycA (12.03%). The mediating effect pattern was generally similar in the sensitivity analysis without adjustment for cell type proportion (**Table S24**) or further adjustment for diabetes and blood pressure (**Table S25**).

Because the association of metabolites with GrimAA, and the association of GrimAA with ASCVD were both stronger in men. We performed mediation analysis in men only and found that GrimAA mediated the effect of various-sized VLDL and IDL particle concentrations and their cholesterol and TG, TG in LDL and small-sized HDL on the risk of incident ASCVD (all P_{ACME} <0.05, **Table S26)** in men. The maximum mediating proportion was 21.68% for ApoB/A1 (P_{ACME} = 3.2E-02). Such mediation effects were not observed in all participants after adjustment for cell type proportion (**Tables S27-S28**). For the other three DNAm aging indicators, no statistically significant mediation effect was observed (**Tables S29-S34**).

Prediction of incident ASCVD based on NMR-measured metabolites and DNAm age acceleration

The AUC increased from 0.688 to 0.707 (P=8.0E-03, Delong's test) by adding LiAA to the reference model; the continuous NRI, categorical NRI and IDI was 24.6% (11.4%, 37.7%), 4.6% (0.2%, 9.0%) and 1.8% (0.9%, 2.6%) correspondingly (**Table 4, Table S35**). The

calibration performance showed modest change before and after the addition of the LiAA (**Figure S9**). The increment of AUC was not statistically significant by adding other DNAm AA indicators to the reference model.

The AUC increased from 0.688 to 0.734 by adding the first 17 PCs of metabolites to the reference model (P=1.8E-04). PCs of the NMR-measured metabolites also improved risk discrimination and reclassification of ASCVD risk (continuous NRI: 44.4% [31.4%, 57.4%], categorical NRI: 8.0% [2.0%, 14.1%], IDI: 5.9% [4.3%, 7.5%]) (**Table 4**). The addition of PCs of metabolites improved the calibration performance of the model (**Figure S10**). We also added the metabolites one by one to the reference model. We observed a statistically significant increment of AUC by adding albumin to the reference model (P=1.1E-02, **Table S36**); but not for other metabolites (all P > 0.05, **Table S36-38**).

Based on the reference model plus 17 PCs of metabolites, further adding DNAm AA did not yield significant categorical NRI (all P > 0.05, **Table S39**), although significant continuous NRI and IDI were observed for Grim, Li, and Horvath AA. In sensitivity analysis, the change of C index for survival data was generally similar by adding DNAm AA or PCs of metabolites (**Table S40**).

Discussion

This study comprehensively investigated the role of alterations in the metabolite profiles (including lipoprotein particles, lipid constituents, and multiple circulating metabolites), and accelerated epigenetic age in the aging process of the Chinese population. Importantly, LiAA, the first-generation methylation age estimator, was strongly associated with cardiovascular aging, specifically time-to-incident ASCVD. In contrast, GrimAA, the second-generation

methylation age estimator, was strongly associated with general aging, such as frailty and total death. Individuals with accelerated GrimAA also had an unfavourable lipidomic and proinflammatory profile. Epigenetic clocks partly mediated (up to 20%) the effects of metabolites on the various aging outcomes.

Interestingly, the present results show that TG and cholesterol carried within lipoproteins of varying sizes and densities had contrasting impacts on epigenetic aging. Findings from previous studies on the association of lipids with epigenetic aging were too inconsistent to draw conclusions. Many associations between lipids and epigenetic aging could subsequently be explained by lipid-related changes in the proportions of white blood cell types.[27] For example, Horvath AA exhibited a positive association with TG, a negative association with HDL-C, and no association with LDL-C in postmenopausal women (N=4173, mostly African, European, and Hispanic ancestry); however, after adjusting for cell type proportions, Horvath AA was only associated with TG with much weaker associations.[15] Similarly, Hannum AA was found to be negatively associated with HDL particles and positively associated with TG in 830 participants of European ancestry. The observed associations were largely attenuated by adjusting for estimated cell type proportion.[16] These findings supported that DNAm age acceleration is driven by lipid-related changes in cell composition and are against the true effect of lipids on DNAm aging.

However, previous studies used conventional lipid measures and thus failed to distinguish complex lipid profiles, which have been proven to be associated with aging differently.[15-17] One study demonstrated significant associations of neutral lipids and phospholipids with DNAm aging after adjusting for cell type proportion, but not

sphingolipids.[17] This previous study indicated that the composition of lipoprotein particles might have contrasting impacts on DNAm aging. Our findings expanded on the previous report by showing that the key determinants of influence on epigenetic aging were the size and constituents of lipoprotein particles. Our findings highlight the importance of accounting for potentially contrasting effects of the size and constituents of lipoprotein particles on biological aging.

Most individuals tend to develop a chronic low-grade proinflammatory state as they age.[12] Despite the intriguing connection between inflammation and epigenetic aging, previous studies yielded inconsistent results. Most of these studies focused on the impact of CPR on epigenetic aging and reported mixed results: null[9,16] or positive associatons[15,28]. The present study measured both high sensitivity CRP and GlycA, a novel NMR-measured biomarker of systemic inflammation. GlycA had a moderate correlation (r=0.61) with the established inflammatory biomarker CRP.[29] Our findings revealed independent impacts of GlycA and CRP on epigenetic aging. GlycA, but not CRP, was found to be influenced by a variety of cardiovascular risk factors, including gut-derived inflammation, physical inactivity, smoking, and low socioeconomic class.[30] CRP, on the other hand, was more strongly influenced by body composition.[30] Taken together, these findings suggested that GlycA and CRP may reflect distinct sources of inflammation.

A recent review demonstrated that epigenetic clocks have varying associations with health outcomes[3], such as total mortality, diabetes, cardiovascular disease, cancer, and Alzheimer’s disease. To the best of our knowledge, this is the first study to use multiple epigenetic clocks and compare their effects on various health outcomes in the same

population. We found that second-generation DNAm age, especially GrimAA, outperformed the first-generation age estimators in predicting general aging outcomes such as frailty and total mortality. First-generation age estimators, on the other hand, performed better in the prediction of incident risk of cardiovascular disease. This disparity could be explained by the fact that the second-generation is trained to estimate general aging-related phenotypes and lifespan (**Table S2**). A previous study showed the statistical inference for the association between PhenoAge and mortality was more than 20 orders of magnitude stronger than with the best first-generation clocks.[7] GrimAA outperforms other biological age estimators in the prediction of all-cause mortality and capturing multisystem dysfunction.[31,32] Our study also noted that GrimAA is associated with ASCVD among men but not among women. A previous study suggested the associations of sociodemographic factors with epigenetic AA vary between women and men.[33] Although the exact reasons are still unclear, these findings highlight the importance of considering sex differences when linking epigenetic aging and clinical outcomes.

Populations of different ethnicities are characterized by different methylation profiles, aging rates, and association effects.[9] Thus, ethnic difference is of great importance in limiting the generalization of the association results across populations/studies.[34] In our study, LiAA (which was established among Chinese populations[6]) exhibited a more robust association with ASCVD incidence. These findings further underscore the importance of developing epigenetic age estimators among multi-ethnic populations.

It has been suggested that metabolic dysfunction accelerates aging partly through epigenetic modification of aging genes. For example, higher level of total cholesterol to total

lipids ratio in IDL particles was found to be associated with the methylation level of cg24881558 mapped to *TERT*,[14] a gene consistently been involved in the aging process.[3,35] For another example, the methylation level of *CHD1L* (a gene related to DNA replication and repair) has been linked to age in previous studies.[5,36,37] Lactate was shown to result in hypermethylated status of cg16525692 mapped to *CHD1L*.[14] Our mediation analysis used a composite epigenetic aging biomarker instead of methylation level of a single gene and revealed several metabolism-epigenetic axes that may contribute to general and cardiovascular aging. These findings are interesting and may help to understand the underlying pathway from unfavourable metabolic profile to faster biological aging.

Longitudinal EWAS revealed that several differentially methylated CpG positions were associated with incident risk of cardiovascular disease.[23,38,39] However, the potential of DNA methylation in predicting incident ASCVD has yet to be fully assessed. A previous study constructed methylation risk score of cardiovascular disease and found the C index increased from 0.691 to 0.695[40] by adding methylation risk score to the model of traditional risk factors. Our study found that the addition of LiAA to the traditional risk factors resulted in a modest yet significant increment of AUC (0.688 to 0.707), indicating the potential predicting role of epigenetic AA in the future risk of cardiovascular disease. However, based on the models that included PCs which explained 95% of the variance in the 225 metabolic markers, LiAA showed modest improvement in the discrimination and reclassification of the ASCVD risk.

We first provided a detailed overview of the differential effects of lipoproteins across a wide range of lipid classes and species, as well as other metabolites, on five widely used epigenetic aging markers. Furthermore, we quantitatively estimated how much of the effects

of unfavourable metabolites on the increased risk of ASCVD and frailty are mediated through DNAm age acceleration. These findings could be leveraged to offer new insight into the pathway behind lipoprotein particles with different sizes and constituents to biological aging. Our findings also highlighted the different mechanisms underlying the five widely used DNAm age estimators. The longitudinal follow-up data allowed us to link the metabolism-epigenetic aging axis to incident risk of ASCVD and the change in frailty. Adjusting for possible risk factors, such as cell compositions, precluded the possibility that DNAm age acceleration is driven by confounders.

Our study has limitations. First, both DNA methylation and NMR-metabolites were measured at baseline, preventing formal evaluation of effect directionality. However, previous longitudinal study suggested that advanced epigenetic age might be a prognostic marker of pathological metabolic processes.[28] Mendelian randomization studies also showed that lipid levels can alter DNAm.[13] It has also been proposed that most causal changes in methylation are a consequence of dyslipidemia rather than a cause of it.[21] The mediation effect was estimated under the framework of counterfactual definitions of direct and indirect effects. With adjustment for possible confounders, the estimated mediating proportions were less likely to be influenced by the uncertainty of the effect directionality of metabolites and DNAm. Future studies using DNAm and metabolites data collected at multiple time points are expected to provide insights into the temporal dynamics of the relationship between lipids and biological aging. Furthermore, collinearity within the metabolomics data exists. Multiple lipid measures may represent subsets of strongly correlated lipid fractions. As a result, the mediating role of a single metabolite in the present study cannot be extrapolated to the

complete metabolomic profile, nor can the individual mediating role of each metabolite be differentiated. Nonetheless, this does not detract from the value of the study that we observe clear differences in the pattern of associations between different-sized lipoproteins and their compositions with DNAm aging. Lastly, future investigations are warranted to replicate our findings in other populations.

This prospective cohort of the Chinese population shows that the key determinants of lipid profile influence on epigenetic aging were the size and constituents of lipoprotein particles. Our findings offer new insight into the different mechanisms that underlie the first and second-generation DNA methylation age in the general and cardiovascular aging process. Our study also adds to the evidence regarding the potential of using multi-omics biomarkers in the prediction of cardiovascular disease.

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Supplementary Material

Supplemental Text
Supplemental Figure 1-10
Supplemental Table 1-40

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1.

Table 1. Baseline characteristics of 980 participants according to GrimAA.

Baseline characteristics	GrimAA				P value
	Q1	Q2	Q3	Q4	
Age, year	50.3	49.5	51.0	51.3	1.3E-01
Female, %	**85.3**	**63.2**	**21.2**	**3.8**	**2.6E-52**
Urban area, %	28.9	18.8	15.8	20.7	1.8E-01
Middle school and above, %	43.9	46.6	40.3	47.2	7.4E-01
Married, %	92.0	90.4	92.5	93.2	6.7E-01
Family history of heart attack, %	4.3	5.4	7.3	6.5	5.2E-01
Fasting time, h	3.8	4.1	3.9	4.2	7.2E-01
Lifestyle factors					
Current daily tobacco smoker*, %	**15.7**	**28.2**	**45.0**	**62.8**	**2.2E-25**
Current daily alcohol drinker, %	**4.7**	**5.8**	**8.1**	**13.2**	**1.3E-04**
Physical activity, MET-h/day	24.2	23.3	22.5	21.8	6.2E-02
Dietary habits					
Days consumed fresh vegetables/week	6.7	6.8	6.7	6.7	8.9E-01
Days consumed fresh fruits/week	**2.3**	**1.8**	**2.0**	**1.7**	**6.0E-03**
Days consumed red meat/week	2.9	2.9	2.8	3.0	9.8E-01
Adiposity					
Body mass index, kg/m^2	23.7	23.6	23.6	23.5	4.8E-01
Waist circumference, cm	80.8	80.7	81.5	80.9	9.1E-01
Waist-to-hip ratio	0.88	0.90	0.90	0.90	7.0E-02
Fat percentage, %	26.1	26.6	26.4	26.6	9.2E-01
Prevalent hypertension, %	36.9	39.6	44.4	44.8	1.4E-01
Prevalent diabetes, %	**3.1**	**8.6**	**8.9**	**10.8**	**2.0E-03**

The results are presented as means or percentages with adjustments for age, sex, and study area. AA=age acceleration; MET = metabolic equivalent of task. The bold text represents statistically different distribution (P value < 0.05) across quartiles of DNA methylation age acceleration (logistic regression for categorical variables, ANOVA for continuous variables).

* To avoid misleadingly elevated risk, former smokers who stopped smoking for illness were categorized as the current smoker.

Figure 1. Associations of cholesterol and triglyceride DNA methylation age acceleration.

Estimates are the increase in DNAm AA associated with 1-SD higher metabolic measures calculated by linear regression models. Results with raw P value < 0.05 are presented in text. *Statistically significant results (false discovery rate–adjusted P value < 0.05, number of testing=225). Red blocks indicate positive associations between metabolites and DNAm AA; blue blocks indicated negative associations. AA = age acceleration; HDL = high-density lipoprotein; IDL = intermediate-density lipoprotein; LDL = low-density lipoprotein; VLDL =very low-density lipoprotein. Multivariable model was adjusted for chronological age, sex, study area, education level, marital status, fasting time, batch, lifestyle factors (smoking status, alcohol drinking, physical activity, average days consuming fresh vegetables, fruits, and red meat per week, and body mass index), and the estimated cell type proportion (CD4+ T cell, CD8+ T cell, B cell, natural killer, monocytes, and granulocyte).

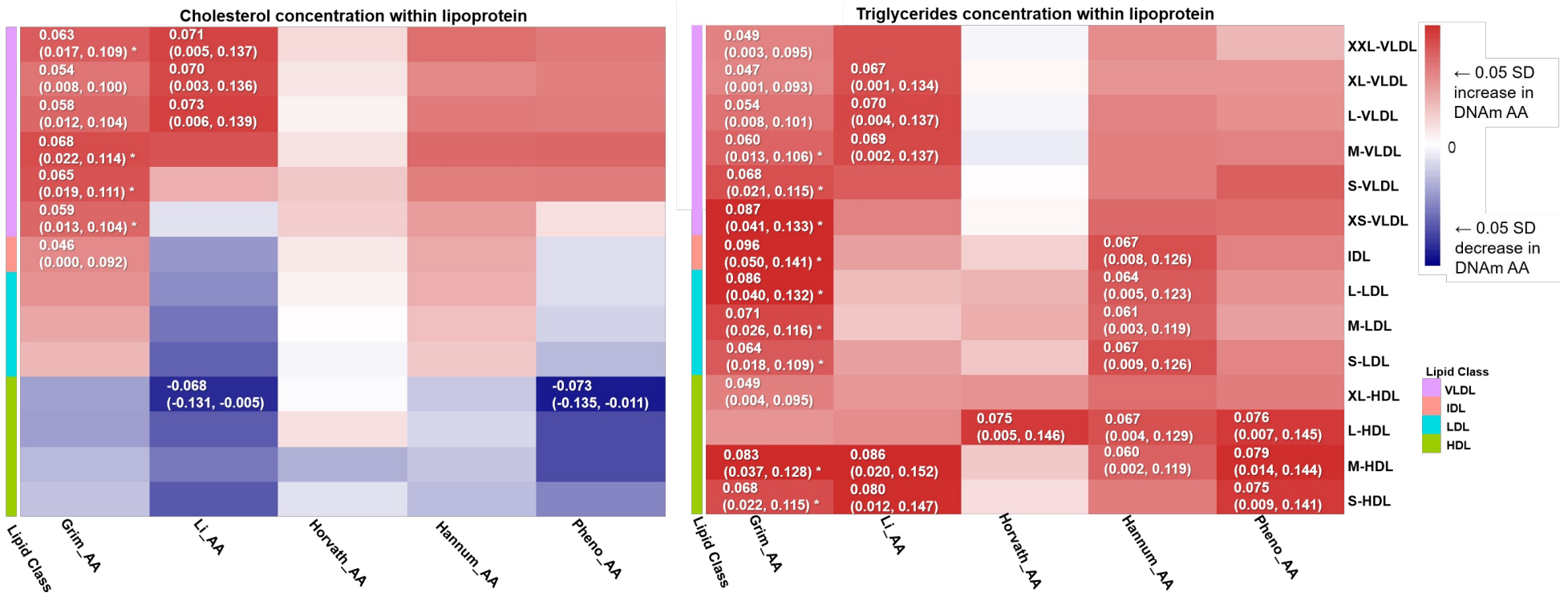
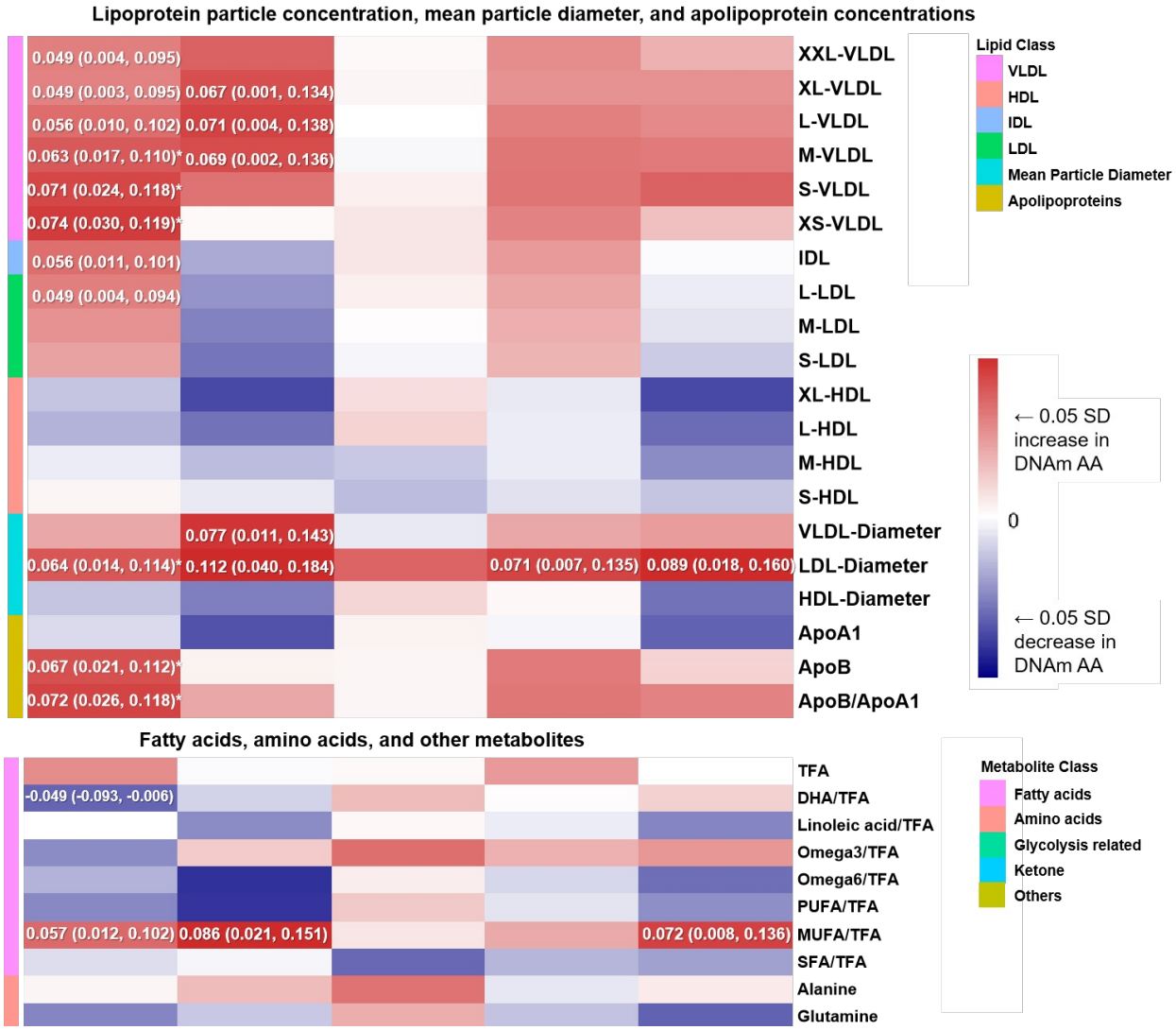

Cholesterol concentration within lipoprotein
0.063 (0.017, 0.109) *
0.071 (0.005, 0.137)
0.054 (0.008, 0.100)
0.070 (0.003, 0.136)
0.058 (0.012, 0.104)
0.073 (0.006, 0.139)
0.068 (0.022, 0.114) *
0.065 (0.019, 0.111) *
0.059 (0.013, 0.104) *
0.046 (0.000, 0.092)
-0.068 (-0.131, -0.005)
-0.073 (-0.135, -0.011)
Lipid Class
Grim_AA
Li_AA
Horvath_AA
Hannum_AA
Pheno_AA
Triglycerides concentration within lipoprotein
0.049 (0.003, 0.095)
0.047 (0.001, 0.093)
0.067 (0.001, 0.134)
0.054 (0.008, 0.101)
0.070 (0.004, 0.137)
0.060 (0.013, 0.106) *
0.069 (0.002, 0.137)
0.068 (0.021, 0.115) *
0.087 (0.041, 0.133) *
0.096 (0.050, 0.141) *
0.067 (0.008, 0.126)
0.086 (0.040, 0.132) *
0.064 (0.005, 0.123)
0.071 (0.026, 0.116) *
0.061 (0.003, 0.119)
0.064 (0.018, 0.109) *
0.067 (0.009, 0.126)
0.049 (0.004, 0.095)
0.075 (0.005, 0.146)
0.067 (0.004, 0.129)
0.076 (0.007, 0.145)
0.083 (0.037, 0.128) *
0.086 (0.020, 0.152)
0.060 (0.002, 0.119)
0.079 (0.014, 0.144)
0.068 (0.022, 0.115) *
0.080 (0.012, 0.147)
0.075 (0.009, 0.141)
XXL-VLDL
XL-VLDL
L-VLDL
M-VLDL
S-VLDL
XS-VLDL
IDL
L-LDL
M-LDL
S-LDL
XL-HDL
L-HDL
M-HDL
S-HDL
← 0.05 SD increase in DNAm AA
0
← 0.05 SD decrease in DNAm AA
Lipid Class
VLDL
IDL
LDL
HDL

Figure 2. Associations of size-specific lipoprotein particle concentrations, mean lipoprotein particle diameter, apolipoprotein concentrations, and other metabolites with DNA methylation age acceleration.

Estimates are the increase in DNAm AA associated with 1-SD higher metabolic measures calculated by linear regression models. Results with raw P value < 0.05 are presented in text. *Statistically significant results (false discovery rate–adjusted P value < 0.05, number of testing=225). Red blocks indicate positive association between metabolites and DNAm AA; blue blocks indicated negative association. AA = age acceleration; ApoA1 = apolipoprotein A1; ApoB = apolipoprotein B; DHA = Docosahexaenoic acid; HDL = high-density lipoprotein; IDL = intermediate-density lipoprotein; LDL = low-density lipoprotein; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; SFA = saturated fatty acid TFA = total fatty acid; VLDL =very low-density lipoprotein. Model adjustment as in Figure 1.


Lipoprotein particle concentration, mean particle diameter, and apolipoprotein concentrations
0.049 (0.004, 0.095)
0.049 (0.003, 0.095) 0.067 (0.001, 0.134)
0.056 (0.010, 0.102) 0.071 (0.004, 0.138)
0.063 (0.017, 0.110)* 0.069 (0.002, 0.136)
0.071 (0.024, 0.118)*
0.074 (0.030, 0.119)*
0.056 (0.011, 0.101)
0.049 (0.004, 0.094)
0.077 (0.011, 0.143)
0.064 (0.014, 0.114)* 0.112 (0.040, 0.184) 0.071 (0.007, 0.135) 0.089 (0.018, 0.160)
0.067 (0.021, 0.112)*
0.072 (0.026, 0.118)*
XXL-VLDL
XL-VLDL
L-VLDL
M-VLDL
S-VLDL
XS-VLDL
IDL
L-LDL
M-LDL
S-LDL
XL-HDL
L-HDL
M-HDL
S-HDL
VLDL-Diameter
LDL-Diameter
HDL-Diameter
ApoA1
ApoB
ApoB/ApoA1
Lipid Class
VLDL
HDL
IDL
LDL
Mean Particle Diameter
Apolipoproteins
← 0.05 SD increase in DNAm AA
0
← 0.05 SD decrease in DNAm AA
Fatty acids, amino acids, and other metabolites
-0.049 (-0.093, -0.006)
0.057 (0.012, 0.102) 0.086 (0.021, 0.151) 0.072 (0.008, 0.136)
TFA
DHA/TFA
Linoleic acid/TFA
Omega3/TFA
Omega6/TFA
PUFA/TFA
MUFA/TFA
SFA/TFA
Alanine
Glutamine
Metabolite Class
Fatty acids
Amino acids
Glycolysis related
Ketone
Others

measured metabolites to frailty index through GrimAA.

	Proportion mediated, %	P_{ACME}		Proportion mediated, %	P_{ACME}		Proportion mediated, %	P_{ACME}
VLDL diameter	0.05	1.1E-01	S-VLDL			LDL diameter	**15.58**	**1.6E-02**
XXL-VLDL			Particle	**18.74**	**4.0E-03**	HDL		
Particle	**14.39**	**2.4E-02**	Cholesterol	**17.51**	**6.0E-03**	XL-HDL cholesterol	-8.46	1.8E-01
Cholesterol	**19.11**	**6.0E-03**	Triglycerides	**19.74**	**6.0E-03**	XL-HDL triglycerides	**9.02**	**3.0E-02**
Triglycerides	**13.75**	**2.8E-02**	XS-VLDL			L-HDL triglycerides	9.15	8.4E-02
XL-VLDL			Particle	**20.82**	**4.0E-03**	M-HDL triglycerides	**20.88**	**4.0E-03**
Particle	**13.52**	**3.0E-02**	Cholesterol	**16.46**	**1.2E-02**	S-HDL triglycerides	**19.91**	**6.0E-03**
Cholesterol	**15.08**	**1.6E-02**	Triglycerides	**21.98**	**6.0E-03**	Apolipoprotein		
Triglycerides	**12.84**	**3.4E-02**	IDL			ApoB	**20.00**	**6.0E-03**
L-VLDL			Particle	**15.70**	**1.4E-02**	ApoB/A1	**20.37**	**4.0E-03**
Particle	**15.33**	**1.2E-02**	Cholesterol	**11.21**	**5.0E-02**	DHA/TFA	-13.77	3.0E-02
Cholesterol	**15.99**	**1.2E-02**	Triglycerides	**14.01**	**8.0E-03**	MUFA/TFA	**16.89**	**1.6E-02**
Triglycerides	**15.07**	**1.8E-02**	LDL			Histidine	11.59	7.8E-02
M-VLDL			L-LDL particle	**12.82**	**3.8E-02**	Glucose	**2.92**	**3.8E-02**
Particle	**17.22**	**6.0E-03**	L-LDL TG	**12.74**	**8.0E-03**	Lactate	**8.48**	**3.6E-02**
Cholesterol	**19.06**	**6.0E-03**	M-LDL TG	**12.30**	**1.0E-02**	Acetate	-10.48	6.2E-02
Triglycerides	**16.21**	**8.0E-03**	S-LDL TG	**13.83**	**1.2E-02**	Glycoprotein acetyls	**15.79**	**8.0E-03**

Metabolites potentially associated with any of the five methylation AA (raw P estimated by linear regression models < 0.05) were analyzed.

Bold text represents statistically significant result (P_{ACME} estimated by causal mediation analyses < 0.05). AA = age acceleration; ACME = average causal mediation effects; ApoA1 = apolipoprotein A1; ApoB = apolipoprotein B; DHA = Docosahexaenoic acid; HDL = high-density lipoprotein; IDL = intermediate-density lipoprotein; LDL = low-density lipoprotein; MUFA = monounsaturated fatty acid; TFA = total fatty acid; VLDL =very low-density lipoprotein.

Covariates included chronological age, sex, study area, education level, marital status, fasting time, batch, lifestyle factors (smoking status, alcohol drinking, physical

activity, average days consuming fresh vegetables, fruits, and red meat per week, and body mass index), and the estimated cell type proportion (CD4+ T cell, CD8+ T cell, B cell, natural killer, monocytes, and granulocyte).

Table 3. The mediating effect from NMR-measured metabolites to the risk of incident ASCVD through LiAA.

	Proportion mediated, %	P_{ACME}		Proportion mediated, %	P_{ACME}		Proportion mediated, %	P_{ACME}
VLDL diameter	**13.11**	**2.4E-02**	S-VLDL			LDL diameter	**15.57**	**1.2E-02**
XXL-VLDL			Particle	7.06	9.0E-02	HDL		
Particle	9.52	5.6E-02	Cholesterol	4.07	2.8E-01	XL-HDL cholesterol	**7.20**	**4.0E-02**
Cholesterol	**8.01**	**3.0E-02**	Triglycerides	8.42	5.6E-02	XL-HDL triglycerides	5.03	2.1E-01
Triglycerides	**10.63**	**4.4E-02**	XS-VLDL			L-HDL triglycerides	6.42	2.2E-01
XL-VLDL			Particle	0.83	8.0E-01	M-HDL triglycerides	**13.42**	**1.6E-02**
Particle	**10.25**	**4.0E-02**	Cholesterol	-0.51	8.6E-01	S-HDL triglycerides	**9.34**	**2.2E-02**
Cholesterol	**8.89**	**3.4E-02**	Triglycerides	6.24	1.2E-01	Apolipoprotein		
Triglycerides	**10.34**	**4.0E-02**	IDL			ApoB	1.14	7.1E-01
L-VLDL			Particle	-2.28	5.3E-01	ApoB/A1	3.05	2.2E-01
Particle	**10.49**	**3.0E-02**	Cholesterol	-3.39	4.0E-01	DHA/TFA	2.52	4.4E-01
Cholesterol	**9.93**	**2.8E-02**	Triglycerides	4.03	2.1E-01	MUFA/TFA	**15.20**	**2.0E-02**
Triglycerides	**10.77**	**3.4E-02**	LDL			Histidine	**10.22**	**2.4E-02**
M-VLDL			L-LDL particle	-3.15	4.1E-01	Glucose	6.23	2.4E-01
Particle	**9.44**	**3.6E-02**	L-LDL TG	2.94	3.5E-01	Lactate	5.33	1.4E-01
Cholesterol	**7.89**	**4.8E-02**	M-LDL TG	2.90	4.3E-01	Acetate	-5.21	2.9E-01
Triglycerides	**9.90**	**3.6E-02**	S-LDL TG	4.87	2.2E-01	Glycoprotein acetyls	**12.03**	**1.6E-02**

Metabolites potentially associated with any of the five methylation AA (ra

Bold text represents statistically significant result (P_{ACME} estimated by causal mediation analyses < 0.05). Covariates and abbreviation same as Table 2.

Table 4. Improvement in risk prediction for incident ASCVD by NMR-measured metabolites and DNA methylation age acceleration (AA).

	AUC	*P* value	cNRI (95% CI)	*P* value	IDI (95% CI)	*P* value
Ref Model: Age, sex, study area, smoking, alcohol consumption, body mass index, prevalent diabetes, SBP, DBP, TC, HDL-C, TG						
			AUC = 0.688			
Ref + DNA methylation AA*						
Grim AA	0.693	3.8E-01	**0.173 (0.040, 0.306)**	**1.1E-02**	**0.008 (0.002, 0.014)**	**1.1E-02**
Li AA	**0.707**	**8.0E-03**	**0.246 (0.114, 0.377)**	**2.5E-04**	**0.018 (0.009, 0.026)**	**4.0E-05**
Horvath AA	0.690	5.0E-01	**0.199 (0.066, 0.331)**	**3.0E-03**	0.003 (-0.001, 0.007)	1.1E-01
Hannum AA	0.688	5.6E-01	-0.034(-0.168, 0.099)	6.1E-01	0.000 (-0.001, 0.001)	6.1E-01
Pheno AA	0.688	1.0E+00	0.075 (-0.058, 0.208)	2.7E-01	0.001 (-0.001, 0.002)	6.2E-01
Ref + metabolites (first 17 principle components explaining > 95% variance of 225 metabolic markers) *						
	0.734	**1.8E-04**	**0.444 (0.314, 0.574)**	**1.2E-06**	**0.059 (0.043, 0.075)**	**5.5E-07**
[Ref + metabolites] + DNA methylation AA†						
Grim AA	0.737	3.5E-01	**0.158 (0.025, 0.291)**	**2.0E-02**	**0.006 (0.001, 0.011)**	**2.9E-02**
Li AA	0.743	7.3E-02	**0.185 (0.052, 0.317)**	**6.0E-03**	**0.011 (0.004, 0.018)**	**1.0E-03**
Horvath AA	0.739	1.1E-01	**0.232 (0.099, 0.364)**	**1.0E-03**	**0.004 (0.000, 0.009)**	**3.7E-02**
Hannum AA	0.734	6.8E-01	-0.044 (-0.018, 0.089)	5.1E-01	0.000 (-0.001, 0.001)	9.0E-01
Pheno AA	0.734	8.9E-01	-0.006 (-0.139, 0.127)	9.3E-01	0.000 (-0.001, 0.001)	9.3E-01

* Models were compared to the reference model.

† Models were compared to the reference model plus 17 principle components of 225 metabolites. AUC = area under ROC curve; CI = confide integrated discrimination improvement; cNRI = continuous net reclassification improvement. AUC, IDI, and cNRI were calculated for logistic regression models. C-index for Cox regression models were provided in Table S40. P values were estimated by Delong's test.

Novelty and Significance

What is known?

- Changes in lipid metabolism and DNA methylation characterize aging.
- Lipid metabolism influences DNA methylation levels at age-related genes, suggesting that lipid metabolites may affect the risk of age-related diseases through epigenetic modifications.
- Epigenetic clocks are valuable tools for estimating biological age, with some studies linking accelerated epigenetic age to age-related conditions, though the specific mechanisms they capture are still unclear.

What new information does this article contribute?

- Size and composition of lipoprotein particles were key determinants in how lipid profiles influence epigenetic aging.
- Epigenetic clocks mediate the association of circulating metabolites with cardiovascular or general aging. Different generations of epigenetic clocks mediate these associations differently, with the first generation (Li clock) being particularly relevant for cardiovascular disease risk, and the second generation (Grim clock) being more closely tied to frailty.
- Li clock and circulating metabolites have the potential to predict the future risk of cardiovascular disease beyond traditional risk factors.
- This research deepens our understanding of how lipid metabolism and epigenetic changes contribute to the aging process, offering valuable insights into the biological mechanisms of aging and potential targets for intervention.

With the global population rapidly aging, understanding the complex mechanisms of aging is essential for developing effective interventions. Changes in lipid metabolism and DNA methylation are key features of aging. Epigenetic clocks are useful for estimating biological age and have been linked to age-related conditions, but their precise mechanisms are not fully understood. This study aimed to investigate how different generations of epigenetic clocks capture various aspects of aging, focusing

on cardiovascular and general aging; and how they reflect the impact of circulating metabolites on these aging processes. We found the size and composition of lipoprotein particles were key determinants in how lipid profiles influence epigenetic aging. Our results highlight that epigenomic mechanisms may mediate the relationship between circulating metabolites and the aging process, with distinct mechanisms underlying the effects of first and second-generation DNA methylation clocks on cardiovascular and general aging. Our results also add to the evidence regarding the potential of using multi-omics biomarkers in the prediction of cardiovascular disease.