Lifestyle factors and genetic variants on two biological age measures: evidence from 94,443 Taiwan Biobank participants

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Abstract

Background:

Biological age (BA) can be estimated by phenotypes and is useful for predicting lifespan and healthspan. Levine *et al.* proposed a PhenoAge and a BioAge to measure BA. Although there have been studies investigating the genetic predisposition to BA acceleration in Europeans, little has been known regarding this topic in Asians.

Methods:

I here estimated PhenoAgeAccel (age-adjusted PhenoAge) and BioAgeAccel (age-adjusted BioAge) of 94,443 Taiwan Biobank (TWB) participants, wherein 25,460 TWB1 subjects formed a discovery cohort and 68,983 TWB2 individuals constructed a replication cohort. Lifestyle factors and genetic variants associated with PhenoAgeAccel and BioAgeAccel were investigated through regression analysis and a genome-wide association study (GWAS).

Results:

A unit (kg/m^2) increase of BMI was associated with a 0.177-year PhenoAgeAccel (95% C.I. = 0.163~0.191, $p = 6.0 \times 10^{-129}$) and 0.171-year BioAgeAccel (95% C.I. = 0.165~0.177, $p = 0$). Smokers on average had a 1.134-year PhenoAgeAccel (95% C.I. = 0.966~1.303, $p = 1.3 \times 10^{-39}$) compared with non-smokers. Drinkers on average had a 0.640-year PhenoAgeAccel (95% C.I. = 0.433~0.847, $p = 1.3 \times 10^{-9}$) and 0.193-year BioAgeAccel (95% C.I. = 0.107~0.279, $p = 1.1 \times 10^{-5}$) relative to non-drinkers. A total of 11 and 4 single-nucleotide polymorphisms (SNPs) were associated with PhenoAgeAccel and BioAgeAccel ($p < 5 \times 10^{-8}$ in both TWB1 and TWB2), respectively.

Conclusions:

A PhenoAgeAccel-associated SNP (rs1260326 in *GCKR*) and two BioAgeAccel-associated

SNPs (rs7412 in *APOE*; rs16998073 near *FGF5*) were consistent with the finding from the UK Biobank. The lifestyle analysis shows that prevention from obesity, cigarette smoking, and alcohol consumption is associated with a slower rate of biological aging.

Keywords: Biomarkers, longevity, genetics.

Introduction

People's biological age (BA) may be different from their chronological age. Biologically young subjects generally have healthier conditions and longer lifespan than biologically old people (1). BA is associated with life expectancy. Slowing the pace of biological aging and extending life expectancy will be an important public health issue (2).

There are various ways to estimate one's BA (2-6). Till today, no single method has been universally accepted as a golden measure for aging process (3). Formulas of BA usually integrate multiple important biomarkers such as estimated glomerular filtration rate (4), creatinine (5, 6), glycosylated hemoglobin (2), diastolic blood pressure (4), systolic blood pressure (SBP) (2, 6), total cholesterol (2, 6), C-reactive protein (2, 5, 6), albumin (2, 5, 6), etc. With the advancement of epigenetics, DNA methylation (DNAm) age, a weighted average of levels at multiple cytosine-phosphate-guanine (CpG) sites, has been proposed as an epigenetic measure of BA (5, 7-9).

Recently, Levine *et al.* analyzed data from the third National Health and Nutrition Examination Survey (NHANES III) with a Cox regularized regression model (5). The hazard of aging-related mortality was regressed on 43 markers, including chronological age and 42 clinical markers. They developed a model for "phenotypic age" (the so-called "PhenoAge") that was a linear combination of chronological age and 9 biomarkers. A total of 4 out of the 9 clinical

biomarkers were related to immunity: lymphocyte percent, **mean (red) cell volume**, red cell distribution width, and **white blood cell count**. Two were associated with liver functions: **albumin** and alkaline phosphatase. Besides, indices reflecting kidney functions (**creatinine**), metabolic condition (**serum fasting glucose**), and inflammation (C-reactive protein) were also included as the predictors of one's PhenoAge. Compared with chronological age, PhenoAge can better reflect one's physiological condition (5). The 5 biomarkers highlighted in bold font type were also measured by the Taiwan Biobank (TWB), whereas the remaining 4 were not.

Another BA measure providing one of the most accurate mortality predictors is Levine's biological age (the so-called "BioAge") (6), which was also trained by the NHANES III data. BioAge was derived by chronological age and 7 biomarkers including **albumin**, alkaline phosphatase, **creatinine**, C-reactive protein, **glycated hemoglobin (HbA1c)**, **SBP**, and **total cholesterol**. The former 4 biomarkers were also components of PhenoAge, whereas the latter 3 were not. The 5 biomarkers highlighted in bold font type were also measured by the TWB, whereas the remaining 2 were not.

A study has shown that PhenoAge captures morbidity and mortality risk across diverse subpopulations of the NHANES IV data. BioAge and PhenoAge were largely comparable, although PhenoAge performed better in healthy people (e.g., those disease-free and with normal body mass index [BMI]) (10).

Recently, Kuo *et al.* performed a genome-wide association study (GWAS) in European-descent individuals from the UK Biobank (UKB), to identify genetic variants that were associated with age-adjusted PhenoAge (PhenoAgeAccel) and age-adjusted BioAge (BioAgeAccel) (11). They found that the strongest signal was observed at the *APOE* gene. However, no lifestyle factors such as cigarette smoking have been discussed in that GWAS (11). Moreover, the investigated ethnicity has been limited to the European descent.

To explore the association of lifestyle factors and genetic variants with biological aging, I first estimated PhenoAge and BioAge of TWB participants according to Levine *et al.*'s formulas (5, 6). Because PhenoAge and BioAge could better reflect physiological conditions (5, 6, 10), I used them to gauge one's BA. I then searched for lifestyle factors and genetic variants that were associated with BA acceleration.

Method

Taiwan Biobank

Since October 2012, TWB has recruited Taiwan residents aged 30 to 70 years and collected their genomic and lifestyle information (12). After signing informed consent, community-based volunteers took physical examinations, and provided their blood and urine samples. TWB researchers further collected lifestyle factors through a face-to-face interview with each participant. TWB was approved by the Institutional Review Board on Biomedical Science Research/IRB-BM, Academia Sinica, and also by the Ethics and Governance Council of Taiwan Biobank, Taiwan. This study received approval from the Research Ethics Committee of National Taiwan University Hospital (NTUH-REC no. 201805050RINB).

Till October 2020, a total of 27,737 and 79,775 subjects have been whole-genome genotyped by the TWB1 and TWB2 genotyping arrays, respectively. PLINK 1.9 (13) was used to assess cryptic relatedness, i.e., PI-HAT = Probability(IBD = 2) + 0.5 × Probability(IBD = 1), where IBD is the genome-wide identity by descent (IBD) sharing coefficients between any two TWB subjects. I removed one individual from each pair with PI-HAT $\geq$ 0.2, which is a cutoff value commonly adopted by many studies (14, 15). After this step, TWB1 and TWB2 cohorts contained 25,460 and 68,983 subjects, respectively.

A majority of TWB subjects were of Han Chinese ancestry (12). The TWB1 genotyping array was run on the Axiom Genome-Wide Array Plate System (Affymetrix, Santa Clara, CA, USA). It was designed for Taiwan's Han Chinese and was released in April 2013. According to next-generation sequencing of ~1,000 TWB individuals and experience of developing TWB1, the TWB2 genotyping array was later released in August 2018.

TWB1 and TWB2 arrays contain 632,172 and 648,611 autosomal single-nucleotide polymorphisms (SNPs), respectively. For TWB1, 27,628 SNPs with genotyping rates < 95% and 6,900 SNPs with Hardy-Weinberg test *P*-values < 5.7×10^{-7} (16) were removed. Regarding TWB2, 26,920 SNPs with genotyping rates < 95% and 15,774 SNPs with Hardy-Weinberg test *P*-values < 5.7×10^{-7} (16) were excluded. Through this quality control process, 597,644 TWB1 SNPs and 605,917 TWB2 SNPs remained in my analysis. I then constructed ancestry principal components (PCs) with these SNPs. A total of 92,870 SNPs were overlapped across TWB1 and TWB2 arrays.

Genotype imputation was performed on the Michigan Imputation Server (https://imputationserver.sph.umich.edu/index.html). The East Asian (EAS) population from the 1000 Genomes Phase 3 v5 was served as the reference panel. I excluded SNPs with low imputation information score (Rsq < 0.8) or with Hardy-Weinberg test *P*-values < 5.7×10^{-7} (16). TWB1 and TWB2 finally included 7,433,014 and 6,521,115 SNPs, respectively.

Estimation for PhenoAge

There are several formulas to estimate BA, yet no consensus has been reached on the measure of one's BA (3). Recently, Ahadi *et al.* indicated that BA is associated with indices in 4 domains: immunity, metabolic, liver dysregulation, and kidney dysregulation (17). In line with this viewpoint, Levine *et al.* (5) developed a model for PhenoAge with indices in these domains.

Therefore, in this study, I estimated BA according to Levine *et al.*'s PhenoAge (5).

Levine *et al.*'s (5) PhenoAge was developed by analyzing the NHANES III data. By regressing the hazard of aging-related mortality on 42 markers and chronological age, Levine *et al.* (5) finally selected 10 markers (including chronological age) to predict PhenoAge. Among these 10 markers, 4 were not measured by TWB. Therefore, in the first step, I analyzed the NHANES III data and developed the relationship between "6markerPhenoAge" (calculated according to 6 markers) and PhenoAge (calculated according to 10 markers).

A total of 20,050 subjects aged from 17 to 90 were recruited by NHANES III, wherein 18,162 were provided with biomarker data. To appropriately apply NHANES III results to TWB, I only analyzed 10,389 NHANES III subjects aged from 30 to 70, corresponding to the age range of TWB individuals. With 10 markers including albumin, creatinine, fasting (serum) glucose, C-reactive protein, lymphocyte percent, mean red cell volume, red cell distribution width, alkaline phosphatase, white blood cell count, and chronological age, Levine *et al.* (5) built a model for PhenoAge, as follows,

$$\text{PhenoAge} = 141.50225 + \frac{log[-0.00553 \times log(1-MortalityScore)]}{0.090165}. \quad (1)$$

$$MortalityScore = c.d.f.(120, \boldsymbol{z}) = 1 - exp\{-exp(\hat{\beta}_0 + \sum_{k=1}^{K} \hat{\beta}_k z_k)[(exp(120\gamma) - 1)/\gamma]\}, \quad (2)$$

where z_k is the k^{th} marker (k = 1, …, 10). $\hat{\beta}_0 = -19.9067$, $\hat{\beta}_k$s (k = 1, …, 10), and $\gamma = 0.0076927$ were provided by Levine *et al.* (5). Model (2) indicated that the cumulative distribution function (*c.d.f.*) of the Gompertz distribution was used to estimate the mortality risk within 120 months of each individual. Among the 10,389 NHANES III subjects, 9,598 had complete records in all the 10 markers. By using K = 10 in model (2), I calculated PhenoAge of these 9,598 NHANES III subjects.

Among the 10 markers, 6 were also measured by TWB, including albumin, creatinine, fasting (serum) glucose, mean red cell volume, white blood cell count, and chronological age. Therefore, I also calculated "6markerPhenoAge" of these 9,598 NHANES III subjects, by using $K = 6$ in model (2). The scatter plots of "6markerPhenoAge", PhenoAge, and chronological age were shown in Figure S1 of Supplementary Material. The Pearson's correlations between "6markerPhenoAge" and PhenoAge were 0.960 in 4,483 NHANES III males and 0.936 in 5,115 NHANES III females. These high linear correlation coefficients suggested the appropriateness of building a simple linear regression. By regressing PhenoAge on 6markerPhenoAge within each sex, I obtained

$$\text{PhenoAge} = 45.846752 + 1.068403 \times \text{6markerPhenoAge} \quad (3)$$

and

$$\text{PhenoAge} = 46.527973 + 1.023272 \times \text{6markerPhenoAge} \quad (4)$$

for males and females, respectively.

The R-squares of models (3) and (4) were 92.16% (= 0.960^2) and 87.61% (= 0.936^2), respectively. This indicated that 6markerPhenoAge accounted for 92.16% and 87.61% variability of PhenoAge for males and females, respectively. Because these R-squares were large, in the following I estimated PhenoAge of TWB subjects according to their 6markerPhenoAge.

Estimation for BioAge

Another validated BA predictor is BioAge (6), which currently provides one of the most accurate mortality predictors (10). According to an algorithm proposed by Klemera and Doubal (18), BioAge was estimated by 7 biomarkers and chronological age, as follows (11),

$$BioAge = \frac{\sum_{j=1}^{J}(x_j - q_j)\left(\frac{k_j}{s_j^2}\right) + \frac{age}{31.63}}{\sum_{j=1}^{J}\left(\frac{k_j}{s_j}\right)^2 + \frac{1}{31.63}}, \quad (5)$$

where x_j is the j^{th} biomarker, the corresponding q_j, k_j, and s_j were listed in Table S1 (j = 1,…, 7), and age represents chronological age. By using J = 7 in model (5), I calculated BioAge of the 9,598 NHANES III subjects. Besides, because alkaline phosphatase and C-reactive protein were not measured by TWB, I deliberately removed these 2 biomarkers and used J = 5 in model (5) to calculate "6markerBioAge" (5 biomarkers and chronological age) of the 9,598 NHANES III subjects.

The scatter plots of "6markerBioAge", BioAge, and chronological age were shown in Figure S2 of Supplementary Material. The Pearson's correlations between "6markerBioAge" and BioAge were 0.999 in 4,483 NHANES III males and 0.998 in 5,115 NHANES III females. These high linear correlation coefficients suggested the appropriateness of building a simple linear regression. By regressing BioAge on 6markerBioAge within each sex, I obtained

$$\text{BioAge} = 0.1741332 + 0.9981360 \times \text{6markerBioAge} \quad (6)$$

and

$$\text{BioAge} = -0.08724734 + 1.00463030 \times \text{6markerBioAge} \quad (7)$$

for males and females, respectively.

The R-squares of models (6) and (7) were 99.80% (= 0.999^2) and 99.60% (= 0.998^2), respectively. This indicated that 6markerBioAge accounted for 99.80% and 99.60% variability of BioAge for males and females, respectively. Because these R-squares were close to 100%, in the following I estimated BioAge of TWB subjects based on their 6markerBioAge.

Biological age acceleration

PhenoAgeAccel (BioAgeAccel) was measured with the residuals of regressing PhenoAge (BioAge) on chronological age, for TWB1 (discovery cohort) and TWB2 (replication cohort), respectively. Instead of using the difference between PhenoAge (BioAge) and chronological age,

I here calculated residuals from regressing PhenoAge (BioAge) on chronological age. In this way, the residuals are robust to various normalization methods and measurement platforms (19). These residuals were used to quantify BA acceleration, with positive values indicating that an individual is biologically older than his/her chronological age (20).

Statistical analysis for lifestyle factors and genetic variants

The 25,460 TWB1 individuals and 68,983 TWB2 subjects were treated as a discovery set and a replication set, respectively. Before considering genetic variants, I regressed PhenoAgeAccel (BioAgeAccel) on 6 factors including sex, BMI (in kg/m^2), educational attainment (a value ranging from 1 to 7), smoking status (yes vs. no), drinking status (yes vs. no), and regular exercise (yes vs. no). The former three are "profile factors" describing the profile of an individual, whereas the latter three are "lifestyle factors" depicting his/her lifestyle.

TWB also surveyed other lifestyle factors such as eating habits. For example, "do you eat more fruits and vegetables", "do you like salty foods", "do you drink coffee or tea", etc. However, only ~40% TWB participants answered these questions and the remaining ~60% participants chose the simplified version of the TWB questionnaire to save their time. Therefore, a large proportion of missing data (~60%) would compromise the statistical power, if other lifestyle factors were also included in the regression analysis. Only the above-mentioned 6 factors were investigated in my analysis because they were surveyed by both the simplified and original versions of the TWB questionnaire.

Smoking was defined as a subject who had smoked for at least 6 months and had not quit smoking at the time his/her phenotypes were examined. Drinking was defined as a subject having a weekly intake of more than 150 cc of alcohol for at least 6 months and having not stopped drinking at the time his/her phenotypes were measured. Regular exercise was defined as engaging

in 30 minutes of "exercise" three times a week. "Exercise" includes leisure-time activities such as swimming, cycling, jogging, etc.

Educational attainment of each subject was surveyed through a face-to-face interview with TWB researchers. It was recorded as a number ranging from 1 to 7, with 1 indicating "illiterate", 2 "no formal education but literate", 3 "primary school graduate", 4 "junior high school graduate", 5 "senior high school graduate", 6 "college graduate", and 7 "Master's or higher degree".

Regarding genetic variants, I first used the GCTA (Genome-wide Complex Trait Analysis) software version 1.93.2beta (21) to estimate the SNP-heritability for PhenoAgeAccel and BioAgeAccel, respectively. SNP-heritability is the proportion of the variance of PhenoAgeAccel (BioAgeAccel) that can be explained by all genome-wide SNPs. Then, using PLINK 1.9 (13), I regressed PhenoAgeAccel (BioAgeAccel) on each of the 7,433,014 TWB1 SNPs, while adjusting the abovementioned 6 factors and the first 10 ancestry principal components (PCs). The commonly used genetic inheritance model, the additive model, was applied to all SNPs. That is, three genotypes at each SNP were coded as 0, 1, and 2, respectively. For SNPs achieving the genome-wide significance level (5×10^{-8}) in TWB1, I further sought replication from TWB2, by regressing PhenoAgeAccel (BioAgeAccel) on each SNP while adjusting for the abovementioned 16 covariates. SNPs achieving the genome-wide significance level (5×10^{-8}) in both TWB1 and TWB2 were claimed to be significant.

To investigate the association of the significant SNPs ($p < 5 \times 10^{-8}$ in both TWB1 and TWB2) with each of the 8 biomarkers (albumin, creatinine, fasting glucose, mean red cell volume, white blood cell count, HbA1c, SBP, and total cholesterol), I regressed every biomarker on each of the significant SNPs, respectively. Chronological age and the abovementioned 16 covariates

were adjusted in the regression models. SNP-biomarker associations with $p < 5 \times 10^{-8}$ in TWB1 and/or TWB2 were summarized as a table, to investigate which biomarker in BA was associated with the SNP.

Association of tissue-specific gene expressions with PhenoAgeAccel (BioAgeAccel)

To identify the association of gene expressions with PhenoAgeAccel (BioAgeAccel), I first predicted tissue-specific gene expressions via SNPs, by using PrediXcan (22) and the Genotype-Tissue Expression (GTEx) library (23). I then regressed PhenoAgeAccel (BioAgeAccel) on the predicted tissue-specific expression of each gene, respectively, while adjusting for the abovementioned 16 covariates. Expression-PhenoAgeAccel (BioAgeAccel) associations with $p < 2 \times 10^{-6} = 0.05/25000$ in both TWB1 and TWB2 were summarized as a table. The significance level of 2×10^{-6} was determined by the Bonferroni correction while considering 25,000 genes across the genome.

[Table 1 is approximately here]

Results

Effects of non-genetic factors

Table 1 presents sex-specific characteristics of TWB1 (*N* = 25,460) and TWB2 participants (*N* = 68,983), respectively. The characteristics of TWB1 and TWB2 individuals were similar, suggesting the appropriateness of using TWB2 as the replication cohort.

[Table 2 is approximately here]

Table 2 presents the results of regressing PhenoAgeAccel (BioAgeAccel) on 6 factors. Based on the TWB1 data, males on average had a 2.189-year PhenoAgeAccel (95% confidence

interval [C.I.] = 2.076~2.302, $p = 8.0 \times 10^{-309}$) and a 0.879-year BioAgeAccel (95% C.I. = 0.832~0.926, $p = 3.2 \times 10^{-289}$) relative to females. A unit (kg/m^2) increase of BMI was associated with a 0.177-year PhenoAgeAccel (95% C.I. = 0.163~0.191, $p = 6.0 \times 10^{-129}$) and a 0.171-year BioAgeAccel (95% C.I. = 0.165~0.177, $p = 0$). Smokers on average had a 1.134-year PhenoAgeAccel (95% C.I. = 0.966~1.303, $p = 1.3 \times 10^{-39}$) compared with non-smokers. Drinkers on average had a 0.640-year PhenoAgeAccel (95% C.I. = 0.433~0.847, $p = 1.3 \times 10^{-9}$) and a 0.193-year BioAgeAccel (95% confidence interval [C.I.] = 0.107~0.279, $p = 1.1 \times 10^{-5}$) relative to non-drinkers. Acquiring a higher educational degree was associated with a 0.213-year PhenoAge deceleration (PhenoAgeDecel) (95% C.I. = 0.159~0.267, $p = 1.4 \times 10^{-14}$). The above results relating to the 5 factors were well replicated by the TWB2 cohort.

Performing regular exercise was associated with a 0.121-year PhenoAgeDecel (95% C.I. = 0.017~0.225, $p = 0.023$). This result of borderline significance in TWB1 was not replicated by TWB2 ($p = 0.327$). This did not represent that exercise is of little importance. The effect of exercise on PhenoAgeDecel or BioAgeDecel was partly explained by BMI. If I remove BMI from the model of Table 2, exercise is associated with a 0.196-year PhenoAgeDecel ($p = 2.7 \times 10^{-4}$) in TWB1 and a 0.102-year PhenoAgeDecel ($p = 1.2 \times 10^{-3}$) in TWB2; a 0.127-year BioAgeDecel ($p = 6.0 \times 10^{-8}$) in TWB1 and a 0.099-year BioAgeDecel ($p = 3.1 \times 10^{-12}$) in TWB2. Therefore, performing regular exercise is still recommended because it is associated with BA deceleration.

Effects of genetic variants

In TWB1, the SNP-heritability was 14.03% (standard error [S.E.] = 1.49%) for PhenoAgeAccel, which was close to that estimated from the UKB (14.45%, S.E. = 0.95%) (11). The SNP-heritability was 14.66% (S.E. = 1.52%) for BioAgeAccel, which was a bit larger than

that estimated from the UKB (12.39%, S.E. = 0.95%) (11). The quantile-quantile (Q-Q) plots for TWB1 GWAS analyses were shown in Figure S3 of Supplementary Material. We can see that larger *p*-values ($p > 10^{-3}$) generally followed the uniform [0, 1] distribution, implying no inflation of statistics. Moreover, the genomic-control inflation factor was close to 1 (no inflation of statistics), i.e., $\lambda_{GC} = 1.03$ for PhenoAgeAccel and $\lambda_{GC} = 1.04$ for BioAgeAccel. As shown in Figure S3, some SNPs providing very small *p*-values suggested that strong association exists between them and PhenoAgeAccel or BioAgeAccel. These figures were plotted based on the GWAS of TWB1. Because TWB2 was regarded as the replication set, only significant SNPs identified from TWB1 would be further tested in TWB2.

With the clumping procedure in PLINK 1.9 (13), a total of 78 and 67 significant ($p < 5 \times 10^{-8}$) and nearly independent ($r^2 < 0.5$) SNPs were identified from among the 7,433,014 TWB1 SNPs, for PhenoAgeAccel and BioAgeAccel, respectively. Totally 11 out of the 78 PhenoAgeAccel-associated SNPs and 4 out of the 67 BioAgeAccel-associated SNPs could be replicated by TWB2 at the genome-wide significance level ($p < 5 \times 10^{-8}$). Table 3 lists the 11 PhenoAgeAccel-associated SNPs ($p < 5 \times 10^{-8}$ in both TWB1 and TWB2), which locate in the *GCKR* (*glucokinase regulator*), *OR51B5* (*olfactory receptor family 51 subfamily B member 5*), *LUC7L* (*LUC7 like*), *FAM234A* (*family with sequence similarity 234 member A*), *RGS11* (*regulator of G protein signaling 11*), and *AXIN1* (*axin 1*) genes, respectively. The 4 BioAgeAccel-associated SNPs locate in/near the *APOE* (apolipoprotein E), *FGF5* (fibroblast growth factor 5), and *ATP2B1* (ATPase plasma membrane Ca2+ transporting 1) genes.

[Table 3 is approximately here]

A total of 240 SNP-biomarker associations were then analyzed (the above-mentioned 15 SNPs, 5 biomarkers in PhenoAge and 3 additional biomarkers in BioAge, and 2 cohorts). Table 4

lists the significant results with $p < 5 \times 10^{-8}$ in TWB1 and/or TWB2. The SNP in *GCKR*, rs1260326, was associated with albumin (TWB1 and TWB2) and fasting glucose (TWB2). The other 10 SNPs were all significantly associated with mean red cell volume.

[Table 4 is approximately here]

Consistently, the SNP in *GCKR*, rs1260326, was also identified as a PhenoAgeAccel-associated SNP by the UKB study (11). Per T-allele of rs1260326 was associated with a 0.201 ($p = 4.8 \times 10^{-8}$), 0.174 ($p = 2.7 \times 10^{-16}$), and 0.130 ($p = 2.3 \times 10^{-9}$) year PhenoAgeDecel in TWB1, TWB2, and the UKB (11), respectively. This T-allele was previously found to be associated with a lower fasting glucose and thus was protective against type 2 diabetes in a French population (24). In line with that finding, the T-allele of rs1260326 was associated with a lower fasting glucose in TWB1 ($p = 8.6 \times 10^{-7}$) and TWB2 ($p = 1.0 \times 10^{-13}$), as shown in Table 4. This result is reasonable, because a lower level of fasting glucose is associated with a decreased PhenoAge. The positive weight of fasting glucose on PhenoAge, 0.1953 (as shown in Table 1), indicated that fasting glucose is positively associated with PhenoAge.

SNP rs76038336 at the *AXIN1* gene was consistently the most significant genetic variant in both the TWB1 and TWB2 cohorts. Per C-allele at rs76038336 was associated with 1.515 ($p = 6.8 \times 10^{-92}$) and 1.847 ($p = 0$) year PhenoAgeDecel in TWB1 and TWB2, respectively. It was associated with a decreased level of mean red cell volume by 4.978 ($p = 1.5 \times 10^{-257}$) and 5.626 ($p = 0$) fL, in TWB1 and TWB2, respectively.

Among the 4 BioAgeAccel-associated SNPs, rs7412 in the *APOE* gene and rs16998073 near the *FGF5* gene were consistently identified as BioAgeAccel-associated SNPs by the UKB study (11). Per T-allele of rs7412 was associated with a 0.227 ($p = 1.1 \times 10^{-14}$), 0.258 ($p = 4.2 \times$

10^{-48}), and 0.260 ($p = 3.2 \times 10^{-60}$) year BioAgeDecel in TWB1, TWB2, and the UKB (11), respectively. This T-allele was associated with a lower total cholesterol by 11.757 mg/dL ($p = 2.0 \times 10^{-86}$) in TWB1 and 12.723 mg/dL ($p = 1.2 \times 10^{-272}$) in TWB2, as shown in Table 4. Regarding the remaining 3 BioAgeAccel-associated SNPs, their associations with BioAgeAccel were all driven by their associations with SBP, as shown by Table 4. Alleles associated with a lower level of SBP were linked to BioAgeDecel.

[Table 5 is approximately here]

Expression-PhenoAgeAccel associations

Table 5 lists significant expression-PhenoAgeAccel associations with $p < 2 \times 10^{-6}$ in both TWB1 and TWB2. Expressions of the *FAM234A* gene in 4 tissues were significantly associated with PhenoAgeAccel, including visceral adipose, tibial artery, lung, and spleen. Expressions of the *RGS11* gene in 12 tissues were significantly associated with PhenoAgeAccel, including lung, aorta and tibial artery, etc. This suggested that the predicted expressions of the two genes (*FAM234A* & *RGS11*) were related to PhenoAgeAccel. On the other hand, no significant expression-BioAgeAccel associations were detected.

Discussion

There are several ways to gauge one's BA (2-5). I here calculated BA according to Levine *et al.*'s PhenoAge (5) and BioAge (6). PhenoAge (5) was chosen because it is composed of indices in important domains of human physiological conditions. In addition to chronological age, the 9 biomarkers constructing Levine *et al.*'s (5) PhenoAge included indices in 5 domains: immunity, metabolic, liver, kidney, and inflammation conditions. Although TWB did not measure all the 9 biomarkers, the available 5 biomarkers covered 4 important domains: immunity (mean red cell

volume, white blood cell count), metabolic (serum glucose), liver (albumin) and kidney functions (creatinine).

Recently, Ahadi *et al.* indicated that biological aging is associated with the abovementioned 4 domains: immunity, metabolic, liver and kidney conditions (17). My approach to estimate BA with indices in these 4 domains is in line with Ahadi *et al.*'s finding. Based on my analysis in NHANES III data, 6markerPhenoAge accounted for 92.16% and 87.61% variability of PhenoAge for males and females, respectively. Therefore, it may not lose much information by using 6markerPhenoAge to estimate PhenoAge.

BioAge (6) was chosen because it is one of the most accurate mortality predictors (10). It was largely comparable to PhenoAge, although PhenoAge performed better in healthy people (10). Figure S4 of Supplementary Material presents the scatter plots of PhenoAge and chronological age in the TWB cohorts, whereas Figure S5 depicts the scatter plots of BioAge and chronological age in the TWB cohorts. Both PhenoAge and BioAge were highly correlated with chronological age (all Pearson's correlation coefficients > 0.93).

In addition to phenotype data, TWB submitted the blood samples of 2,313 randomly selected participants (1,164 males and 1,149 females) for DNAm quantification. Blood DNAm levels were analyzed with the Illumina Infinium MethylationEPIC BeadChip (Illumina, Inc., San Diego, CA) that covered ~860,000 CpG sites. With these data, I calculated the methylation age of the 2,313 subjects according to Levine's DNAmAge (5) and Lu's GrimAge (9). Figure S6 of Supplementary Material shows that both PhenoAge and BioAge largely corresponded to the two measures of methylation age (all Pearson's correlation coefficients $\geq$ 0.85).

BA can reflect life expectancy. Populations with slower aging process usually have longer life expectancy (1). Life expectancy at birth for the Taiwan population was 80.9 years in 2020 (77.7 for males and 84.2 for females), according to Taiwan's Ministry of the Interior. Taiwan men

had 7.5 years and Taiwan women had 9.2 years longer at life expectancy compared with the average worldwide. Women's longer life expectancy (relative to men) corresponded to my findings in PhenoAgeAccel and BioAgeAccel. Table 2 shows that on average Taiwan women had 2.189-year PhenoAgeDecel and 0.879-year BioAgeDecel compared with Taiwan men.

Genetic variants associated with PhenoAgeAccel and BioAgeAccel have been investigated for UKB participants of European descent (11). However, no lifestyle factors have been discussed in that GWAS (11). Besides, like most published GWAS, participants have been limited to the European descent. Studies of genetic association with disorders have been unproportionally focused on European-ancestry people (25), and BA is not an exception.

There are three major strengths of this study. First, different from most published GWAS that were performed in individuals of European ancestry, this work was conducted among participants from Asia. Second, the sample size of 94,443 is relatively large among GWAS from Asian populations. Finally, by linking lifestyle factors to PhenoAgeAccel and BioAgeAccel, I identified three modifiable risk factors significantly associated with BA acceleration. The most significant factor associated with PhenoAgeAccel was obesity, and then were cigarette smoking and alcohol consumption (Table 2). Both the results of TWB1 (discovery cohort) and TWB2 (replication cohort) agreed with this order.

Although it is a consensus that obesity and smoking are not good for health, it remains controversial whether alcohol consumption is harmful to health (26). For example, even given the same definition of alcohol drinking, another TWB study showed that alcohol drinking does not increase the odds of diabetes, with odds ratios of 0.987 (p = 0.88) in TWB1 and 0.890 (p = 0.06) in TWB2 (27). The current study shows that drinking (a weekly intake of more than 150 cc of alcohol for at least 6 months) was significantly associated with both PhenoAgeAccel and BioAgeAccel (Table 2).

Cigarette smoking was significantly associated with PhenoAgeAccel, but not BioAgeAccel (Table 2). However, smoking should still be avoided to prevent from BA acceleration. Because PhenoAge performed better in healthy people than BioAge (10), the results derived from PhenoAge should be put more emphasis when referring to the general population.

Regarding the significant SNPs identified in this study, one PhenoAgeAccel-associated SNP (rs1260326 in the *GCKR* gene) and two BioAgeAccel-associated SNPs (rs7412 in the *APOE* gene; rs16998073 near the *FGF5* gene) are consistent with the finding from the UKB (11). Because BA measures are combinations of biomarkers, we further investigated the association of each biomarker with every identified SNP. Being the SNPs discovered by both UKB and TWB, rs1260326 was significantly associated with albumin (in both TWB1 and TWB2) and fasting glucose (in TWB2), whereas rs7412 was significantly associated with total cholesterol (in both TWB1 and TWB2). The remaining 10 PhenoAgeAccel-associated SNPs were all significantly associated with mean red cell volume (in both TWB1 and TWB2), whereas the other 3 BioAgeAccel-associated SNPs were significantly associated with SBP (in both TWB1 and TWB2, Table 4).

With the genes listed in Table 3, I also performed a Reactome pathway analysis (28) (https://reactome.org/). Reactome showed that the PhenoAgeAccel-associated genes were enriched in the Wnt signaling pathway, whereas the BioAgeAccel-associated genes were enriched in pathways related to lipoprotein metabolism. Some studies have demonstrated a mechanistic link between the Wnt signaling and aging-related phenotypes (29, 30). Moreover, the Wnt signaling is involved in aging-associated heart diseases and heart disorders (31). Besides, emerging studies have shown that lipid metabolism plays an important role in the aging process (32).

A limitation of this study is the association analysis of predicted gene expressions with

PhenoAgeAccel and BioAgeAccel. Gene expressions were predicted from SNP genotypes, according to the PrediXcan (22) and the GTEx library (23). However, almost all individuals in the GTEx library (23) were of the European ancestry, it remains unclear whether the prediction of gene expressions may be applied to Han Chinese (33).

Prevention from obesity, cigarette smoking, and alcohol consumption is associated with a slower rate of biological aging. Further studies to investigate how other modifiable health behaviors affect BA acceleration will be helpful to extend lifespan of human. Besides, gene-behavior interactions on BA acceleration warrant further research.

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Conflict of Interest

None reported.

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Author contributions

Wan-Yu Lin conceived the study design, applied for the Taiwan Biobank data, developed the analysis tool, performed the analyses, interpreted the analysis results, and wrote the manuscript.

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Tables

	Males			Females			*p*-value of comparing TWB1 males and TWB1 females	*p*-value of comparing TWB2 males and TWB2 females
	TWB1	TWB2	*p*-value of comparing TWB1 males and TWB2 males	TWB1	TWB2	*p*-value of comparing TWB1 females and TWB2 females		
Total	12,800	22,625		12,660	46,358			
BMI (kg/m^2)	25.2±3.5	25.5±3.6	1.3×10^{-8}	23.4±3.7	23.6±3.7	2.1×10^{-5}	0	0
Drinking (yes or no) [1]	1,584 (12.4%)	3,014 (13.3%)	0.011	215 (1.7%)	873 (1.9%)	0.182	6.5×10^{-242}	0
Smoking (yes or no) [2]	2,647 (20.7%)	4,724 (20.9%)	0.666	358 (2.8%)	1,402 (3.0%)	0.262	0	0
Regular exercise (yes or no) [3]	5,384 (42.1%)	9,656 (42.7%)	0.265	5,039 (39.8%)	18,416 (39.7%)	0.884	2.6×10^{-4}	1.3×10^{-13}
Educational attainment (1~7) [4]	5.67±0.90	5.71±0.89	9.5×10^{-7}	5.33±1.01	5.40±0.99	7.5×10^{-11}	7.6×10^{-167}	0
Diabetes (yes or no) [5]	1,356 (10.6%)	2,750 (12.2%)	1.1×10^{-5}	851 (6.7%)	3,390 (7.3%)	0.024	6.2×10^{-28}	1.8×10^{-97}
Hypertension (yes or no) [6]	2,694 (21.0%)	6,255 (27.6%)	7.7×10^{-43}	1,130 (8.9%)	5,557 (12.0%)	6.9×10^{-22}	3.8×10^{-161}	0

Obesity (yes or no) [7]			3,393 (26.5%)	6,461 (28.6%)	3.8×10^{-5}	1,945 (15.4%)	7,482 (16.1%)	0.036	1.3×10^{-105}	6.6×10^{-318}
PhenoAge (years)			44.8±12.6	45.7±12.9	6.6×10^{-12}	42.0±11.7	42.6±11.2	2.1×10^{-7}	4.6×10^{-71}	1.5×10^{-205}
BioAge (years)			47.8±10.8	49.7±10.9	1.7×10^{-56}	46.6±11.2	48.1±10.6	3.5×10^{-42}	8.3×10^{-19}	9.5×10^{-75}
	Domain	Weight ($\hat{\beta}_k$ in Equation 2)	6 markers used in PhenoAge							
Chronological age (years)		0.0804	48.9±11.1	50.5±11.2	8.4×10^{-39}	48.9±11.0	50.1±10.4	6.7×10^{-29}	0.860	4.3×10^{-6}
Albumin (g/L)	Liver	-0.0336	46.2±2.4	45.7±2.3	4.8×10^{-71}	45.0±2.3	44.7±2.2	2.7×10^{-49}	3.3×10^{-312}	0
Creatinine (umol/L)	Kidney	0.0095	80.0±33.7	80.3±29.7	0.376	54.2±19.2	54.5±18.6	0.100	0	0
Fasting glucose (mmol/L)	Metabolic	0.1953	5.51±1.30	5.53±1.30	0.297	5.19±0.99	5.22±1.03	0.003	2.9×10^{-109}	1.3×10^{-209}
Mean red cell volume (fL)	Immunity	0.0268	90.3±8.0	87.9±7.0	9.0×10^{-177}	89.5±8.5	87.3±8.1	8.2×10^{-151}	1.2×10^{-14}	4.7×10^{-25}
White blood cell count (1000 cells/uL)	Immunity	0.0554	6.1±1.6	6.0±1.7	7.6×10^{-15}	5.8±1.6	5.7±1.6	5.5×10^{-12}	1.6×10^{-52}	2.8×10^{-94}
			3 additional markers used in BioAge							
HbA1c (%)			5.79±0.88	5.88±0.91	9.9×10^{-19}	5.67±0.71	5.73±0.73	5.9×10^{-17}	6.1×10^{-32}	6.2×10^{-96}

SBP (mmHg)	122.9±16.2	126.6±16.7	1.5×10^{-91}	113.8±17.5	116.8±17.8	5.2×10^{-64}	0	0
Total cholesterol (mg/dL)	191.8±35.0	191.9±35.0	0.726	195.3±36.0	198.2±36.0	6.8×10^{-16}	3.4×10^{-15}	3.1×10^{-106}

Table 1. **Basic characteristics of TWB1 and TWB2 participants**

Data are presented in *n* (%) or mean±SD.

[1] Drinking was defined as a person having a weekly intake of more than 150 mL of alcohol for at least 6 months and having not stopped drinking at the time he/she participated TWB.

[2] Smoking was defined as a person who had smoked cigarettes for at least 6 months and had not quit smoking at the time he/she participated TWB.

[3] Regular exercise was defined as performing 30 minutes of "exercise" three times a week. "Exercise" includes leisure-time activities such as swimming, jogging, cycling, mountain climbing, dancing, weight training, etc.

[4] Educational attainment was recorded as a number ranging from 1 to 7, with 1 indicating "illiterate", 2 "no formal education but literate", 3 "primary school graduate", 4 "junior high school graduate", 5 "senior high school graduate", 6 "college graduate", and 7 "Master's or higher degree".

[5] Subjects with diabetes included those with physician-diagnosed diabetes, or those having fasting glucose > 126 mg/dL (7 mmol/L) or HbA1c > 6.5 % (48 mmol/mol) according to the TWB test results.

[6] Hypertension was defined as diastolic blood pressure > 80 mmHg or SBP > 130 mmHg.

[7] Obesity was defined as BMI ≥ 27 kg/m^2, according to the Ministry of Health and Welfare of Taiwan.

	PhenoAgeAccel						BioAgeAccel					
	TWB1			TWB2			TWB1			TWB2		
	Regression coefficient	95% C.I.	*P*-value	Regression coefficient	95% C.I.	*P*-value	Regression coefficient	95% C.I.	*P*-value	Regression coefficient	95% C.I.	*P*-value
Sex (female vs. male)	-2.189	[-2.302, -2.076]	8.0×10^{-309}	-2.109	[-2.179, -2.039]	0 [1]	-0.879	[-0.926, -0.832]	3.2×10^{-289}	-0.875	[-0.905, -0.845]	0 [1]
BMI (kg/m^2)	0.177	[0.163, 0.191]	6.0×10^{-129}	0.166	[0.158, 0.174]	0 [1]	0.171	[0.165, 0.177]	0 [1]	0.167	[0.163, 0.170]	0 [1]
Education (1~7)	-0.213	[-0.267, -0.159]	1.4×10^{-14}	-0.086	[-0.117, -0.055]	7.2×10^{-8}	-0.027	[-0.049, -0.004]	0.02	-0.030	[-0.044, -0.017]	1.3×10^{-5}
Smoking (yes vs. no)	1.134	[0.966, 1.303]	1.3×10^{-39}	1.193	[1.081, 1.304]	3.9×10^{-97}	-0.038	[-0.108, 0.032]	0.29	-0.035	[-0.084, 0.013]	0.152
Drinking (yes vs. no)	0.640	[0.433, 0.847]	1.3×10^{-9}	0.518	[0.383, 0.652]	4.4×10^{-14}	0.193	[0.107, 0.279]	1.1×10^{-5}	0.254	[0.195, 0.312]	1.4×10^{-17}
Exercise (yes vs. no)	-0.121	[-0.225, -0.017]	0.023	-0.030	[-0.091, 0.030]	0.327	-0.055	[-0.099, -0.012]	0.012	-0.028	[-0.054, -0.001]	0.040
R-square [2]	13.02%			11.86%			20.24%			19.28%		

Table 2. **Results of regressing PhenoAgeAccel and BioAgeAccel on 6 factors (before including SNPs)**

1 A *P*-value of 0 means that the test is extremely significant, i.e., sex and BMI were very significantly associated with PhenoAgeAccel and BioAgeAccel.

2 The R-square (ranging from 0% to 100%) of the regression model, i.e., the percentage of the variation in PhenoAgeAccel or BioAgeAccel that

can be explained by the 6 factors.

SNP	Chromosome	Base pair	Gene	Effect allele	Other allele	Effect allele frequency (TWB1/TWB2)	Regression coefficient (TWB1/TWB2)	Standard error of regression coefficient (TWB1/TWB2)	*P*-value (TWB1/TWB2)
PhenoAgeAccel									
rs1260326	2	27730940	*GCKR*	T	C	0.494/0.494	-0.201/-0.174	0.037/0.021	$4.8 \times 10^{-8}/2.7 \times 10^{-16}$
rs218265	4	55408999	---	C	T	0.326/0.336	0.231/0.151	0.039/0.023	$3.0 \times 10^{-9}/2.6 \times 10^{-11}$
rs11037480	11	5472472	*OR51B5*	C	T	0.013/0.016	-1.137/-0.969	0.164/0.086	$3.9 \times 10^{-12}/1.1 \times 10^{-29}$
rs1203979	16	261866	*LUC7L*	A	T	0.493/0.497	-0.323/-0.235	0.037/0.021	$1.5 \times 10^{-18}/2.7 \times 10^{-28}$
rs966965120	16	279723	*LUC7L*	A	G	0.113/0.111	-1.086/-1.026	0.057/0.034	$4.7 \times 10^{-79}/6.0 \times 10^{-200}$
rs56007737	16	287917	*FAM234A*	G	C	0.281/0.279	-0.381/-0.379	0.041/0.024	$7.8 \times 10^{-21}/3.0 \times 10^{-57}$
rs740000	16	319725	*FAM234A*	C	T	0.444/0.435	-0.285/-0.276	0.037/0.022	$1.3 \times 10^{-14}/1.1 \times 10^{-37}$
rs2685125	16	324403	*RGS11*	G	C	0.380/0.374	0.263/0.217	0.038/0.022	$3.4 \times 10^{-12}/7.1 \times 10^{-23}$
rs76038336	16	359611	*AXIN1*	C	G	0.065/0.060	-1.515/-1.847	0.074/0.044	$6.8 \times 10^{-92}/0$
rs1057209	16	381716	*AXIN1*	C	G	0.160/0.157	-0.591/-0.681	0.050/0.029	$3.0 \times 10^{-32}/6.0 \times 10^{-120}$
rs7206286	16	386179	*AXIN1*	G	A	0.349/0.340	-0.267/-0.285	0.038/0.022	$3.4 \times 10^{-12}/1.2 \times 10^{-36}$
BioAgeAccel									
rs7556898	2	165008513	---	T	C	0.420/0.494	-0.114/-0.080	0.015/0.009	$1.3 \times 10^{-13}/1.6 \times 10^{-17}$
rs16998073	4	81184341	*PRDM8 -FGF5*	T	A	0.418/0.416	0.092/0.099	0.015/0.009	$3.2 \times 10^{-9}/6.0 \times 10^{-26}$
rs10858917	12	90088790	*ATP2B1*	G	A	0.319/0.319	-0.093/-0.071	0.016/0.010	$1.2 \times 10^{-8}/7.1 \times 10^{-13}$
rs7412	19	45412079	*APOE*	T	C	0.073/0.073	-0.227/-0.258	0.029/0.018	$1.1 \times 10^{-14}/4.2 \times 10^{-48}$

Table 3. SNPs associated with PhenoAgeAccel or BioAgeAccel ($p < \mathbf{5 \times 10^{-8}}$ in both TWB1 and TWB2)

SNP	Chr.	Base pair	Gene	Effect allele	Other allele	Effect allele frequency (TWB1/TWB2)	Phenotype	Regression coefficient (TWB1/TWB2)	Standard error of regression coefficient (TWB1/TWB2)	*P*-value (TWB1/TWB2)
PhenoAgeAccel										
rs1260326	2	27730940	*GCKR*	T	C	0.494/0.494	Albumin (g/L)	0.019/0.019	0.002/0.001	$2.3 \times 10^{-20}/7.8 \times 10^{-57}$
							Fasting glucose (mmol/L)	-0.049/-0.043	0.010/0.006	$8.6 \times 10^{-7}/1.0 \times 10^{-13}$
rs218265	4	55408999	---	C	T	0.326/0.336	Mean red cell volume (fL)	0.592/0.501	0.076/0.043	$9.1 \times 10^{-15}/3.5 \times 10^{-31}$
rs11037480	11	5472472	*OR51B5*	C	T	0.013/0.016	Mean red cell volume (fL)	-3.897/-2.748	0.321/0.163	$7.8 \times 10^{-34}/1.9 \times 10^{-63}$
rs1203979	16	261866	*LUC7L*	A	T	0.493/0.497	Mean red cell volume (fL)	-1.130/-0.931	0.072/0.041	$1.7 \times 10^{-55}/5.7 \times 10^{-116}$
rs966965120	16	279723	*LUC7L*	A	G	0.113/0.111	Mean red cell volume (fL)	-3.594/-3.263	0.111/0.064	$9.0 \times 10^{-224}/0$
rs56007737	16	287917	*FAM234A*	G	C	0.281/0.279	Mean red cell volume (fL)	-1.229/-1.162	0.080/0.045	$1.5 \times 10^{-53}/1.1 \times 10^{-144}$
rs740000	16	319725	*FAM234A*	C	T	0.444/0.435	Mean red cell volume (fL)	-0.856/-0.747	0.072/0.041	$3.3 \times 10^{-32}/5.5 \times 10^{-74}$
rs2685125	16	324403	*RGS11*	G	C	0.380/0.374	Mean red cell volume (fL)	0.766/0.678	0.074/0.042	$5.5 \times 10^{-25}/2.6 \times 10^{-58}$
rs76038336	16	359611	*AXIN1*	C	G	0.065/0.060	Mean red cell	-4.978/-5.626	0.144/0.083	$1.5 \times 10^{-257}/0$

							volume (fL)			
rs1057209	16	381716	*AXIN1*	C	G	0.160/0.157	Mean red cell volume (fL)	-1.945/-2.069	0.097/0.055	$5.7 \times 10^{-88}/2.1 \times 10^{-302}$
rs7206286	16	386179	*AXIN1*	G	A	0.349/0.340	Mean red cell volume (fL)	-0.901/-0.912	0.075/0.043	$5.2 \times 10^{-33}/3.3 \times 10^{-100}$
BioAgeAccel										
rs7556898	2	165008513	---	T	C	0.420/0.494	SBP (mmHg)	-1.070/-0.817	0.133/0.084	$8.9 \times 10^{-16}/2.3 \times 10^{-22}$
rs16998073	4	81184341	*PRDM8 -FGF5*	T	A	0.418/0.416	SBP (mmHg)	1.205/1.237	0.133/0.084	$1.9 \times 10^{-19}/7.1 \times 10^{-49}$
rs10858917	12	90088790	*ATP2B1*	G	A	0.319/0.319	SBP (mmHg)	-1.095/-0.801	0.142/0.089	$1.1 \times 10^{-14}/2.3 \times 10^{-19}$
rs7412	19	45412079	*APOE*	T	C	0.073/0.073	Total cholesterol (mg/dL)	-11.757/-12.723	0.594/0.359	$2.0 \times 10^{-86}/1.2 \times 10^{-272}$

Table 4. **SNP-biomarker associations ($p < 5 \times 10^{-8}$ in TWB1 and/or TWB2)**

Gene	Tissue	*p*-value in TWB1	*p*-value in TWB2
FAM234A	Visceral adipose (Omentum)	2.4×10^{-14}	2.0×10^{-43}
FAM234A	Tibial artery	4.5×10^{-9}	1.5×10^{-35}
FAM234A	Lung	5.9×10^{-8}	2.0×10^{-35}
FAM234A	Spleen	2.6×10^{-12}	1.4×10^{-31}
RGS11	Adrenal gland	3.6×10^{-25}	6.0×10^{-35}
RGS11	Aorta artery	5.5×10^{-15}	6.0×10^{-37}
RGS11	Coronary artery	4.1×10^{-8}	7.8×10^{-7}
RGS11	Tibial artery	6.0×10^{-9}	2.4×10^{-28}
RGS11	Cells transformed fibroblasts	5.8×10^{-20}	1.7×10^{-28}
RGS11	Colon transverse	3.0×10^{-13}	2.7×10^{-21}
RGS11	Esophagus mucosa	9.9×10^{-9}	2.4×10^{-13}
RGS11	Left heart ventricle	4.0×10^{-7}	2.6×10^{-19}
RGS11	Lung	2.2×10^{-7}	2.3×10^{-22}
RGS11	Tibial nerve	1.4×10^{-15}	8.0×10^{-21}
RGS11	Skin-Not Sun Exposed (Suprapubic)	1.0×10^{-14}	1.1×10^{-21}
RGS11	Uterus	7.3×10^{-9}	1.3×10^{-17}

Table 5. **Expression-PhenoAgeAccel associations ($p < 2 \times 10^{-6}$ in TWB1 and TWB2)**