Gene-environment interactions and gene-gene interactions on two biological age measures: evidence from Taiwan Biobank participants

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Abstract

PhenoAge and BioAge are two commonly used biological age (BA) measures. I here searched for gene-environment interactions (GxE) and gene-gene interactions (GxG) on PhenoAgeAccel (age-adjusted PhenoAge) and BioAgeAccel (age-adjusted BioAge) of 111,996 Taiwan Biobank (TWB) participants, including a discovery set of 86,536 TWB2 individuals and a replication set of 25,460 TWB1 individuals. Searching for variance quantitative trait loci (vQTLs) provides a convenient way to evaluate GxE and GxG. A total of 4 nearly independent (linkage disequilibrium measure $r^2 < 0.01$) PhenoAgeAccel-vQTLs were identified from 5,303,039 autosomal TWB2 SNPs ($p < 5E-8$), whereas no vQTLs were found from BioAgeAccel. These 4 PhenoAgeAccel-vQTLs (rs35276921, rs141927875, rs10903013, and rs76038336) were further replicated by TWB1 ($p < 5E-8$). They were located in the *OR51B5*, *FAM234A*, and *AXIN1* genes. All 4 PhenoAgeAccel-vQTLs were significantly associated with PhenoAgeAccel ($p < 5E-8$). A phylogenetic heat map of the GxE analyses showed that smoking exacerbated the PhenoAgeAccel-vQTLs' aging effects, while higher educational attainment attenuated the PhenoAgeAccel-vQTLs' aging effects. Body mass index, chronological age, alcohol consumption, and sex did not prominently modulate PhenoAgeAccel-vQTLs' aging effects. Based on the vQTL results, I also detected rs141927875-rs35276921 interaction ($p = 4.7E-61$) and rs76038336-rs10903013 interaction ($p = 3.3E-116$) on PhenoAgeAccel.

Keywords: Biomarkers, longevity, genetics, scale test.

1 Introduction

Biological age (BA) measures can provide critical information for human health and physiological conditions [1]. Biologically young individuals generally have longer lifespans and

healthier conditions than biologically old individuals [2]. Slowing the rate of biological aging will help prevent various age-related disorders and cancers [3]. However, there is no consensus on how to measure one's BA. Recently, with the big health data collected by biobanks of some countries, BA can be estimated through phenotype data [4-9].

Several phenotypes are highly related to one's healthspan and lifespan [4-9]. To be specific, BA is usually estimated by integration of multiple vital biomarkers such as C-reactive protein [5,8,9], albumin [5,8,9], creatinine [8,9], total cholesterol [5,9], systolic blood pressure (SBP) [5,9], diastolic blood pressure [7], glycated hemoglobin (HbA1c) [5], estimated glomerular filtration rate [7], etc.

Recently, BA was reported to be critically associated with indices in the following four domains: immunity, metabolic, and functions of the kidney and liver [10]. Consistent with this viewpoint, Levine *et al.* [8] proposed a PhenoAge including 9 phenotypes and chronological age. These 9 biomarkers covered indices in these critical domains, including immunity: **mean corpuscular volume (MCV), white blood cell count,** red cell distribution width, and lymphocyte percent (4 biomarkers); metabolic condition: **serum fasting glucose** (1 biomarker); kidney function: **creatinine** (1 biomarker); liver function: **albumin** and alkaline phosphatase (2 biomarkers); and inflammation: C-reactive protein (1 biomarker).

The five clinical markers presented in bold font were measured by the Taiwan Biobank (TWB), whereas the other four were not. Although TWB did not collect all nine biomarkers, I calculated the PhenoAge of TWB participants by imputing it based on six markers (chronological age and the abovementioned five bold-font markers) [11].

In addition to PhenoAge, BioAge is another commonly used BA measure that provides one of the most accurate mortality predictors [9]. BioAge was estimated by chronological age and seven biomarkers in four domains, including metabolic condition: **HbA1c**, **SBP**, and **total cholesterol** (3 biomarkers); kidney function: **creatinine** (1 biomarker); liver function: **albumin** and alkaline

phosphatase (2 biomarkers); and inflammation: C-reactive protein (1 biomarker).

TWB measured the abovementioned five bold-font markers, whereas the other two were not. Although TWB did not collect all seven biomarkers, I previously calculated the BioAge of TWB participants by imputing it based on six markers (chronological age and the abovementioned five bold-font markers) [4]. Formulas of PhenoAge and BioAge aggregated essential health indicators. Therefore, they can reflect one's health and physiological conditions [8].

After measuring BA, BA acceleration (BAA) can be obtained through the residuals of regressing BA on chronological age, i.e., PhenoAgeAccel (age-adjusted PhenoAge) and BioAgeAccel (age-adjusted BioAge) [4,12]. In this work, I explored gene-environment interactions (GxE) and gene-gene interactions (GxG) on PhenoAgeAccel and BioAgeAccel according to analyses of ~111,996 TWB participants. Through this, I investigated whether environmental factors or other genetic variants can modulate the effects of aging-associated genes.

2 Experimental procedures

2.1 Taiwan Biobank data

Since October 2012, TWB has recruited Taiwan residents aged 30 to 70 and collected their genomic and lifestyle factors [13]. After signing informed consent, community-based volunteers took physical examinations and provided blood and urine samples. TWB health professionals further collected lifestyle information 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 the Ethics and Governance Council of Taiwan Biobank, Taiwan. TWB approved my application to access the data on February 18, 2020 (application number: TWBR10810-07). The current work further received approval from the

Research Ethics Committee of the National Taiwan University Hospital (NTUH-REC no. 201805050RINB).

As of March 2022, 27,719 and 103,332 individuals (aged 30-70 years) have been whole-genome genotyped by the TWB 1.0 and TWB 2.0 genotyping arrays, respectively. The TWB 1.0 array was designed for Taiwan's Han Chinese, running on the Axiom Genome-Wide Array Plate System (Affymetrix, Santa Clara, CA). The TWB 2.0 array was developed according to the experience of designing the TWB 1.0 array and the next-generation sequencing of ~1,000 TWB individuals. These two arrays were released in April 2013 and August 2018, respectively. Individuals genotyped by the TWB 1.0 and TWB 2.0 arrays formed a discovery set (called "the TWB1 cohort") and a replication set (called "the TWB2 cohort"), separately.

A total of 1,462 individuals were genotyped by both arrays. To ensure that the replication set was independent of the discovery set, I removed these 1,462 individuals from the TWB2 cohort. I also tried to exclude individuals with more than 10% missing in their genotype calls, where 10% is a commonly adopted cutoff in quality control [14]. Nonetheless, no individuals were removed according to this criterion.

To assess cryptic relatedness, I calculated PI-HAT = Probability(IBD = 2) + 0.5 × Probability(IBD = 1) by PLINK 1.9 [15], where IBD is the genome-wide identity by descent sharing coefficients between any two TWB individuals. I removed one individual from each pair with PI-HAT $\geq$ 0.2, a cutoff value commonly chosen by some studies [16,17] and our previous works [4,18,19]. After this step, the TWB1 and TWB2 cohorts comprised 25,460 and 86,536 individuals, respectively.

TWB 1.0 and TWB 2.0 arrays covered 632,172 and 648,611 autosomal SNPs, respectively. I removed 6,900 SNPs with Hardy-Weinberg test p values < 5.7×10^{-7} [20] and 27,628 SNPs with genotyping rates < 95% from the TWB1 cohort and excluded 17,419 SNPs with Hardy-Weinberg

test p-values < 5.7×10^{-7} [20] and 22,614 SNPs with genotyping rates < 95% from the TWB2 cohort. The remaining 597,644 TWB1 SNPs and 608,578 TWB2 SNPs were used to construct ancestry principal components (PCs). A total of 92,021 SNPs overlapped across the two SNP sets (597,644 TWB1 SNPs and 608,578 TWB2 SNPs).

The Michigan Imputation Server (https://imputationserver.sph.umich.edu/index.html) was further used to impute genotypes. The East Asian population from the 1000 Genomes Phase 3 v5 was set as the reference panel. I removed SNPs with low imputation information scores (R-square < 0.8), with imputation rates < 95%, or with Hardy-Weinberg test p-values < 5.7×10^{-7} [20]. The TWB1 and TWB2 individuals were finally genotyped (or imputed) on 7,433,014 and 6,546,183 autosomal SNPs, respectively. I analyzed the 5,303,039 SNPs overlapping the two SNP sets (7,433,014 TWB1 SNPs and 6,546,183 TWB2 SNPs).

2.2 Principles of the scale test

Let G be the number of minor alleles at an SNP (the assumption of "additive allelic effects" is used here to simplify the conceptual derivation); E be the environmental factor that can be continuous or dichotomous. Without loss of generality, modeling a phenotype (denoted as "Y") with an SNP (G) and an environmental factor (E) can be expressed as follows,

$$Y = \beta_0 + \beta_G G + \beta_E E + \beta_{INT} G \times E + \varepsilon\,, \tag{1}$$

where ε is the random error term. By assuming G and E are independent of each other, Pare *et al.* derived the variance of Y conditional on the genotype as [21]

$$Var(Y|G=g) = Var(\beta_0 + \beta_G G + \beta_E E + \beta_{INT} G \times E + \varepsilon | G=g)$$

$$= Var(\beta_0) + Var(\beta_G G|G=g) + Var(\beta_E E) + Var(\beta_{INT} G \times E|G=g)$$

$$+ 2Cov(\beta_E E, \beta_{INT} G \times E|G=g) + Var(\varepsilon)$$

$$= 0 + 0 + \beta_E^2 Var(E) + \beta_{INT}^2 g^2 Var(E) + 2\beta_E \beta_{INT} g Var(E) + Var(\varepsilon)$$

$$= (\beta_E + \beta_{INT} g)^2 Var(E) + Var(\varepsilon) \quad (2)$$

I obtained $Var(Y|G = g) = (\beta_E + \beta_{INT} g)^2 Var(E) + Var(\varepsilon)$. In the absence of GxE, $\beta_{INT} = 0$ and $Var(Y|G = g) = \beta_E^2 Var(E) + Var(\varepsilon)$, representing the variance of *Y* remains constant across the three genotypes (g = 0, 1, and 2). Therefore, I investigated GxE by testing equal variance (homoscedasticity) of BAA (PhenoAgeAccel and BioAgeAccel) across the three genotype groups. Likewise, *E* in equation (1) can be substituted with another genetic variant "G_2". Non-constant phenotypic variance can result from GxE or GxG.

2.3 Statistical Analysis

2.3.1 Genome-wide variance quantitative trait loci (vQTL) search for BAA

Soave and Sun proposed a two-stage regression framework to perform the vQTL search while adjusting for covariates [22]. To provide results robust to outliers and the distributions of BAA, I performed the "rank-based inverse normal transformation" (RINT) [23] on BAA (PhenoAgeAccel and BioAgeAccel) prior to the analysis. With this step, RINT-BAA was normally distributed, and therefore, I could obtain "genotypes-and-covariates adjusted RINT-BAA" through the residuals of regressing RINT-BAA on genotypes and covariates. For each SNP, I first adjusted RINT-BAA with genotype effects (as two dummy variables, without assuming "additive allelic effects") and covariates, including sex (male vs. female), chronological age (in years), BMI (in kg/m^2), performing regular exercise (yes vs. no), educational attainment (an integer from 1 to 10), smoking status (yes vs. no), drinking status (yes vs. no), and the first 10 ancestry PCs. These profile or lifestyle factors were associated with PhenoAgeAccel and BioAgeAccel (Table 2 of my previous work [11]), and therefore they were chosen as covariates. This model was called the "larger model" because more covariates were adjusted. The definition

of each covariate was described under Table 1.

Through the above step, I obtained the "genotypes-and-covariates adjusted RINT-BAA", denoted as "e_i" for the *i*th individual. The dispersion of e_i was then calculated by $D_i = (e_i - \tilde{e})^2$, where $\tilde{e}$ was the sample median of e_i across all *n* individuals (the sample median is more robust than the sample mean). Subsequently, I regressed RINT-D_i on the two dummy variables for genotype coding to check whether the dispersion of e_i was dependent on different genotypes. The significance of the *F*-statistic of this regression model meant that the dispersion of "genotypes-and-covariates adjusted RINT-BAA" (e_i) varied with different genotypes, which was a clue of GxE or GxG according to the derivation of equation (2). This *F*-statistic is called the "scale test", which is a right-tailed test [24,25].

To avoid potential collider bias, I also considered a "smaller model", where only sex (male vs. female), chronological age (in years), and the first 10 ancestry PCs were adjusted. I analyzed the 5,303,039 SNPs overlapping the TWB1 and TWB2 SNP sets. *P*-values of the scale test < 5E-8 (the commonly used genome-wide significance level [26]) in both the smaller and larger models were considered significant. Significant SNPs identified from the TWB2 cohort (*n* = 86,536) were further analyzed using the TWB1 cohort (*n* = 25,460). SNPs with *P*-values of the scale test < 5E-8 in the TWB1 cohort were considered successfully replicated. These SNPs were called vQTLs. All analyses were performed using the statistical software R (version 4.2.2).

2.3.2 GxE analysis for vQTLs

Subsequently, I combined the TWB1 and TWB2 cohorts to investigate which E enriched the GxE signal (*n* = 25,460 + 86,536 = 111,996). For each vQTL, RINT-BAA was regressed on the number of aging alleles (0, 1, or 2), sex (male vs. female), chronological age (in years), BMI (in kg/m^2), performing regular exercise (yes vs. no), educational attainment (an integer from 1 to 10),

smoking status (yes vs. no), drinking status (yes vs. no), and the first 10 ancestry PCs. In this regression model, I added the interaction term of the number of aging alleles and one of the seven Es: sex, chronological age, BMI, performing regular exercise, educational attainment, smoking status, and drinking status. I used a phylogenetic heat map to present the interaction two-sided *p*-values and the GxE directions (i.e., synergistic interactions or antagonistic interactions). A positive interaction effect indicates that the E exacerbates the vQTL's aging effect, while a negative interaction effect suggests that the E attenuates the vQTL's aging effect.

2.3.3 GxG analysis for nearly independent vQTLs

By aggregating the TWB1 and TWB2 cohorts (n = 25,460 + 86,536 = 111,996), I also investigated which vQTL modulated the effects of other vQTLs. A GxG search requires more than one vQTL to be put into a regression model. To prevent the collinearity problem, I looked for nearly independent vQTLs with the linkage disequilibrium measure $r^2 < 0.01$ using the PLINK clumping procedure [15]. Subsequently, RINT-BAA was regressed on the numbers of aging alleles (0, 1, or 2) for any two nearly independent vQTLs and their interaction term (i.e., product term) while controlling the covariates mentioned in the larger model. Two-sided *p*-values of the interaction term < 3.6E-15 [0.05/C(5303039, 2)] were considered significant, where $C(n, r)$ was the combination notation, and 5303039 was the number of SNPs analyzed in this study.

[Table 1 is approximately here]

3 Results

3.1 Characteristics of the TWB1 and TWB2 participants

Table 1 presents the characteristics of the TWB1 and TWB2 participants stratified by sex.

The TWB1 and TWB2 participants had similar characteristics. Compared with female participants, male participants had higher percentages of drinking alcoholic beverages, cigarette smoking, and performing regular exercise. Moreover, males had higher body mass index (BMI) and educational attainment than females. Except for total cholesterol, males had higher mean levels in the other seven biomarkers (albumin, creatinine, fasting glucose, MCV, white blood cell count, HbA1c, and systolic blood pressure) than females.

The top row of Figure S1 (in the supporting information) shows the boxplots of BAA (PhenoAgeAccel and BioAgeAccel). The numbers shown under each plot mark the range of PhenoAgeAccel and BioAgeAccel (minimum ~ maximum). Because PhenoAgeAccel (or BioAgeAccel) was obtained from the residuals of regressing PhenoAge (or BioAge) on chronological age, PhenoAgeAccel and BioAgeAccel centered around 0 behaving like residuals. However, both the two measures of BAA were skewed to the right. To generate results robust to outliers and the distributions of BAA, I performed the RINT transformation [23] on BAA before the analysis. RINT-PhenoAgeAccel and RINT-BioAgeAccel were normally distributed, as shown in the bottom row of Figure S1.

Figure S2 presents the Pearson's correlation coefficients between PhenoAge, BioAge, chronological age, and the eight biomarkers constituting PhenoAge or BioAge. The correlation plots were stratified by the discovery (TWB2)/replication (TWB1) cohorts and sex. Both PhenoAge and BioAge were highly correlated with chronological age (correlation > 0.9). The correlations between biomarkers were consistent across the two cohorts (TWB1 and TWB2).

3.2 Tests of scale and location

Typical genome-wide association studies (GWAS) investigate mean phenotypic differences between genotypes, while vQTL searches assess phenotypic variability across genotypes.

Inspired by Soave *et al.* [24] and Staley *et al.* [25], examining phenotypic mean and variance can be termed "location" and "scale" tests, respectively. The results of these two tests for RINT-BAA were presented as follows.

[Figure 1 is approximately here]

[Scale test] Figure 1 shows the quantile-quantile (Q-Q) plots and histograms of the vQTLs' *p*-values. I detected stronger signals of vQTLs from PhenoAgeAccel (Figure 1) than from BioAgeAccel (Figure S3). A total of 154 PhenoAgeAccel-vQTL SNPs were identified from 5,303,039 SNPs ($p < 5E-8$ in TWB2's and TWB1's smaller and larger models, Supplementary Table S1 columns J-M), whereas no vQTLs were found from BioAgeAccel. They were located in the *OR51B5* (on chromosome [chr.] 11), *LUC7L* (on chr. 16), *FAM234A* (on chr. 16), *RGS11* (on chr. 16), *AXIN1* (on chr. 16), *MRPL28* (on chr. 16), and *RAB11FIP3* (on chr. 16) genes, respectively (Figure 2 and Supplementary Table S1).

[Figure 2 is approximately here]

I also performed the scale test on the eight biomarkers constituting PhenoAgeAccel (MCV, white blood cell count, serum fasting glucose, creatinine, and albumin) and BioAgeAccel (HbA1c, SBP, and total cholesterol), respectively. A total of 155 MCV-vQTL SNPs were identified from 5,303,039 SNPs ($p < 5E-8$ in TWB2's and TWB1's smaller and larger models). They were located in or around the PhenoAgeAccel-vQTL genes (*OR51B5*, *LUC7L*, *FAM234A*, *RGS11*, *AXIN1*, *MRPL28*, and *RAB11FIP3*). Supplementary Table S2 columns J-M present the scale *p*-values of the 154 PhenoAgeAccel-vQTL SNPs when the phenotype is RINT-MCV. The scale $p < 5E-8$ for all TWB2's smaller and larger models (Table S2 columns J-K), and the scale $p < 2E-5$ for all TWB1's smaller and larger models (Table S2 columns L-M). On the contrary, no vQTLs were found from the other seven biomarkers. The 154 PhenoAgeAccel-vQTL SNPs were mainly detected through the signals of MCV.

[Location test] All the 154 PhenoAgeAccel-vQTL SNPs were associated with PhenoAgeAccel. When regressing RINT-PhenoAgeAccel on these 154 PhenoAgeAccel-vQTL SNPs (coded as 0, 1, or 2, representing the number of aging alleles) while adjusting for the covariates (sex, chronological age, BMI, performing regular exercise, educational attainment, smoking status, drinking status, and the first 10 ancestry PCs), the genetic main effects (Table S1 column H) of all the 154 SNPs were significant (location $p < 5E\text{-}8$, Table S1 column I). For each of the 154 PhenoAgeAccel-vQTL SNPs, Supplementary Table S1 lists the aging allele that was positively associated with PhenoAgeAccel (Table S1 column E) together with its frequency (Table S1 column G) and the other allele (negatively associated with PhenoAgeAccel, Table S1 column F).

[Figure 3 is approximately here]

3.3 Analysis results of GxE

Figures 3 and 4 show the phylogenetic heat maps of the GxE analyses for PhenoAgeAccel and MCV, respectively. Smoking exacerbated PhenoAgeAccel-vQTLs' aging effects (Figure 3, red color), while higher educational attainment attenuated the PhenoAgeAccel-vQTLs' aging effects (Figure 3, blue color). BMI, chronological age, alcohol consumption, and sex did not prominently modulate PhenoAgeAccel-vQTLs' aging effects (Figure 3). Chronological age exacerbated MCV-vQTLs' genetic effects (Figure 4, red color), while higher educational attainment and larger BMI attenuated the MCV-vQTLs' genetic effects (Figure 4, blue color).

[Figures 4-5 are approximately here]

3.4 Analysis results of GxG

The GxG analysis could not be performed on all 154 PhenoAgeAccel-vQTL SNPs because most of them were highly correlated. To address this issue, I first used the PLINK clumping

procedure [15] to find 4 nearly independent vQTLs (out of the 154 PhenoAgeAccel-vQTL SNPs) with linkage disequilibrium measure $r^2 < 0.01$, including rs35276921 (near the *OR51B5* gene on chr. 11), rs141927875 (in the *OR51B5* gene on chr. 11), rs10903013 (in the *FAM234A* gene on chr. 16), and rs76038336 (in the *AXIN1* gene on chr. 16). Among the 4 independent PhenoAgeAccel-vQTLs, rs76038336 has been reported as PhenoAgeAccel-QTL in my previous work [11]. Using rs141927875 and rs76038336 as examples, Figure 5 demonstrates a visual representation of how the vQTLs (i.e., increased RINT-PhenoAgeAccel variance with more "T" or "C" alleles) and QTLs appear (i.e., decreased RINT-PhenoAgeAccel mean with more "T" or "C" alleles).

[Table 2 is approximately here]

RINT-BAA was regressed on the numbers of aging alleles (0, 1, or 2) for any two independent vQTLs and their interaction term (i.e., product term) while controlling the covariates including sex (male vs. female), chronological age (in years), BMI (in kg/m^2), performing regular exercise (yes vs. no), educational attainment (an integer from 1 to 10), smoking status (yes vs. no), drinking status (yes vs. no), and the first 10 ancestry PCs. Table 2 shows the GxG *p*-values among the 4 nearly independent PhenoAgeAccel-vQTLs. rs141927875-rs35276921 and rs76038336-rs10903013 interactions were significant with *p*-values < 3.6E-15 = 0.05/C(5303039, 2), where $C(n, r)$ was the combination notation, and 5303039 was the number of SNPs analyzed in this study. No multicollinearity problems have been detected in the 6 GxG regression models. The variance inflation factors (VIFs) were all controlled under 2. Figure 6 presents the mean RINT-PhenoAgeAccel of each genotype combination, in which clear evidence of GxG can be seen. I also performed the GxG analysis on the eight biomarkers constituting PhenoAgeAccel (MCV, white blood cell count, serum fasting glucose, creatinine, and albumin) and BioAgeAccel (HbA1c, SBP, and total cholesterol). Like the GxE results, MCV was the main indicator

responsible for the significant GxG finding for PhenoAgeAccel.

[Figure 6 is approximately here]

4 Discussion

GxE has received much attention because this topic is related to how lifestyle factors modify the effects of hereditary materials [27]. Not only GxE but GxG can also lead to the "non-constant variance" (heteroscedasticity) of a phenotype across different genotypes of an SNP [28]. The scale test aims to explore biologically interesting SNPs (with GxE or GxG) without specifying any Es (or other Gs), which can alleviate the harsh penalty on multiple testing.

The results herein indicated that PhenoAgeAccel and its component (MCV) demonstrated evidence of GxE and GxG. Some studies have found that cigarette smoking shortens human life expectancy [29,30]. Figure 3 shows that smoking exacerbates PhenoAgeAccel-vQTLs' aging effects (red color). Moreover, well-educated individuals are more likely to live longer. Data from the United States revealed that the remaining life expectancy at age 25 is approximately 10 years longer for people with a college degree than those without a high school degree [31]. Consistently, Figure 3 demonstrates that higher educational attainment attenuates the PhenoAgeAccel-vQTLs' aging effects (blue color).

MCV measures the average volume of a red blood cell (RBC), which is calculated as 10×hematocrit/RBC. High MCV (or macrocytosis) is associated with a deficiency in folic acid and vitamin B12, a pathologic condition of older people due to decreased absorption [32]. Moreover, a high MCV is linked to increased cancer mortality and all-cause mortality [33]. Figure 4 shows that chronological age exacerbates MCV-vQTLs' genetic effects (red color). Furthermore, higher educational attainment and larger BMI are usually related to a higher income and better

nutritional intake [34]. Figure 4 demonstrates that these two factors attenuate the MCV-vQTLs' genetic effects (blue color).

Although the genetic associations for the two measures of BAA (PhenoAgeAccel and BioAgeAccel) have been investigated in individuals of European [12] and Asian [11] ancestries, no studies have discussed how Es modulate the effects of aging alleles till now. This work has shown that some non-genetic factors (i.e., smoking and education) can modify the impact of aging-associated genes.

Through the era of GWAS, "missing heritability" remains an unsolved problem [35]. Genetic variants identified from GWAS cannot fully explain the heritability of most complex diseases [36]. An explanation for missing heritability is that genetic variants may present differential effects on humans according to other genetic factors, the so-called GxG. In a GWAS, the "SNP heritability" quantifies the proportion of phenotypic variance explained by all measured SNPs [37]. The SNP heritability of PhenoAgeAccel was estimated at 14.45% and 14.03% from the UK Biobank [12] and the TWB individuals [11] , respectively. As a tiny part of millions of SNPs, the 11 previously published QTLs explained 3.21% variability of PhenoAgeAccel [11], whereas the 4 vQTLs identified in this work explained 2.65% variability of PhenoAgeAccel. Moreover, according to the significant GxG results, if I included the product terms of rs141927875 * rs35276921 and rs76038336 * rs10903013 in addition to the 4 vQTLs (i.e., 6 explanatory variables), the total R^2 explained could be increased to 3.22%. To sum up, the vQTL search facilitates the discoveries of GxE and GxG for PhenoAgeAccel, which can help predict the aging rate.

5 Conclusion

154 PhenoAgeAccel-vQTL SNPs were identified from TWB2 and replicated by TWB1 ($p <$

5E-8 in both cohorts' smaller and larger models). Four were nearly independent with the linkage disequilibrium measure $r^2 < 0.01$, including rs35276921, rs141927875, rs10903013, and rs76038336. These 4 vQTLs were located in or near the *OR51B5*, *FAM234A*, and *AXIN1* genes (Supplementary Table S1). The subsequent GxE analysis showed that smoking exacerbated the vQTLs' aging effects, while higher educational attainment attenuated the vQTLs' aging effects. Body mass index, chronological age, alcohol consumption, and sex did not significantly modulate PhenoAgeAccel-vQTLs' aging effects. Moreover, rs141927875-rs35276921 (both on chr. 11) and rs76038336- rs10903013 (both on chr. 16) interactions on PhenoAgeAccel were discovered (Table 2). Synergistic interactions among the PhenoAgeAccel-vQTLs on the same chromosome were observed (Figure 6). The combined effects of biological-age-deceleration (BAD) alleles were greater than that predicted by their respective impacts.

Supporting Information

Supporting Information is available from the Wiley Online Library.

Acknowledgments

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Conflict of Interest

The author declares no conflict of interest.

Data availability statement

The individual-level Taiwan Biobank data supporting the findings in this study are available upon application to Taiwan Biobank (https://www.twbiobank.org.tw/new_web/). Taiwan Biobank approved my application to access the data on February 18, 2020 (application number: TWBR10810-07; principal investigator: Wan-Yu Lin).

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Tables

	Male participants		Female participants	
	TWB2 (discovery)	**TWB1 (replication)**	**TWB2 (discovery)**	**TWB1 (replication)**
Total	29,453	12,800	57,083	12,660
Chronological age (years)	50.1±11.3	48.9±11.1	49.8±10.5	48.9±11.0
PhenoAge (years)	45.3±12.9	44.8±12.6	42.3±11.3	42.0±11.7
BioAge (years)	49.3±10.9	47.8±10.8	47.8±10.6	46.6±11.2
Body mass index (kg/m^2)	25.5±3.6	25.2±3.5	23.6±3.8	23.4±3.7
Drinking (yes or no) [1]	3,916 (13.3%)	1,584 (12.4%)	1,138 (2.0%)	215 (1.7%)
Smoking (yes or no) [2]	6,072 (20.6%)	2,647 (20.7%)	1,829 (3.2%)	358 (2.8%)
Regular exercise (yes or no) [3]	12,258 (41.6%)	5,384 (42.1%)	22,153 (38.8%)	5,039 (39.8%)
Educational attainment (1~7) [4]	5.75±0.88	5.67±0.90	5.45±0.97	5.33±1.01
Albumin (g/L)	45.8±2.3	46.2±2.4	44.7±2.2	45.0±2.3
Creatinine (μmol/L)	80.7±30.2	80.0±33.7	54.7±18.8	54.2±19.2
Fasting glucose (mmol/L)	5.52±1.29	5.51±1.30	5.23±1.05	5.19±0.99
Mean corpuscular volume (fL)	87.9±6.8	90.3±8.0	87.2±8.0	89.5±8.5
White blood cell count (1000 cells/μL)	5.9±1.6	6.1±1.6	5.7±1.6	5.8±1.6
HbA1c (%)	5.87±0.91	5.79±0.88	5.73±0.74	5.67±0.71
Systolic blood pressure (mmHg)	126.8±16.6	122.9±16.2	116.8±17.9	113.8±17.5
Total cholesterol (mg/dL)	192.3±35.3	191.8±34.6	198.3±36.2	195.3±36.0

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

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

[1] Drinking was defined as a person who had a weekly intake of more than 150 mL of alcoholic beverages for at least 6 months and had not stopped drinking when participating in TWB.

[2] Smoking was defined as a person who had smoked cigarettes for at least 6 months and had not quit smoking when participating in TWB.

[3] Regular exercise was defined as performing exercise for 30 min thrice a week". 'Exercise' included 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".

GxG *p*-value	*rs35276921*	*rs141927875*	*rs10903013*	*rs76038336*
rs35276921	---	**4.7E-61**	0.65	3.5E-4
rs141927875	---	---	0.91	0.35
rs10903013	---	---	---	**3.3E-116**
rs76038336	---	---	---	---

Table 2. **GxG *p*-values of the 4 nearly independent PhenoAgeAccel-vQTLs**

RINT-BAA was regressed on the numbers of aging alleles (0, 1, or 2) for any two independent vQTLs and their interaction term (i.e., product term) while controlling the covariates mentioned in the larger model. *P*-values less than 3.6E-15 [0.05/*C*(5303039, 2)] were highlighted in bold, where *C*(*n*, *r*) was the combination notation, and 5303039 was the number of SNPs analyzed in this study.

Figure legends

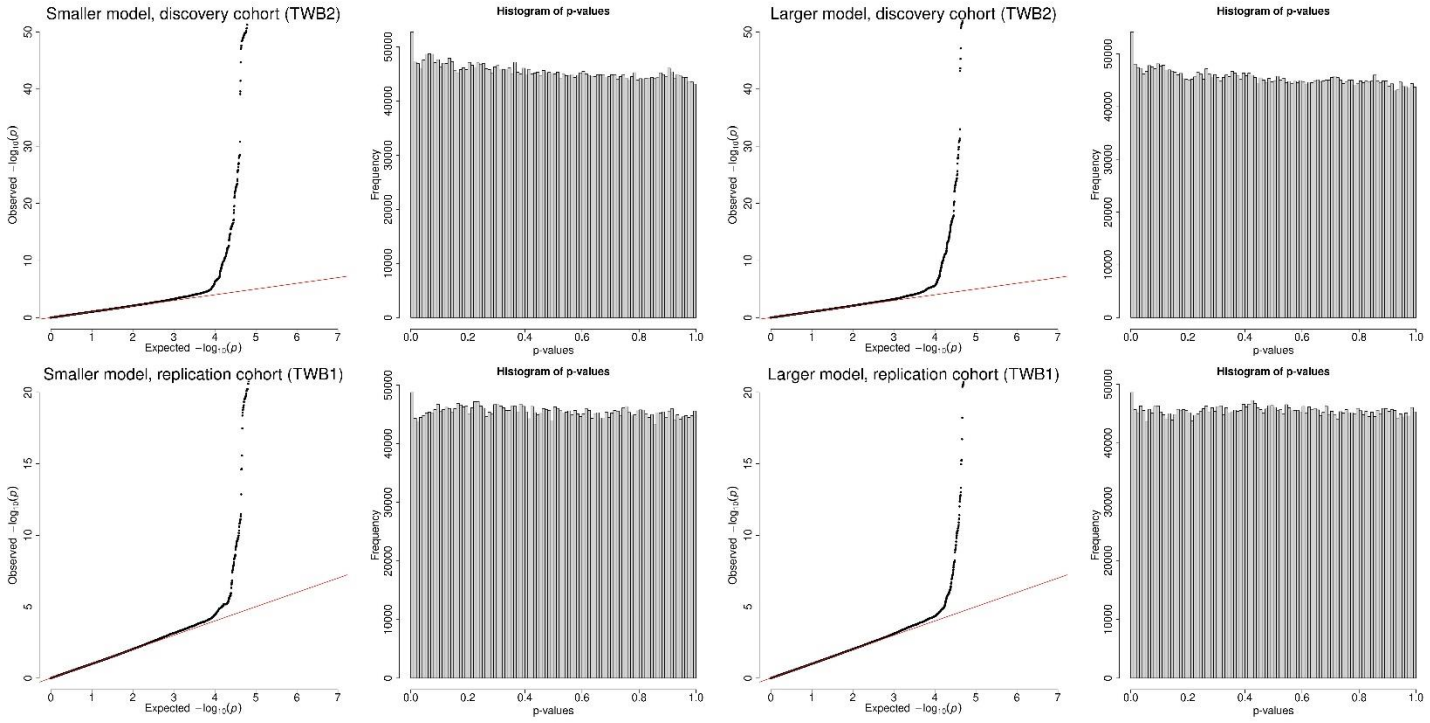

Figure 1. **The quantile-quantile (Q-Q) plots and histograms of the vQTLs' *p*-values.** In the Q-Q plots, the red lines depict that the observed *p*-values (of the scale test) correspond to the expected *p*-values (of the scale test). The discovery (TWB2) and replication (TWB1) cohorts comprised 86,536 and 25,460 individuals, respectively. The histograms at the right of the Q-Q plots demonstrate the distributions of the original scale of *p*-values.

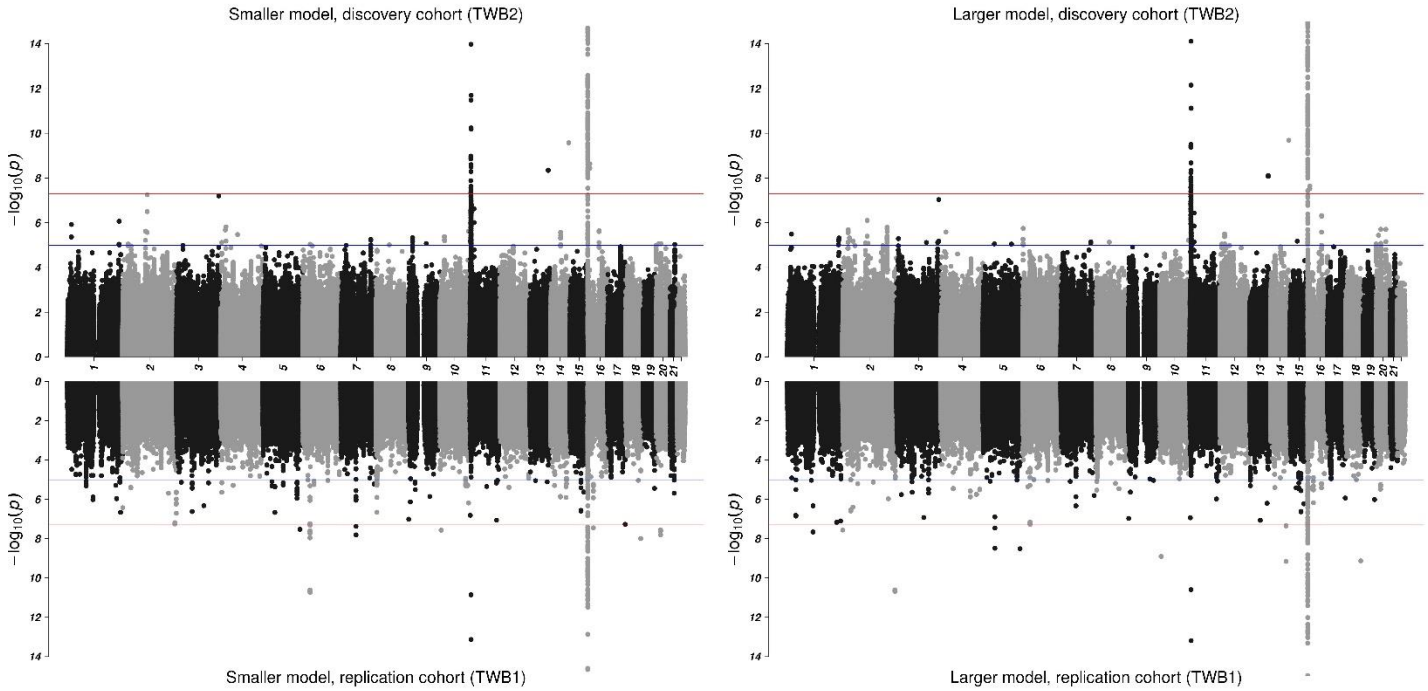

Figure 2. **The Miami plots for the vQTL analyses.** The left and right columns show the results from the smaller and larger models, respectively. The top and bottom rows present the results from the discovery (TWB2, n = 86,536) and replication (TWB1, n = 25,460) cohorts. The horizontal red and blue lines mark the genome-wide significance level (5E-8) and the suggestive significance level (1E-5).

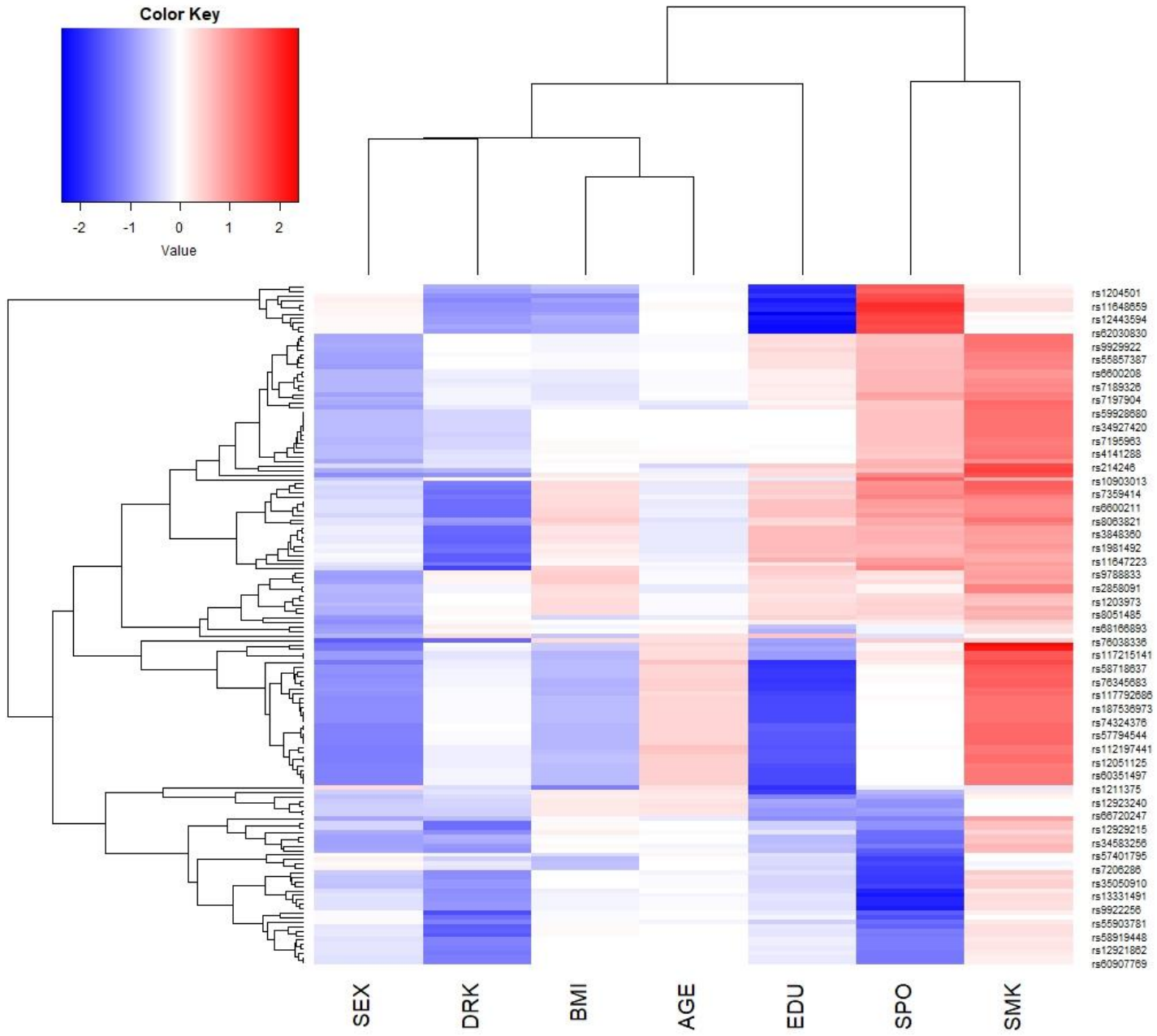

Figure 3. **The phylogenetic heat map of the gene-environment interaction analyses for PhenoAgeAccel.** The magnitude of the value represents $-\log_{10}$(two-sided *p*-value of the SNP-E interaction), which is always positive. However, I deliberately added a positive/negative sign in front of the magnitude. A positive sign indicates that the environmental factor (E) exacerbates the PhenoAgeAccel-vQTLs' aging effects. In contrast, a negative sign suggests that the E attenuates the PhenoAgeAccel-vQTLs' aging effects (detailed values listed in Table S1 columns N-T). The *x*-axis lists the 7 Es, including SEX (female vs. male), DRK (alcohol consumption, yes vs. no), BMI (in kg/m^2), AGE (chronological age, in years), EDU (educational attainment, an integer from 1 to 7), SPO (performing regular exercise, yes vs. no), and SMK (cigarette smoking status, yes vs. no). The total

sample size was n = 25,460 + 86,536 = 111,996.

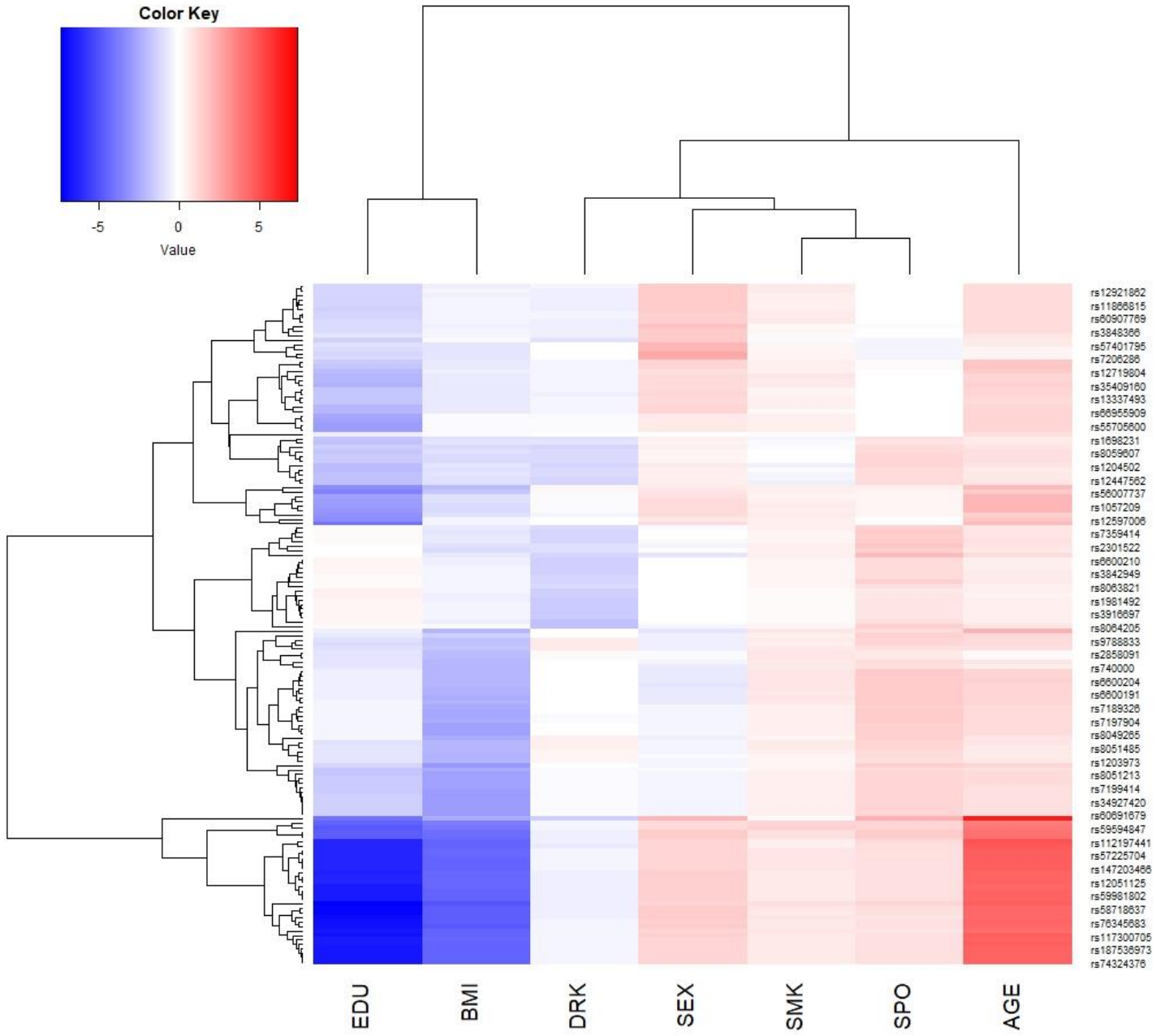

Figure 4. **The phylogenetic heat map of the gene-environment interaction analyses for mean corpuscular volume (MCV).** The magnitude of the value represents $-\log_{10}$(two-sided p-value of the SNP-E interaction), which is always positive. However, I deliberately added a positive/negative sign in front of the magnitude. A positive sign indicates that the environmental factor (E) exacerbates the MCV-vQTLs' effects. In contrast, a negative sign suggests that the E attenuates the MCV-vQTLs' effects (detailed values listed in Table S2 columns N-T). The x-axis lists the 7 Es, including SEX (female vs. male), DRK (alcohol consumption, yes vs. no), BMI (in kg/m^2), AGE (chronological age, in years), EDU (educational attainment, an integer from 1 to

7), SPO (performing regular exercise, yes vs. no), and SMK (cigarette smoking status, yes vs. no). The total sample size was n = 25,460 + 86,536 = 111,996.

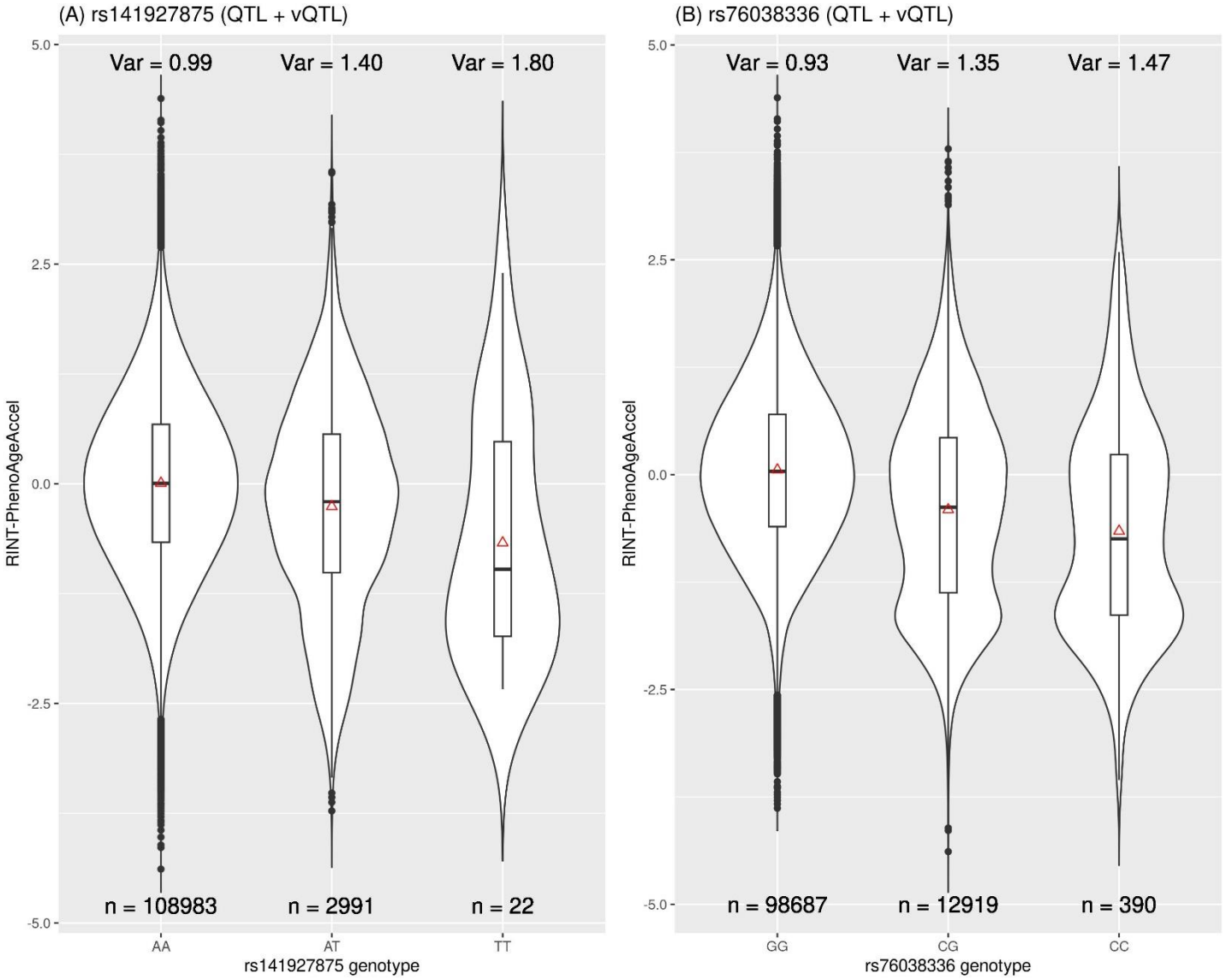

Figure 5. **The violin plots (combining box plots and kernel density plots) of RINT-PhenoAgeAccel according to the genotypes of rs141927875 and rs76038336.** The red triangles mark the mean RINT-PhenoAgeAccel of each genotype group, whereas the black segments inside the boxes are the medians of RINT-PhenoAgeAccel of each genotype group. The numbers at the bottom of the figure are the sample sizes of the three genotypes. The total sample size was n = 25,460 + 86,536 = 111,996.

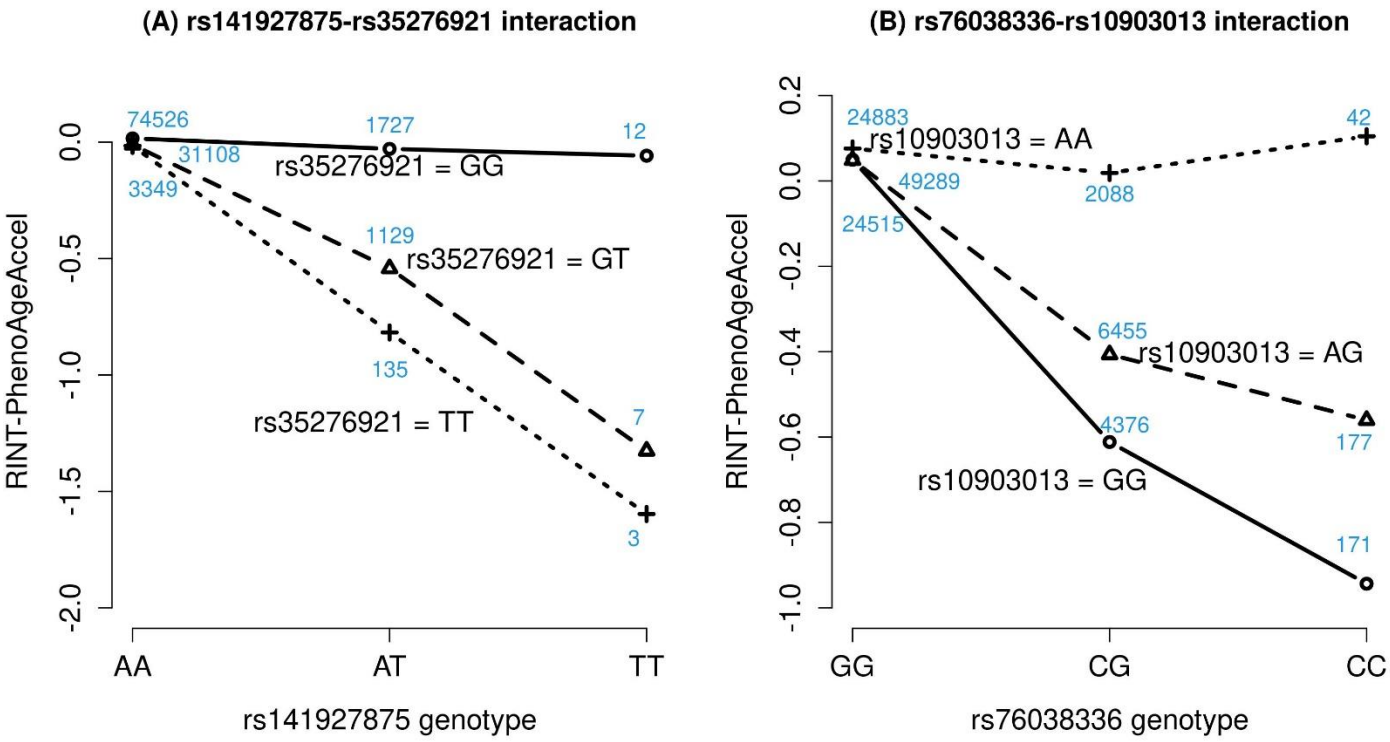

Figure 6. **rs141927875-rs35276921 and rs76038336-rs10903013 interaction plots.** Each point is the mean RINT-PhenoAgeAccel of a genotype combination. The blue numbers shown around points are the sample sizes of the genotype combinations. The total sample size was n = 25,460 + 86,536 = 111,996.