Clinical Epigenetics

RESEARCH

Open Access

Influence of physical activity on the epigenetic clock: evidence from a Japanese cross-sectional study

Masatoshi Nagata[1], Shohei Komaki[1], Yuichiro Nishida[2], Hideki Ohmomo[1], Megumi Hara[2], Keitaro Tanaka[2] and Atsushi Shimizu[1*]

Abstract

Background Biological age, especially epigenetic age derived from the epigenetic clock, is a significant measure of aging, considering the differences in aging rates among individuals. The epigenetic clock, a machine learning-based algorithm, uses DNA methylation states to estimate biological age. Previous studies have reported inconsistent associations between physical activity (PA) and the epigenetic clock, especially second-generation clocks such as PhenoAge and GrimAge. This study aimed to clarify this relationship using cross-sectional data from Japanese participants aged 40–69.

Methods We used two datasets from the Saga J-MICC study, of which 867 samples were available for analysis. DNA methylation data from peripheral blood samples were used to calculate the epigenetic age using the epigenetic clocks PhenoAge and GrimAge. PA and sedentary time were measured using a single-axis accelerometer, while self-reported PA, sedentary time, and covariates were assessed using a self-administered questionnaire. The association between PA or sedentary time and epigenetic age acceleration was assessed using multiple linear regression.

Results Pearson's correlation coefficients between accelerometer-based and self-reported PA variables ranged from 0.09 to 0.20. Multivariable regression analysis showed that accelerometer-based PA and sedentary time were associated with epigenetic age decelerations and accelerations, respectively. However, self-reported PA was not associated with the epigenetic age accelerations.

Conclusions These results indicate that reducing sedentary time and increasing PA were associated with slowing both PhenoAge and GrimAge, even in East Asian populations with different exercise habits, body shapes, and lifestyles. This study highlights the potential of objective second-generation epigenetic age acceleration as an outcome index for healthcare interventions and clinical applications.

Keywords Epigenetic clock, Age acceleration, Physical activity, Sedentary, Accelerometer-based measurement

Background

Biological age is an important indicator of aging because the aging rate varies among individuals [1–4]. Many types of biological age predictors have been proposed, and epigenetic age based on the epigenetic clock has received particular attention in recent years as the most reliable biological age predictor [5–7]. The epigenetic clock is a machine learning-based algorithm for calculating

*Correspondence:
Atsushi Shimizu
ashimizu@iwate-med.ac.jp
[1] Division of Biomedical Information Analysis, Institute for Biomedical Sciences of Iwate Medical University, 1-1-1 Idaidori, Yahaba, Shiwa, Iwate 028-3694, Japan
[2] Department of Preventive Medicine, Faculty of Medicine, Saga University, Saga, Japan

epigenetic age using several DNA methylation states of cytosine–phosphate–guanine (CpG) sites. Chronological age-based models, called first-generation clocks, are represented by the Horvath and Hannum clocks [8, 9]. More recently, second-generation clocks trained to reflect age-related diseases and physiological conditions were developed by Levine et al. and Lu et al., and are commonly referred to as PhenoAge and GrimAge, respectively [10, 11]. Epigenetic clocks are expected to be useful for health promotion and disease prevention.

Many studies have shown a relationship between the epigenetic clock and lifestyle factors [12–15]. In particular, its association with physical activity (PA) is important because it is a good candidate for behavioral change interventions in public health [16, 17]. It reduces stress, improves immune and cognitive functions, and has anti-inflammatory effects [18–20]. Therefore, understanding the causal associations between PA and the epigenetic clock is expected to facilitate healthcare intervention and clinical application of epigenetic clocks as an outcome index of aging [21]. Several studies have reported an association between PA and the epigenetic clock, primarily in second-generation clocks; however, the extent and direction of this association are inconsistent [12, 21–26]. This could be because PA is usually assessed based on self-reporting and lacks objectivity and quantifiability [27]. Recent studies using accelerometers showed that second-generation clocks, especially GrimAge, were negatively associated with PA [25, 26]. However, these studies have mainly been conducted in European populations, and there are few studies with large sample sizes in East Asian populations, including Japanese [28]. According to international comparative studies, insufficient PA and sedentary behaviors are highly prevalent among Japanese people, even though they are relatively less obese and have longer life expectancies [29–31]. It is unclear how PA and sedentary time are associated with epigenetic aging in Japanese people of different races and lifestyles compared to those in Western countries.

This study aimed to elucidate the relationship between epigenetic age and accelerometer-based PA/sedentary time using cross-sectional data from Japanese participants aged 40–69. To this end, we primarily investigated the differences in sedentary time and total PA, and secondarily, its components: light-intensity PA (LPA) and moderate-to-vigorous-intensity PA (MVPA). Furthermore, we explored the effect on epigenetic age acceleration (AgeAccel) by replacing one PA with another using an isotemporal substitution model [32]. We mainly used the second-generation clocks PhenoAge and GrimAge, with a focus on epigenetic AgeAccel, because, as noted above, these models have been previously suggested to be associated with PA. Additionally, the Horvath and Hannum clocks were calculated to compare correlations among epigenetic clocks.

Methods

Study population

Study participants were selected from the Saga J-MICC study, which is part of the Japan Multi-Institutional Collaborative Cohort Study. The study participants were aged 40–69 years at the time of the baseline survey (2005–2007) [33]. The subjects and methods of the Saga J-MICC Study have been previously described [33]. Written informed consent was obtained from all participants, and the study protocol was approved by the Ethics Committee of the Saga University Faculty of Medicine and Iwate Medical University. Two types of baseline surveys from the Saga J-MICC study, a random sample and a case–control study, were selected for DNA methylation analysis. The case–control study samples were subjects of a previously conducted nested case–control stroke study [34]. All individuals were classified as stroke-free at the time of data collection during the baseline survey. Consequently, all samples were included in this study.

Assessment of peripheral blood DNA methylation levels

DNA methylation data were obtained from Nishida et al. [35]. Briefly, a DNA sample (> 500 ng) was subjected to bisulfite treatment using a commercial kit (EZ DNA Methylation™ Kit; Zymo Research Corporation, CA, USA). In the random sample ($n = 507$), the Infinium MethylationEPIC BeadChip (EPIC array; Illumina, Inc., San Diego, CA) was used, while in the case–control study sample ($n = 391$), the Infinium HumanMethylation450 BeadChip (HM450 array; Illumina, Inc.) was used. The CpG sites on sex chromosomes and those with a high frequency of missing data (call rate < 95%) were excluded from the total number of CpG sites targeted by the EPIC array (865,859 CpG sites). DNA methylation levels at 840,178 CpG sites were analyzed using an EPIC array. Similarly, in the case of the HM450 array (485,512 CpG sites), CpG sites on sex chromosomes (11,648 CpG sites) and those with a call rate < 95% (3,147 CpG sites) were excluded. Consequently, the DNA methylation levels at 470,717 CpG sites were analyzed using the HM450 array.

Epigenetic age calculation

The epigenetic age of each individual was calculated using DNA methylation data from the Horvath Lab online calculator (https://dnamage.genetics.ucla.edu/). Methylation imputation was performed for some missing CpGs to calculate the epigenetic age in the calculator. AgeAccel was calculated as the residual from the regression of the epigenetic age on the chronological age. For further

validation, additional experiments were conducted using GrimAge version 2 in the same manner as GrimAge [36].

Assessment of accelerometer-based PA and sedentary time

The PA and covariates were measured as previously described [37–40]. In the baseline survey of the Saga J-MICC study, habitual PA was measured using a single-axis accelerometer (Life-Corder; Suzuken Co., Ltd., Nagoya, Japan), which was validated in a previous study [41]. The participants wore an accelerometer on their waist for 10 days, except during bathing and sleeping, and were instructed to maintain their normal lifestyle. Data from the first 3 days were excluded to account for potential changes in physical activity due to increased motivation from wearing the accelerometers. The remaining 7 days were used for analysis, with valid data defined as wearing the accelerometer for at least 8 h per day for at least 4 days. These conditions were similar to those used in previous studies [37–40]. The accelerometer measured 11 acceleration intensity levels (0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, and 9). In this study, sedentary time was defined as an acceleration intensity of 0.5, equivalent to < 1.8 metabolic equivalents of tasks (METs), including activities such as postural changes or light deskwork. Although not identical, this definition is similar to the general definition of < 1.5 METs. LPA (< 3 METs) was defined as the daily PA time (hours per day) with an acceleration intensity level of 1–3, and MVPA (> 3 METs) was defined as the daily PA time (hours per day) with an acceleration intensity level of 4–9. Daily total PA (in MET hours per day), which included light, moderate, and vigorous PA, was calculated by multiplying METs by the time spent at each intensity level. Accelerometer wear time is the sum of sedentary time, LPA, and MVPA.

Assessment of self-reported PA, sedentary time, and covariates

Self-reported PA, sedentary time, and covariates were assessed as previously described [42]. Briefly, the participants received a self-administered questionnaire before the baseline survey, which collected comprehensive data on various factors including cigarette smoking, alcohol consumption, medication, disease history, diet, physical measurements, sleeping time, and PA. Specific details were provided for current smokers and drinkers, and ethanol consumption by drinkers was estimated based on the type of alcoholic beverage consumed. Height and body weight were measured to calculate body mass index (BMI).

To assess sedentary behavior and PA time, we calculated sedentary time, LPA, and MVPA corresponding to an activity intensity of < 1.8, ≥ 1.8 and < 3, and ≥ 3 METs, respectively. This was determined by summing daily life PA and leisure-time PA, as evaluated using a self-administered PA questionnaire (PAQ) originally developed as part of the J-MICC Study baseline questionnaire. Although the validity and reproducibility of this PAQ are limited [43], it follows a format similar to the International PA Questionnaire (IPAQ), which is used as an international standard. Consequently, this PAQ has been employed in many previous studies [42, 44]. For daily life PA, each self-reported activity was assigned a specific MET value based on the Compendium of Physical Activities [45]. The PAQ categorized activities into four levels: sedentary behavior (allocated 1.5 METs), standing (2.0 METs), walking (3.3 METs), and hard labor (4.5 METs). For example, walking (assumed speed of 4.86 km/h) is assigned as 3.3 METs. Time spent on each activity per day was categorized into eight groups (assigned average hours per day): none (0), < 1 (0.5), 1 to < 3 (2), 3 to < 5 (4), 5 to < 7 (6), 7 to < 9 (8), 9 to < 11 (10), and ≥ 11 (12) h per day. For leisure-time PA, the PAQ closely resembled the IPAQ's format and included all PA types. It was categorized into three intensity levels: light (3.3 METs), moderate (4.0 METs), and vigorous (8.0 METs). The questionnaire also asked about activity frequency and duration. Thus, the daily activity time was determined for sedentary time, LPA, and MVPA in hours per day. Furthermore, total PA (MET·h/day) was estimated by summing the daily life and leisure-time PA, which was calculated by multiplying daily frequency, duration, and intensity of activities.

Statistical analysis

Statistical analyses were performed using the R software (version 4.1.3, R Foundation). The mean and standard deviation for continuous variables, and the number and percentage of categorical variables were calculated. The participants' ages were calculated based on the date on which the survey was conducted and converted to age in years.

Pearson's correlation coefficients were calculated for the correlation analysis between the accelerometer-based and self-reported variables. To align the time available for an individual's activity, each variable was divided by wear time or awake time.

The association between PA or sedentary time and epigenetic AgeAccel (outcome) was assessed using multiple linear regression as a single-factor model, with adjustments for wear time. Two models with different covariates were used to account for confounding effects. We adjusted for age [continuous (years)], sex [categorical], number of daily smoking [continuous (pieces)], alcohol consumption [continuous (g of ethanol/day)], years of education [continuous (years)], microarray platform [categorical], and wear time [continuous (hours)] (Model 1).

To assess the self-reported activity variable, awake time (continuous [hours]), which was calculated by subtracting the sleeping time from 24 h, was used instead of the wear time. Associations were further adjusted for BMI (continuous [kg/m^2]), waist circumference (continuous [cm]), and energy intake (continuous [kcal/day]) (Model 2) because these are possible intermediate factors for epigenetic aging. Previous studies have adjusted for blood cell type composition as a covariate by calculating cell type proportions using specific DNA methylation data [23, 25, 26]. However, because exercise can alter the immune cell composition, we did not include cellular composition as a confounding variable because these changes are also an indicator of aging.

In the multiple linear regression analysis, a hierarchical testing procedure was used to control for Type I errors. We primarily investigated the association of AgeAccel with sedentary time and total PA. If the association with total PA was significant, we further tested its components, LPA and MVPA, using Bonferroni correction. Thus, if the total PA was not significant, subsequent tests of its components were considered insignificant. A two-sided p-value less than 0.05 was considered statistically significant. To examine whether there was a quadratic association between PA and epigenetic aging, we tested an additional quadratic term for PA. We also tested the interaction term, PA × sedentary time. The variance inflation factor (VIF) was calculated to check for multicollinearity.

As an exploratory post hoc analysis, isotemporal substitution analysis was performed using both accelerometer-based and self-reported regression models [32]. In this analysis, two of the three variables (sedentary time, LPA, and MVPA) were included in the model to assess changes in activity from the remaining variable to the variable of interest, while holding one of them constant. For example, when LPA time and MVPA are included in the model as variables, the partial regression coefficient for MVPA shows the amount of change when one unit (1 h per day) of activity increases from sedentary time to MVPA, while keeping the LPA fixed.

Results

Participant characteristics

Two datasets from the Saga J-MICC study were used: a case–control study sample and a random sample. A case–control study sample was measured using HM450, and a random sample was measured using EPIC. In total, 867 samples were available for data analysis (Fig. 1). The overall characteristics are listed in Table 1, and the details of each dataset are listed in Additional file 2: Table S1. Approximately 30% of males and 20% of females had a body mass index (BMI) > 25. Additionally, the average sedentary time was 11.5 h, whereas the average time spent on PA (the sum of LPA and MVPA time) was 1.3 h per day.

Comparison of accelerometer-based and self-reported variables

To ascertain the similarity between accelerometer-based and self-reported variables, Pearson's correlations were calculated and compared (Fig. 2). As anticipated from the definition, LPA and MVPA exhibited strong correlations with total PA, specifically 0.7 and 0.88, respectively. The correlation coefficients showed similar trends in the accelerometer-based and self-reported categories. For

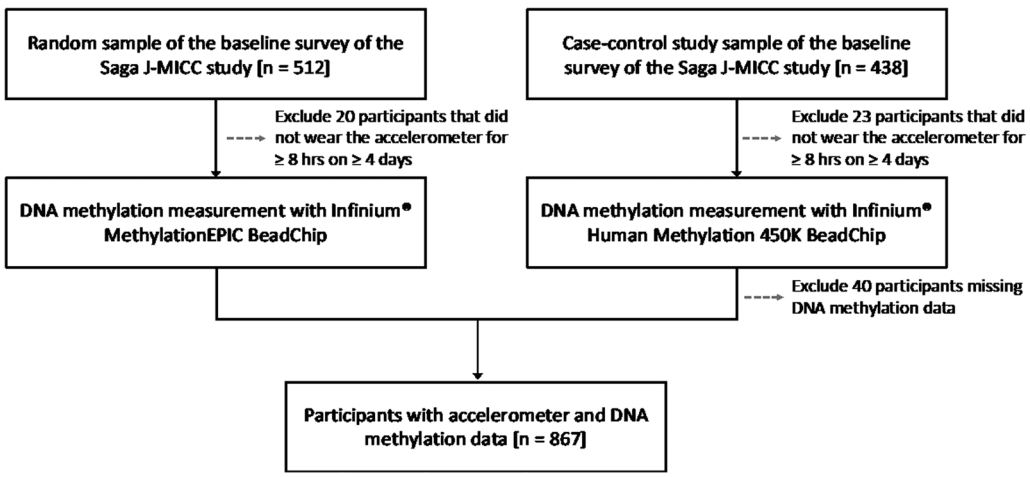

Fig. 1 Flowchart of the study design. In the Saga J-MICC study baseline survey, habitual PA was measured using a single-axis accelerometer. The random sample was measured with EPIC BeadChip, and the case–control study sample was measured with HM450. The valid data from participants who wore the accelerometer on their waist for at least 8 h per day for at least 4 d were used

Table 1 Demographic characteristics of the participants

Characteristics	All participants ($n = 867$) Mean (SD) or *n* (%)
Age, years	58.3 (8.0)
Female, number of participants (%)	392 (45.2%)
Education, years	13.2 (2.1)
Smoking status, cigarettes day^{-1}	4.8 (10.3)
Number of current smokers (%)	194 (22.4%)
Alcohol drinking, g ethanol day^{-1}	15.8 (23.3)
Number of participants consuming 23 g of ethanol daily	219 (25.3%)
BMI, kg m^{-2}	23.1 (3.0)
Number of participants with a BMI over 25 (%)	226 (26.1%)
Waist, cm	84.3 (8.9)
Total energy intake, kcal day^{-1}	1,751.6 (376.9)
Wear time, hour day^{-1}	12.9 (1.7)
Total PA, MET hour day^{-1}	3.7 (1.7)
LPA, hour day^{-1}	1.0 (0.4)
MVPA, hour day^{-1}	0.3 (0.3)
Sedentary time, hour day^{-1}	11.5 (1.7)
Self-reported total PA, MET hour day^{-1}	21.4 (11.8)
Self-reported LPA, hour day^{-1}	3.5 (2.5)
Self-reported MVPA, hour day^{-1}	4.2 (2.7)
Self-reported sedentary time, hour day^{-1}	6.5 (3.1)
Horvath age, years	56.1 (7.8)
Hannum age, years	52.3 (11.5)
PhenoAge, years	44.8 (10.7)
GrimAge, years	61.9 (7.8)
HorvathAgeAccel, years	0.0 (4.3)
HannumAgeAccel, years	0.0 (4.4)
PhenoAgeAccel, years	0.0 (5.5)
GrimAgeAccel, years	−0.1 (4.9)

BMI Body mass index, *MET* Metabolic equivalents, *PA* Physical activity, *LPA* Light-intensity physical activity, *MVPA* Moderate-to-vigorous-intensity physical activity

each corresponding variable, the correlation coefficient was calculated as 0.20 for total PA, 0.15 for LPA, 0.09 for MVPA, and 0.19 for sedentary time.

Epigenetic ages and epigenetic age accelerations

Four epigenetic clocks were used to calculate the epigenetic age. In the EPIC array samples, 19 of 353 CpGs in the Horvath clock, six CpGs of 71 CpGs in the Hannum clock, and two CpGs of 513 CpGs in PhenoAge were missed. In the HM450 array data, no CpGs were missing in any of the three clocks. For GrimAge, the clock probe contained 1,030 CpGs, although the actual probe IDs were not available; therefore, it is not clear how many missing CpGs were in our data. Each epigenetic age strongly correlated with the chronological age, with Pearson's correlation coefficients ranging from 0.72 to 0.88. Although epigenetic ages differed slightly by gender and platform, the correlation coefficients were above 0.59 for each combination (Additional files 1: Figure S1 and S2). The highest correlations among the AgeAccels were observed between HannumAgeAccel and PhenoAgeAccel, whereas the lowest correlations were observed between HorvathAgeAccel and GrimAgeAccel.

Associations between PA and epigenetic age acceleration

A single-factor model was employed in the regression analysis, and two models with different covariates were analyzed to account for confounding effects (Table 2). Model 1 was adjusted for age, sex, smoking, alcohol consumption, education, microarray platform, and wearing or awake time. In Model 2, the covariates were further adjusted for BMI, waist circumference, and energy intake. Among the accelerometer-based variables, PhenoAgeAccel and GrimAgeAccel showed significant negative associations with accelerometer-based total PA (Fig. 3a). PhenoAgeAccel also showed a negative association with

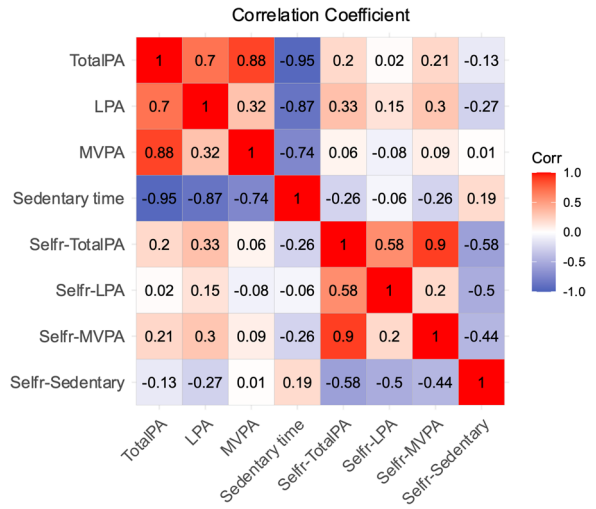

Fig. 2 Heat map of pairwise correlation of PA/sedentary time variables. *PA* Physical activity, *LPA* Light-intensity physical activity, *MVPA* Moderate-to-vigorous-intensity physical activity, *Selfr* Self-reported

LPA. For accelerometer-based sedentary time, positive associations were observed with PhenoAgeAccel and GrimAgeAccel in both models, although GrimAgeAccel did not reach significance in Model 2. However, none of the self-reported variables was associated with PhenoAgeAccel or GrimAgeAccel in either model (Fig. 3b). Similar results were obtained using GrimAge version 2 (Additional file 2: Table S2), although 111 of 1,331 probes were missing CpGs. In this regression analysis, there was a possibility that PA/sedentary time had a quadratic association with epigenetic AgeAccel, as Fox et al. reported [26]; thus, we tested the quadratic term by adding it to the regression formula. Only LPA showed a tendency toward a quadratic association with PhenoAgeAccel, although we did not consider it significant because the total PA was not significant (Additional file 2: Table S3). Furthermore, we tested the interaction between PA and sedentary time by adding a PA×sedentary term and found no association with AgeAccel (Additional file 2: Table S4). The VIF exceeded 11 in the accelerometer-based single-factor model when the LPA was included. In all other cases, VIF was less than five, indicating no multicollinearity.

Exploring activity substitution effects on epigenetic aging

To investigate the impact of replacing one PA with another on epigenetic AgeAccel, we applied an isotemporal substitution model for each sex subgroup because

Table 2 Association between PA/sedentary time and epigenetic age acceleration by multivariable regression analysis of the single-factor model

	PhenoAgeAccel		GrimAgeAccel	
	β (95% CI)	***p*-value**	**β (95% CI)**	***p*-value**
(Model 1)				
Total PA	**− 0.27 (− 0.49– − 0.04)**	**0.020***	**− 0.16 (− 0.30– − 0.02)**	**0.025***
LPA	**− 1.20 (− 2.22– − 0.18)**	**0.022***	− 0.51 (− 1.16–0.14)	0.122
MVPA	− 1.08 (− 2.51–0.36)	0.141	− 0.86 (− 1.77–0.05)	0.064
Sedentary time	**0.89 (0.16–1.62)**	**0.017***	**0.48 (0.02–0.95)**	**0.041***
Self-reported total PA	0.01 (− 0.02–0.04)	0.550	− 0.00 (− 0.02–0.02)	0.757
Self-reported LPA	0.06 (− 0.09–0.21)	0.420	− 0.01 (− 0.11–0.08)	0.801
Self-reported MVPA	0.04 (− 0.10–0.17)	0.603	− 0.00 (− 0.09–0.08)	0.958
Self-reported sedentary time	− 0.07 (− 0.20–0.05)	0.241	− 0.04 (− 0.12–0.04)	0.312
(Model 2)				
Total PA	**− 0.26 (− 0.48– − 0.03)**	**0.028***	**− 0.16 (− 0.30– − 0.01)**	**0.032***
LPA	**− 1.23 (− 2.29– − 0.18)**	**0.022***	− 0.46 (− 1.13–0.21)	0.180
MVPA	− 0.97 (− 2.43–0.48)	0.189	− 0.87 (− 1.80–0.05)	0.063
Sedentary time	**0.88 (0.13–1.62)**	**0.022***	0.46 (− 0.01–0.94)	0.057
Self-reported total PA	0.01 (− 0.02–0.04)	0.540	− 0.00 (− 0.02–0.02)	0.839
Self-reported LPA	0.06 (− 0.09–0.21)	0.420	− 0.01 (− 0.11–0.09)	0.835
Self-reported MVPA	0.04 (− 0.10–0.18)	0.583	0.00 (− 0.08–0.09)	0.936
Self-reported sedentary time	− 0.08 (− 0.20–0.05)	0.223	− 0.05 (− 0.12–0.03)	0.245

Model 1: adjusted for age, sex, years of education, alcohol consumption (g/day), smoking status (cigarettes/day), array type, and wear or awake time

Model 2: additionally adjusted for BMI ($kg.m^{-2}$), waist circumference (cm), and energy intake (kcal/day)

Bold values indicate statistical significance

PA Physical activity, *LPA* Light-intensity physical activity, *MVPA* Moderate-to-vigorous-intensity physical activity

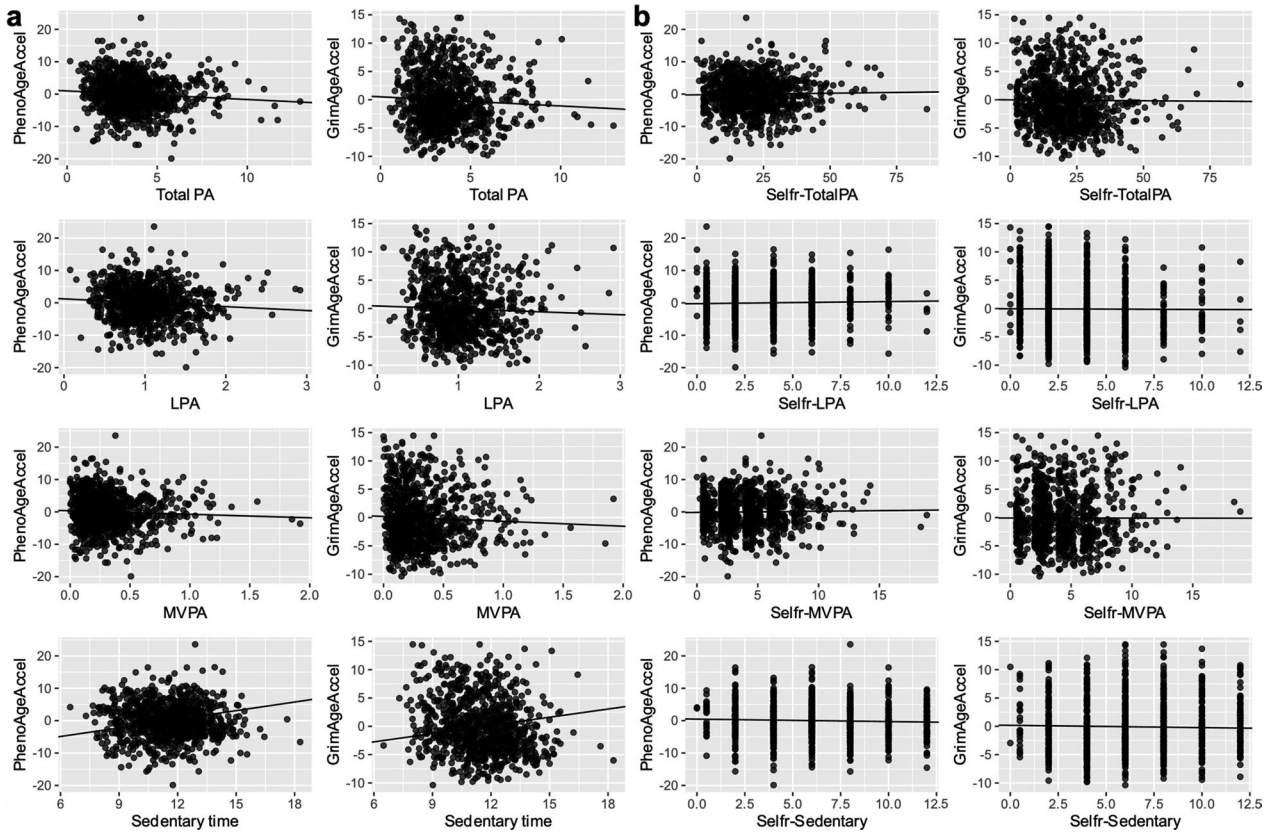

Fig. 3 Scatterplot of association between PA/sedentary time and epigenetic age acceleration. Regression lines were adjusted by age, sex, number of daily smoking, alcohol drinking, years of education, microarray platform, and wear or awake time. **a**, accelerometer-based variables. **b**, self-reported variables. *PA* Physical activity, *LPA* Light-intensity physical activity, *MVPA* Moderate-to-vigorous-intensity physical activity, *Selfr* Self-reported

physical body compositions, such as BMI and waist circumference, generally differ by sex. This analysis calculated the associations with AgeAccel by replacing one unit (one hour) of sedentary time with MVPA or LPA and LPA with MVPA (Table 3). In both models, replacing sedentary individuals with LPA was negatively associated with the GrimAgeAccel in females. Replacing sedentary activity with LPA and LPA with MVPA was also negatively associated with GrimAgeAccel in males in Model 2. The VIF was greater than 26 in the accelerometer-based isotemporal substitution analysis when LPA was included in the model. In other cases, the VIF was less than five and could be regarded as having no multicollinearity.

Discussion

In the current study, we demonstrated the association between accelerometer-based PA and the second-generation epigenetic clock using Japanese cross-sectional data, supporting the preliminary evidence that objectively measured PA is negatively associated with the second-generation epigenetic clock, even in East Asians, whereas self-reported PA showed no significant association with epigenetic age acceleration.

We compared the PA data of accelerometer-based and self-report, and the correlation was 0.09 to 0.20 among each variable. For accelerometer-based variables, total PA was calculated by multiplying metabolic equivalents (METs) by the total activity time, which was the sum of LPA, MVPA, and sedentary time. Moreover, in the self-reported variables, the sum of the activity time should have matched the awake time, which was calculated by subtracting the sleeping time from 24 h, but the deviation was large. It might be possible to reduce the disparity between the accelerometer and self-reported data depending on the questionnaire used, although this was not the case with our data. Skender et al. reported that the Pearson correlation of objective and subjective PA was approximately zero to 0.5, by analyzing 57 articles that measured PA using accelerometry and questionnaires [27]. It is important to ensure validity when evaluating epigenetic age using self-reported activity levels as covariates.

Table 3 Association between PA/sedentary time and epigenetic age acceleration by multivariable regression analysis of the isotemporal substitution model

	PhenoAgeAccel (Male)		PhenoAgeAccel (Female)		GrimAgeAccel (Male)		GrimAgeAccel (Female)	
	β (95% CI)	*p*-value	*β* (95% CI)	*p*-value	*β* (95% CI)	*p*-value	*β* (95% CI)	*p*-value
(Model 1)								
Sedentary to MVPA	−0.26 (−2.08–1.55)	0.777	−1.29 (−4.03–1.45)	0.355	−1.05 (−2.33–0.22)	0.105	−0.42 (−1.85–1.01)	0.563
Sedentary to LPA	−1.14 (−2.45–0.18)	0.091	−0.86 (−2.76–1.05)	0.378	0.28 (−0.65–1.20)	0.559	−1.11 (−2.10–0.12)	0.029
LPA to MVPA	0.87 (−1.66–3.41)	0.499	−0.43 (−4.31–3.44)	0.826	−1.33 (−3.11–0.45)	0.143	0.69 (−1.33–2.71)	0.503
(Model 2)								
Sedentary to MVPA	−0.27 (−2.14–1.59)	0.775	−1.09 (−3.84–1.65)	0.434	−1.41 (−2.71–−0.11)	0.034	−0.27 (−1.68–1.15)	0.713
Sedentary to LPA	−1.2 (−2.58–0.19)	0.090	−0.77 (−2.69–1.15)	0.432	0.58 (−0.39–1.54)	0.243	−1.09 (−2.09–−0.10)	0.031
LPA to MVPA	0.92 (−1.72–3.56)	0.492	−0.32 (−4.21–3.56)	0.870	−1.99 (−3.83–−0.14)	0.035	0.83 (−1.18–2.84)	0.419

Model 1: adjusted for age, sex, years of education, alcohol consumption (g/day), smoking status (cigarettes/day), array type, and wear or awake time

Model 2: additionally adjusted for BMI ($kg.m^{-2}$), waist circumference (cm), and energy intake (kcal/day)

PA Physical activity, *LPA* Light-intensity physical activity, *MVPA* Moderate-to-vigorous-intensity physical activity

The epigenetic clocks used in this study have been previously validated in Asian populations [46]. Our results showed a similar trend in the correlation between AgeAccel (e.g., the correlation coefficient with HorvathAgeAccel is HannumAgeAccel > PhenoAgeAccel > GrimAgeAccel) in samples from Western country people samples [47]. This correlation is similar to that reported by Kawamura et al. for multiple epigenetic clocks in Japanese individuals [28]. Therefore, the results of the epigenetic clock AgeAccel are valid in our study.

Multivariable regression analysis using a single-factor model revealed a significant association between total PA and sedentary time and PhenoAge and GrimAge accelerations. We used two models with different covariates, and the results of the partial regression coefficients were almost identical. This indicates that the relationship between PA/sedentary time and AgeAccel is not substantially influenced by additional variables such as BMI, waist circumference, or energy intake calories, suggesting that PA/sedentary time and AgeAccel are closely related. Furthermore, isotemporal substitution analysis by sex showed that GrimAgeAccel slows down by replacing sedentary time with LPA in females and sedentary time with LPA or LPA with MVPA in males. These results suggest that effective behaviors differ between men and women or that the effects vary based on age and exercise habits. However, the isotemporal substitution analysis was an exploratory trial and we did not adjust for multiple comparisons; thus, the results should be interpreted with caution.

Previous studies using activity trackers have reported a negative relationship between PA and GrimAge. Although Fox et al. reported a quadratic association with PA [26], our data did not show a significant quadratic association. This difference may be attributed to the variations in the extent to which highly active individuals were included in the samples. Our results of single-factor Model 1 showed that an increase of one METs hour per day was associated with a two-month lower GrimAge, and a one-hour increase in sedentary time per day was associated with a half-year higher GrimAge, which is similar to a previous report [25]. However, studies on the association between objective PA and the PhenoAge were limited. Several studies reported that the PhenoAge clock is susceptible to short-term fluctuations [48, 49]. Sensitivity of the epigenetic clock may vary depending on the type and frequency of physical activity.

This study had several limitations. First, the measurement period of PA with the accelerometer device was at most 10 days, and the true amount of activity may not have been reflected because of restrictions on the amount of time the device was worn. Second, there are biases when calculating the epigenetic age. Owing to the differences in demographic characteristics between the case–control study sample (HM450) and random sample (EPIC), as well as variations in the missing CpGs between

HM450 and EPIC, it is conceivable that there were different biases related to epigenetic age in both cases. The number of missing CpGs in GrimAge was unknown, although 111 of the 1,333 CpGs were missing in GrimAge version 2, implying that a similar number of CpGs might have been missed. However, because AgeAccel is calculated as the residual of regressing epigenetic age on chronological age, it is estimated to be robust against missing CpG sites [50, 51]. In addition, our results for GrimAge version 2 were similar to those for GrimAge version 1, suggesting that the effect of bias due to missing CpG sites was small. Finally, our dataset comprised a limited sample of Japanese participants aged 40–69 from the Saga region. Further validation is needed in terms of ethnicity, age range of participants, sex proportion, and longitudinal studies.

Conclusions

In the current study, we demonstrated that accelerometer-based PA had a negative association and sedentary time was positively associated with both PhenoAge and GrimAge accelerations in the Japanese population, whereas self-reported PA had no significant association. Studies of the relationship between PA and epigenetic aging using accelerometers are limited. However, we demonstrated a negative association between AgeAccel of the second-generation epigenetic clock and PA in the East Asian population, which is characterized by distinct races and lifestyles compared to people in Western countries. This suggests that reducing sedentary time and engaging in regular physical activity may have antiaging effects.

Abbreviations

AgeAccel	Epigenetic age acceleration
BMI	Body mass index
CpG	Cytosine–phosphate–guanine
EPIC	Illumina MethylationEPIC BeadChip
HM450	Illumina HumanMethylation450 BeadChip
LPA	Light-intensity physical activity
MET	Metabolic equivalent of task
MVPA	Moderate-to-vigorous-intensity physical activity
PA	Physical activity
PAQ	Physical activity questionnaire
VIF	Variance inflation factor

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13148-024-01756-1.

Additional file1

Additional file2

Acknowledgements

We thank the study participants, including those who provided the study specimens and data. We appreciate the efforts of those involved in the application process, study recruitment, sample management, and data cleaning. We would also like to thank Editage (www.editage.jp) for the English language editing.

Author contributions

This study was conceptualized by MN, SK, and AS, and supervised by KT and AS. The analytical design was developed by MN, SK, YN, HO, and AS. Data curation was performed by SK, HO, and YN. Data analysis was performed by MN. All the authors interpreted the results. The manuscript was written by MN and reviewed by all authors. All the authors have read and approved the final version of this manuscript.

Funding

This study was supported by Grants-in-Aid for Scientific Research (Grant Numbers 17015018, 221S0001, 18390182, 20249038, 16H06277, 17H01554, 22H03468, and 22H04923 [CoBiA]) from the Japanese Ministry of Education, Culture, Sports, Science and Technology.

Availability of data and materials

Individual-level data cannot be made publicly available owing to informed consent.

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committees of Iwate Medical University (approval ID: MH2022-045), Saga University Faculty of Medicine (approval no. 17–11), and Nagoya University Graduate School of Medicine (approval no. 253). Throughout the study, only DNA methylation datasets that had already been processed and anonymized were accessed.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 29 February 2024 Accepted: 1 October 2024
Published online: 15 October 2024

References

1. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013;153(6):1194–217.
2. Jones MJ, Goodman SJ, Kobor MS. DNA methylation and healthy human aging. Aging Cell. 2015;14(6):924–32.
3. Bell CG, Lowe R, Adams PD, Baccarelli AA, Beck S, Bell JT, et al. DNA methylation aging clocks: challenges and recommendations. Genome Biol. 2019;20:1–24.
4. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. Hallmarks of aging: An expanding universe. Cell. 2023;186(2):243–78.
5. Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. Nat Rev Genet. 2018;19(6):371–84.
6. Li X, Ploner A, Wang Y, Magnusson PK, Reynolds C, Finkel D, et al. Longitudinal trajectories, correlations and mortality associations of nine biological ages across 20-years follow-up. Elife. 2020;9:51507.
7. Oblak L, Zaag J, Higgins-Chen AT, Levine ME, Boks MP. A systematic review of biological, social and environmental factors associated with epigenetic clock acceleration. Ageing Res Rev. 2021;69:101348.
8. Horvath S. DNA methylation age of human tissues and cell types. Genome Bio. 2013;14(10):1–20.
9. Hannum G, Guinney J, Zhao L, Zhang L, Hughes G, Sadda S, et al. Genome-wide methylation profiles reveal quantitative views of human aging rates. Mol Cell. 2013;49(2):359–67.
10. Levine ME, Lu AT, Quach A, Chen BH, Assimes TL, Bandinelli S, et al. An epigenetic biomarker of aging for lifespan and healthspan. Aging (Albany NY). 2018;10(4):573.

11. Lu AT, Quach A, Wilson JG, Reiner AP, Aviv A, Raj K, et al. DNA methylation GrimAge strongly predicts lifespan and healthspan. Aging (Albany NY). 2019;11(2):303.
12. Quach A, Levine ME, Tanaka T, Lu AT, Chen BH, Ferrucci L, et al. Epigenetic clock analysis of diet, exercise, education, and lifestyle factors. Aging (Albany NY). 2017;9(2):419.
13. Kim K, Zheng Y, Joyce BT, Jiang H, Greenland P, Jacobs DR Jr, et al. Relative contributions of six lifestyle-and health-related exposures to epigenetic aging: the coronary artery risk development in young adults (CARDIA) study. Clin Epigenet. 2022;14(1):85.
14. Galkin F, Kovalchuk O, Koldasbayeva D, Zhavoronkov A, Bischof E. Stress, diet, exercise: common environmental factors and their impact on epigenetic age. Ageing Res Rev. 2023;88:101956.
15. Si J, Chen L, Yu C, Guo Y, Sun D, Pang Y, et al. Healthy lifestyle, DNA methylation age acceleration, and incident risk of coronary heart disease. Clin Epigenet. 2023;15(1):52.
16. Lee I-M, Shiroma EJ, Lobelo F, Puska P, Blair SN, Katzmarzyk PT. Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. Lancet. 2012;380(9838):219–29.
17. Nieman DC, Wentz LM. The compelling link between physical activity and the body's defense system. J Sport Health Sci. 2019;8(3):201–17.
18. Ekelund U, Steene-Johannessen J, Brown WJ, Fagerland MW, Owen N, Powell KE, et al. Does physical activity attenuate, or even eliminate, the detrimental association of sitting time with mortality? A harmonised meta-analysis of data from more than 1 million men and women. Lancet. 2016;388(10051):1302–10.
19. Simpson RJ, Lowder TW, Spielmann G, Bigley AB, LaVoy EC, Kunz H. Exercise and the aging immune system. Aging Res Rev. 2012;11(3):404–20.
20. Hillman CH, Erickson KI, Kramer AF. Be smart, exercise your heart: exercise effects on brain and cognition. Nat Rev Neurosci. 2008;9(1):58–65.
21. Sillanpää E, Heikkinen A, Kankaanpää A, Paavilainen A, Kujala UM, Tammelin TH, et al. Blood and skeletal muscle ageing determined by epigenetic clocks and their associations with physical activity and functioning. Clin Epigenet. 2021;13(1):110.
22. Gale CR, Marioni RE, Cukiíc I, Chastin SF, Dall PM, Dontje ML, et al. The epigenetic clock and objectively measured sedentary and walking behavior in older adults: the Lothian Birth Cohort 1936. Clin Epigenet. 2018;10:1–6.
23. Kresovich JK, Garval EL, Martinez Lopez AM, Xu Z, Niehoff NM, White AJ, et al. Associations of body composition and physical activity level with multiple measures of epigenetic age acceleration. Am J Epidemiol. 2021;190(6):984–93.
24. Kankaanpää A, Tolvanen A, Bollepalli S, Leskinen T, Kujala UM, Kaprio J, Ollikainen M, Sillanpää E. Leisure-time and occupational physical activity associates differently with epigenetic aging. Med Sci Sports Exerc. 2021;53(3):487–95.
25. Spartano NL, Wang R, Yang Q, Chernofsky A, Murabito JM, Vasan RS, et al. Association of accelerometer-measured physical activity and sedentary time with epigenetic markers of aging. Med Sci Sports Exerc. 2023;55(2):264–72.
26. Fox FA, Liu D, Breteler MM, Aziz NA. Physical activity is associated with slower epigenetic ageing—findings from the Rhineland study. Aging Cell. 2023;22(6):13828.
27. Skender S, Ose J, Chang-Claude J, Paskow M, Brühmann B, Siegel EM, et al. Accelerometry and physical activity questionnaires-a systematic review. BMC Public Health. 2016;16(1):1–10.
28. Kawamura T, Radak Z, Tabata H, Akiyama H, Nakamura N, Kawakami R, et al. Associations between cardiorespiratory fitness and lifestyle-related factors with DNA methylation-based ageing clocks in older men: WASEDA'S Health Study. Aging Cell. 2023;23(1):13960.
29. Bauman A, Bull F, Chey T, Craig CL, Ainsworth BE, Sallis JF, et al. The international prevalence study on physical activity: results from 20 countries. Int J Behav Nutr Phys Act. 2009;6(1):1–11.
30. Bauman A, Ainsworth BE, Sallis JF, Hagströmer M, Craig CL, Bull FC, et al. The descriptive epidemiology of sitting: a 20-country comparison using the international physical activity questionnaire (IPAQ). Am J Prev Med. 2011;41(2):228–35.
31. Kitayama A, Koohsari MJ, Ishii K, Shibata A, Oka K. Sedentary time in a nationally representative sample of adults in Japan: prevalence and sociodemographic correlates. Prev Med Rep. 2021;23:101439.
32. Mekary RA, Willett WC, Hu FB, Ding EL. Isotemporal substitution paradigm for physical activity epidemiology and weight change. Am J Epidemiol. 2009;170(4):519–27.
33. Hara M, Higaki Y, Imaizumi T, Taguchi N, Nakamura K, Nanri H, et al. Factors influencing participation rate in a baseline survey of a genetic cohort in Japan. J Epidemiol. 2010;20(1):40–5.
34. Hachiya T, Kamatani Y, Takahashi A, Hata J, Furukawa R, Shiwa Y, et al. Genetic predisposition to ischemic stroke: a polygenic risk score. Stroke. 2017;48(2):253–8.
35. Nishida Y, Hara M, Ohmomo H, Ono K, Shimizu A, Horita M, et al. Epigenome-wide association study identified VTI1A DNA methylation associated with accelerometer-assessed physical activity. Med Sci Sports Exerc. 2022;54(11):1879–88.
36. Lu AT, Binder AM, Zhang J, Yan Q, Reiner AP, Cox SR, et al. DNA methylation GrimAge version 2. Aging (Albany NY). 2022;14(23):9484.
37. Nishida Y, Hara M, Higaki Y, Taguchi N, Nakamura K, Nanri H, et al. Habitual light-intensity physical activity and ASC methylation in a middle-aged population. Int J Sports Med. 2019;40(10):670–7.
38. Nishida Y, Higaki Y, Taguchi N, Hara M, Nakamura K, Nanri H, et al. Intensity-specific and modified effects of physical activity on serum adiponectin in a middle-aged population. J Endocr Soc. 2019;3(1):13–26.
39. Nishida Y, Hara M, Fuku N, Taguchi N, Horita M, Shimanoe C, et al. The interaction between mitochondrial haplogroups (M7a/D) and physical activity on adiponectin in a Japanese population. Mitochondrion. 2020;53:234–42.
40. Nishida Y, Hara M, Higaki Y, Taguchi N, Nakamura K, Nanri H, et al. Sedentary time, physical activity, and serum SPARC in a middle-aged population. Eur J Sport Sci. 2022;22(11):1786–94.
41. Kumahara H, Schutz Y, Ayabe M, Yoshioka M, Yoshitake Y, Shindo M, Ishii K, Tanaka H. The use of uniaxial accelerometry for the assessment of physical-activity-related energy expenditure: a validation study against whole-body indirect calorimetry. Br J Nutr. 2004;91(2):235–43.
42. Hara M, Nishida Y, Tanaka K, Shimanoe C, Koga K, Furukawa T, et al. Moderate-to-vigorous physical activity and sedentary behavior are independently associated with renal function: a cross-sectional study. J Epidemiol. 2023;33(6):285–93.
43. Fujii H, Yamamoto S, Takeda-Imai F, Inoue M, Tsugane S, Kadowaki T, Noda M. Validity and applicability of a simple questionnaire for the estimation of total and domain-specific physical activity. Diabetol Int. 2011;2(2):47–54.
44. Nishida Y, Hachiya T, Hara M, Shimanoe C, Tanaka K, Sutoh Y, et al. Japan Multi-institutional collaborative cohort study group. The interaction between ABCA1 polymorphism and physical activity on the HDL-cholesterol levels in a Japanese population. J Lipid Res. 2020;61:86–94.
45. Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, et al. Compendium of physical activities: an update of activity codes and MET intensities. Med Sci Sports Exerc. 2000;32(9 Suppl):S498-504.
46. Lin W-Y. Epigenetic clocks derived from western samples differentially reflect Taiwanese health outcomes. Front Genet. 2023;14:1089819.
47. Crimmins EM, Thyagarajan B, Levine ME, Weir DR, Faul J. Associations of age, sex, race/ethnicity, and education with 13 epigenetic clocks in a nationally representative US sample: the health and retirement study. J Gerontol A Biol Sci Med Sci. 2021;76(6):1117–23.
48. Xiao C, Beitler JJ, Peng G, Levine ME, Conneely KN, Zhao H, et al. Epigenetic age acceleration, fatigue, and inflammation in patients undergoing radiation therapy for head and neck cancer: a longitudinal study. Cancer. 2021;127(18):3361–71.
49. Komaki S, Ohmomo H, Hachiya T, Sutoh Y, Ono K, Furukawa R, et al. Evaluation of short-term epigenetic age fluctuation. Clin Epigenet. 2022;14(1):1–6.
50. McEwen LM, Jones MJ, Lin DTS, Edgar RD, Husquin LT, MacIsaac JL, et al. Systematic evaluation of DNA methylation age estimation with common preprocessing methods and the Infinium MethylationEPIC BeadChip array. Clin Epigenet. 2018;10(1):1–9.
51. Dhingra R, Kwee LC, Diaz-Sanchez D, Devlin RB, Cascio W, Hauser ER, et al. Evaluating DNA methylation age on the illumina MethylationEPIC bead chip. PLoS ONE. 2018;14(4):0207834.

Publisher's Note