보건학석사 학위논문

DNA Methylation Clock Analysis and Comparison in Korean with Type 2 Diabetes

제2형 당뇨병을 가진 한국인에서 DNA 메틸레이션 시계 분석 및 비교

2023년 2월

서울대학교 보건대학원

보건학과 유전체역학 전공

이 승 은

DNA Methylation Clock Analysis and Comparison in Korean with Type 2 Diabetes

제2형 당뇨병을 가진 한국인에서 DNA 메틸레이션 시계 분석 및 비교

지도교수 성 주 헌

이 논문을 보건학 석사 학위논문으로 제출함

2023년 2월

서울대학교 보건대학원

보건학과 유전체역학 전공

이 승 은

이승은의 석사 학위논문을 인준함

2023년 2월

위 원 장 원 성 호 (Seal)

부위원장 김 은 애 (Seal)

위 원 성 주 헌 (Seal)

Abstract

Background: Diabetes mellitus, a chronic disease whose prevalence is increasing worldwide, is important to manage because it causes systemic and complex complications. It is also known that the prevalence of diabetes tends to increase with age, and age-related diseases appear earlier in diabetic patients. Among them, type 2 diabetes is an important type of diabetes, accounting for 90% of all diabetes patients. On the other hand, DNA methylation is an emerging tool for studying the epigenetic aging mechanism. It is known to play an essential role in developing various diseases because it reflects the effects of the individual's environment and lifestyle and causes changes in gene expression. In addition, it can function as a clinically and epidemiologically important biomarker because it is stable, has low invasiveness, and is reversible.

Estimating age according to DNA methylated loci is called DNA methylation age (mAge), also called epigenetic biomarker of aging. The basic principle is to select hypermethylated or hypomethylated CpGs based on various health risks related to aging, such as cancer, obesity, and cardiovascular disease, and measure aging linearly through this. The modeling calculating the methylation age is called a DNA methylation clock, or a methylation clock. Meanwhile, DNA methylation clock tends to be underestimated in those aged 60-65 years and older. Furthermore, it lacks ethnic diversity, especially in Asians, so the tendency to underestimate the methylation age in the Korean diabetic cohort or the relationship between diabetes-related factors and methylation age was not confirmed yet.

Therefore, in order to find out the relationship between Koreans with type

2 diabetes and aging through the correlation between methylation age and the Korean diabetic cohort, this study investigated a relationship among nine DNA methylation clocks (Horvath, Hannum, PhenoAge, GrimAge, AAHorvath, AAHannum, AAPheno, AAGrim, and DunedinPACE), and part of the Seoul National University Hospital T2D cohort (SNUH), and part of the Ansan/Anseong Community-based Cohort (AS) of the Korean Genome and Epidemiology Study (KoGES).

Methods: The SNUH cohort consisted of 429 patients (232 patients with type 2 diabetes and 197 patients in the control group), and the AS cohort consisted of 400 patients (200 patients with type 2 diabetes and 200 patients in the control group). After receiving the raw DNA methylation data of the two groups, the methylation density was calculated as a beta value, from which the methylation age and acceleration were obtained. In addition, according to diabetes status, duration of diabetes, and stage of CKD, groups were divided as follows: diabetes group/control group, long duration group (more than 10 years)/short duration group (less than 10 years)/non-diabetic group, and CKD stage 5/CKD stage 4/CKD stage 3 or less with type 2 diabetes groups. Each group's methylation age and acceleration were plotted according to age, and the mean and variance were compared. By applying a multiple linear regression equation, it was confirmed that the clock significantly reflects fasting glucose, an index reflecting the results of diabetes and the primary criterion for diagnosing diabetes. In addition, the AICs of the multiple linear regression models for each clock in the entire cohort were compared. Through a Bland-Altman plot, it was confirmed that the Korean diabetic cohort also shows underestimation tendency of DNA methylation clocks. In addition, it was confirmed whether CpGs known to be related to type 2 diabetes were included in the clocks used in this study. Among those CpGs, CpGs that showed significant differences between groups in

this study were summarized.

Results: As a result of applying nine epigenetic clocks in the Korean diabetic cohort and examining them in various ways, the clocks in which diabetes status and progression of CKD stage in diabetic patients were reflected in aging were Horvath mAge/EAA, Hannum mAge/EAA, and DunedinPACE. In addition, in the group with more patients with accelerated aging due to aggravated progression of T2D, the glucose variable in the multiple linear regression model appeared significantly, and the underestimation tendency was prominent. The above indicated an association between the Korean diabetic cohort and mAge/EAA. Therefore, this study provides a basis for using mAge/EAA to study aging and complications in Korean diabetic patients. On the other hand, it was found that T2D-related CpGs with significant differences between the subgroups in the Korean diabetic cohort were not included more than 10 in the clocks. Therefore, to study aging and complications in Korean with T2D, we propose to create a next-generation clock that includes them. However, since the two cohorts differ in the composition of diabetes severity and the whole sample size is small, follow-up studies are needed to support this result. If a large sample size is obtained, it is proposed to conduct a study to see the difference in absolute values as well as the difference in slope in the plot of mAges/EAAs by fixing the age of the diabetic group and the control group equally.

Keywords: Type 2 Diabetes, Aging, DNA methylation clock, Epigenetic clock, Methylation age, Korean cohort

Student Number: 2021-23644

Table of Contents

List of Tables

List of Figures

I. Introduction

1. Study Background

1.1. Type 2 diabetes and aging

As of 2020, the prevalence of diabetes among adults aged 18 years and older in the United States is 14.7% (Below, the numbers in parentheses are the 95% confidence interval, 13.2-16.4), and the prevalence of diabetes among adults aged 65 years and older is 29.2% (26.4-32.1) (**Figure 1**). Among diabetic patients aged 18 years or older, 77.0% were diagnosed and knew about it [1]. In the same year, the prevalence of diabetes among adults aged 19 and older in Korea was 13.9% (13.3-14.5), and the prevalence of diabetes among adults aged 65 and older was 30.1% (28.8-31.4) (**Figure 2**). Among adults aged 30 years or older, 61.4% (59.9-62.9) of those who were diagnosed and were taking medication, and 24.5% (23.3-25.7) of those who reached the control standard for diabetes (HbA1c <6.5%) [2]. In both Korea and the United States, the prevalence of diabetes has been increasing over the years, and the prevalence tends to increase with age.

Diabetes is a type of metabolic disease in which insulin is insufficient in secretion, or insulin does not function normally, is characterized by high blood glucose concentration and causes various symptoms and signs due to hyperglycemia. In particular, type 2 diabetes (T2D, Type 2 Diabetes) is a common form among diabetes types, accounting for nearly 90% of the world's diabetes prevalence [3].

In T2D patients, age-related diseases, ranging from osteoporosis and bladder dysfunction to Alzheimer's disease, kidney dysfunction, and frailty, occur at

a younger age, which implies that T2D itself can indicate the progression of aging [4]. Diabetic microenvironment, such as high circulating glucose and altered lipid metabolism, increases the burden of SnC (senescent cells), which drives age-related morbidity and mortality, and SnC contributes to the pathogenesis of diabetes through a direct effect on pancreatic β-cell function, participation in adipose tissue dysfunction, and peripheral tissue damage [5].

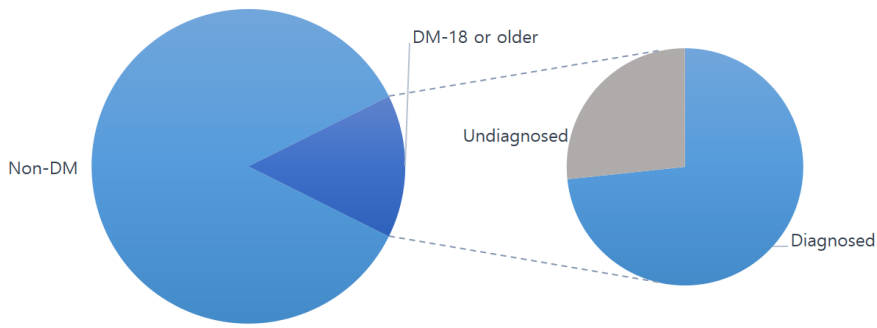

Figure 1. Prevalence of diabetes in Americans aged 18 years or older (left), and diagnosed rates in diabetic patients (right).

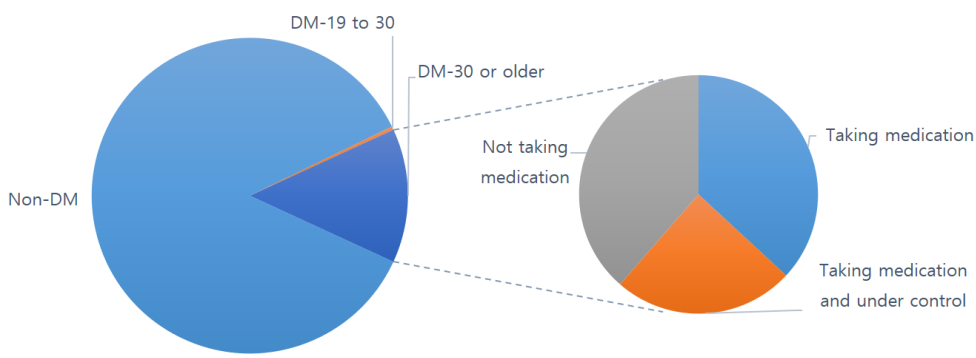

Figure 2. Prevalence of diabetes in Koreans aged 19 years or older (left) and among them, medication intake and control rates in diabetic patients aged 30 years or older (right).

However, the epigenetic pathophysiological causal relationship in this kind of mechanism on T2D still has many unclear areas [6]. Therefore, the importance of epigenetic research in the mechanism study of T2D is growing.

1.2. Epigenetics and DNA methylation

The hallmarks of aging have been reported as key factors causing cell damage, including genomic instability, telomere attrition, deregulated nutrient-sensing, and epigenetic alterations [7]. That is various epigenetic changes cause aging-related functional changes and diseases.

The most widely studied epigenetic mechanism is DNA methylation (DNAm), which mainly refers to adding of methyl groups to cytosine among CpG dinucleotides. DNA methylation plays an essential role in the development of various diseases because it may cause changes in gene expression, such as inhibiting gene expression by interacting with histone deacetylase or interfering binding with transcription factors [8]. In other words, it is possible to track the effects of various environmental and lifestyle factors through DNAm.

Moreover, DNAm is very stable and less invasive compared to RNA or protein-based biomarkers [9]. Given the difficulty of accessing key tissues involved in T2D in clinical studies (such as pancreatic cells), the development of non-invasive examinations will have significant implications [8]. It is also important that most epigenetic changes are intrinsically reversible [10]. Epigenetic changes can be

modified and reversed through diet, drug, and lifestyle improvements [11]. This is why new ideas with DNAm for monitoring, prevention and early intervention of T2D high-risk individuals and development of therapeutic drug targets has clinical and epidemiological implications [12].

Some of the various changes in DNA methylation patterns related to T2D reported so far are as follows: In a NIT-1 (the cell line derived from mice beta cell) experiment, cyclic adenosine monophosphate (cAMP) response element (CRE) inhibited about 90% of the gene expression of reporter gene operated by the insulin promoter when the CpG site of Ins2 was methylated, which is known to be similar to the insulin gene INS in humans [13]. It was also found that the CpG site of CRE, which is known to act on insulin promoters and play an important role in insulin gene regulation [14], independently inhibits the activity of insulin promoters by nearly 50% [13]. In addition, it has been found that nutritional problems from fetus to adulthood can lead to changes in DNA methylation that affect obesity and the risk of T2D development [8].

1.3. DNA Methylation age as the new biomarker in aging and age-related diseases

DNA methylation age (mAge) is the age estimated by DNA methylation loci, also known as epigenetic biomarker of aging. It means that while chronological age does not actually reflect changes in biological function, mAge can estimate relative age on a biological basis by calculating aging-related health risks such as cancer, obesity,

cardiovascular disease, and lung function [12]. A function that calculates mAge is commonly called a DNA methylation clock. The main idea of the clock is to measure biological aging by selecting CpG whose hypermethylation or hypomethylation is related to aging.

Recent studies have reported that mAge is a useful biomarker for predicting the physical and mental health of the elderly [15] and is not only a useful tool for the early diagnosis of cancer but also a useful source of data for understanding the underlying mechanisms of pathogenesis and patient prognosis [11]. For example, CpG sites and regions related to type 2 diabetes have the potential to be used as prognostic biomarkers of disease development or complications, as well as helping to understand the mechanism of pathogenesis, and methylation age calculated with these CpG sites appears to be associated with type 2 diabetes [12].

The method of calculating DNA methylation age that reflects actual aging changes associated with various diseases, health conditions, and healthy life expectancy through DNA methylation is called the epigenetic clock. One review concluded that the epigenetic clock emerged as the most promising model among six potential biological age predictors: epigenetic clocks, telomere length, transcriptomic predictors, proteomic predictors, metabolomics-based predictors, and composite biomarker predictors [16].

DNA methylation clock, meanwhile, has several limitations. First, mAge tends to be underestimated in samples aged approximately 60-65 years and older. Although its exact mechanism has yet to be identified, it is assumed that it will be improved by modifying CpGs in the clock's calculation or the modeling of the dataset [17]. Second, ethnic diversity is lacking in most clocks, especially in Asians, as the

clocks are made in a cohort composed mostly of Europeans [18-22]. However, the underestimation tendency of mAge in the Korean diabetic cohort or the relationship between T2D-related factors and mAge in Korean diabetic patients has not yet been identified.

DNA methylation clock is a new field that began to be studied in earnest, starting with Horvath in 2013, and a standardized research method has yet to be developed for about 10 years. Therefore, it is still necessary to consider its possibility and clinical applicability. The results of a literature review of the relationship between T2D and the clocks are as follows. In one paper [23], it was found that log2 (glucose) significantly increased IEAA and EEAA, age accelerations, in a multiple linear regression consisting of diet, exercise, education, and lifestyle factors. In this observational study, metformin, a first-line drug for treating type 2 diabetes, did not delay epigenetic aging. As for the ethnicity of the samples used in this study, the InCHIANTI samples are composed of European, while the WHI sample was 49% Caucasian and only 3% Asian or Pacific Islander.

In addition, in a study that predicted the incidence and prevalence of various diseases in Horvath, Hannum, PhenoAge, GrimAge, and DunedinPoAM (previous version of DunedinPACE) [24], PhenoAge and GrimAge predicted the incidence of T2D. They used a discovery cohort of 4450 and a replication cohort of 2578 from a Scottish cohort, Generation Scotland: Scottish Family Health Study (GS:SFHS or GS). Race information was not disclosed, but in the raw data of GS [25], it is said to consist of 99% of the 23,960 were white.

In 40 obese patients who visited Maastricht University Medical Center in Maastricht, the Netherlands, a study confirmed that Horvath mAge/EAA decreased

as well as fasting glucose, HbA1c, and BMI after bariatric surgery [26], and ethnicity information was not disclosed in this study.

In another study [27], the difference in mAge slope over time between the case group and the control group was not so steep. However, because mAge value was older in the case group than in the control group, biological aging concluded to be related to the development of T2D. In addition, the difference in slope of EAA was not large (Horvath acceleration) but rather decreased in the case group and increased in the control group (Hannum acceleration, PhenoAge acceleration, GrimAge acceleration). Nevertheless, EAA of the case group was positive, and EAA of the control group was negative, suggesting that aging was accelerated in the control group. This study used the Doetinchem cohort study, a population-based study from Doetinchem in the Netherlands, which has a limitation of the lack of ethnic subgroups.

2. Objective

In this study, the relationship between T2D-related factors of the Korean cohort and nine clocks (Horvath, Hannum, PhenoAge, GrimAge, AAHorvath, AAHannum, AAPheno, AAGrim, and DunedinPACE) was investigated, and the relationship between aging defined by each clock and T2D was inferred. To this end, the tendency was confirmed by dividing the subgroups according to DM status, DM duration, and CKD stages. Then, check the mAge clocks in which glucose is significantly reflected by the subgroups and see how much clocks reflect T2D progress. In order to confirm that the aging status of each situation is reflected in the clock when the patient is in a diabetic state when the duration of diabetes is long, and when renal complications due to diabetes are exacerbated, analyzed as follows for each subgroup by the clocks: plot mAge/EAA by age plot, compare the mean and variance of mAge/EAA of the subgroups, check how significant the variable glucose - the main factor that directly reflects insulin dysfunction - is in multiple linear regression model, calculate AIC for each model used at this time, confirm the nature of the clock in terms of CpGs by checking how much T2D-related CpGs are included in the clock formula. Also, found out the T2D-related CpGs showed significant differences by the subgroups of the cohort. Since each methylation age clock focuses on various aspects of aging, the association with a Korean cohort with diabetes was examined through overall trends rather than a specific single indicator.

As far as we know, this study is the second to use a Korean-only cohort for mAge studies after the first, which was conducted with the middle-aged metabolic syndrome cohort [28]. In addition, this study is the second study using DNA mAge as a T2D subject [27]. Considering that racial/ethnic differences in T2D have been consistently reported [29], and some studies even show differences between inter-

East Asians [30], the lack of racial diversity in DNA mAge in T2D studies is fatal to T2D studies, so this study is expected to add racial diversity to the study, and it is also expected to show the possibility of using DNA mAge in Korean T2D research.

II. Materials and Method

1. Study population

Two cohorts were used in this study, the Seoul National University Hospital T2D cohort (SNUH) and the Ansan/Anseong Community-based Cohort (AS) of the Korean Genome and Epidemiology Study (KoGES).

SNUH is part of the Seoul National University Hospital Diabetes Clinic cohort, which started in January 2001 and is ongoing. 429 Korean samples, including 232 T2D patients and 197 non-T2D controls from 2016 to 2018 were used [31]. AS consists of incident T2D cases and sex-matched controls, as a part of the Anseong/Ansan community-based cohort of the Korean Genome and Epidemiology Study (KoGES) conducted from 2001 to the 8th follow-up in 2018. 400 Korean samples consisting of 200 T2D patients and 200 non-T2D controls in 2009-2010 (the 5th follow-up) were used [32]. This data is based on a community-based cohort (Ansan and Ansung study) of human resources distributed by the National Human Resources Bank of Korea Centers for Disease Control and Prevention.

2. Data collection

In the SNUH cohort, age and sex were derived from social security number, a SNUH doctor diagnosed diabetes status, and diabetes prevalence was determined from the patient's oral response or SNUH treatment period. Height, weight, BMI, waist

circumference, systolic and diastolic blood pressure, HbA1c, fasting glucose, eGFR was calculated from the test results in SNUH.

From the AS cohort, 19 items out of 1932 items (age, sex, height, weight, waist circumference (measured 3 times), diabetes diagnosis, diabetes duration, HbA1c, fasting glucose, Glucose tolerance test (2 hour) glucose, systolic blood pressure (measured 3 times), diastolic blood pressure (measured 3 times), and creatinine) were extracted. These generated 13 items identical to the SNUH. The process of extracting 13 items from 17 items is as follows.

In the DM group, those who answered that they had been diagnosed by a doctor (64 people), or according to the recent American Diabetes Association guidelines [33], those whose HbA1c was 6.5% or more (200 people), fasting glucose after fasting for 8 hours 126 mg/dL or more (135 people), or glucose level after 2 hours of 75 g oral glucose loading of 200 mg/dL or more (129 people, total 200 people above) was included.

For 140 people, including 136 who said they had never been diagnosed with DM and 4 who said they had been diagnosed but did not answer the year of diagnosis, the previous follow-up (2001-2009, 1-4th follow-up) data was used to calculate the duration of DM. If you answered 'I have been diagnosed with T2D' or 'I am being treated for T2D' in 1-4th follow-up, the duration of T2D is converted according to the year of collection of the research data of the sample or from the year of the first answer to the year of collection of the research data (19 patients), and if you answered no to 'diagnosed with T2D' or 'under treatment with T2D' in all follow-ups, the period from the first year of meeting the T2D criteria to the year of collection of study data (5th follow-up) was applied (121 persons). In addition, if the responses of

the previous and subsequent riders were inconsistent, the first response was adopted.

eGFR was calculated using the MDRD method proposed by Levey et al (2006) [34]. CKD (chronic kidney disease) stage was classified according to Kidney Disease: Improving Global Outcomes (KDIGO) guideline as stage 1 for eGFR (mL/min/1.73m^2) over 90, stage 2 for more than 60, stage 3 for more than 30, stage 4 for more than 15, and stage 5 (kidney failure) for those less than 15 [35].

3. DNA methylation clocks used in study

Chronological age has a constant acceleration, but mAge has irregular acceleration due to the influence of environmental exposures, poor diet, social stressors, economic stress, and infections [12]. EAA (Epigenetic Age Acceleration) is the calculation of only the acceleration, excluding the influence of age. In the first and the second generation clocks, mAge is mainly defined as the residual of a simple linear regression model regressed on chronological age [36]. On the other hand, DunedinPACE, the third-generation clock, calculates EAA from the beginning without extracting EAA from mAge [22].

Since the definition of aging (age) varies from clock to clock, the calculation method and the specific CpGs the clock sees differ. It is also different to interpret what disease and health indicators the results are related to. Therefore, it is essential to understand the characteristics of each clock.

In this study, 4 mAges and 5 EAAs were used. All clocks are summarized

below (**Table 1**). AAHorvath, AAHan, AAPheno, and AAGrim refer to EAAs made as residuals for the chronological age of Horvath, Hannum, PhenoAge, and GrimAge, respectively. The clocks were selected because Horvath and Hannum are the clocks that measure chronological age relatively accurately, PhenoAge is related to increased activation of pro-inflammatory and interferon pathways, GrimAge is associated with time-to-death, time-to-coronary heart disease, time-to-cancer, computed tomography data for fatty liver/excess visceral fat, and DunedinPACE showed a higher acceleration in young adults with prenatal/childhood deficits [18-22]. Also, these clocks were chosen because Horvath, Hannum, PhenoAge, and GrimAge are known to have an association with insulin level [37], and DunedinPACE has an association with type 2 diabetes morbidity [22].

The first-generation clock used information about individuals given by chronological age, and the second-generation clock selected CpGs related to clinical biomarkers to obtain a linear regression model [38]. The first-generation clocks include Horvath and Hannum, and the second-generation clocks include PhenoAge and GrimAge.

Horvath mAge is one of the most representative epigenetic clocks to calculate mAge based on DNA methylation (DNAm) profiles by collecting 353 CpGs common to Illumina 27K and 450K from 51 tissues of 3,931 individuals. It has calibrated with chronological age. Among Horvath's training datasets, the healthy tissue dataset contains several type 1 Diabetes patient samples [18]. A follow-up study also found that Horvath is significantly associated with incident type 2 diabetes [27].

Clock type	No. of CpGs	Used platform	Tissues/cell types from which methylation data were extracted	Computation	Calibrate with	Ref.
mAge						
Horvath	353	27/450K	51 different tissues	Elastic net regression	Chronological age	[18]
Hannum	71	450K	Whole blood	Elastic net regression	Chronological age	[19]
PhenoAge	513	450K/EPIC	Whole blood	Elastic net regression	Chronological age and 9 clinical laboratory values	[20]
GrimAge	1,030	450K/EPIC	Whole blood	Elastic net regression	Chronological age, plasma biomarkers and packyears of smoking associated with morbidity and mortality	[21]
EAA						
AAHorvath			calculated from Horvath			
AAHannum			calculated from Hannum			
AAPheno			calculated from PhenoAge			
AAGrim			calculated from GrimAge			
DunedinPACE	173	450K/EPIC	Whole blood	Elastic net regression	Methylation patterns reflecting individual differences in age-related decline	[22]

Table 1. mAges and EAAs used in study. 'Used Platform' is the name of the Illumina Infinium Methylation BeadChip kit.

Hannum was created using 71 CpG sites extracted from 482 whole blood samples using the Illumina 450K array. In Hannum's modeling, DM status was used as a covariate [19]. Moreover, there is a follow-up study showing that it is significantly related to incident type 2 diabetes [27].

Levine et al. developed PhenoAge based on 10 clinical measures related to phenotypic age-Albumin, ALP, creatinine, CRP, serum glucose, mean cell volume, lymphocyte percentage, red cell distribution width, white blood cell count and a weighted average of chronological age. PhenoAge used 513 CpGs from 9,926 individuals of methylation data with common CpGs in 450K and EPIC probes [20]. A follow-up study found that PhenoAge is also significantly associated with incident type 2 diabetes [27].

GrimAge by Ake et al. was developed to estimate time to death by selecting CpGs among common CpGs of whole blood-based 450K and EPIC probes from 1,731 individuals. The selected CpGs are related to chronological age, sex, DNAm pack-years, and DNAm-based surrogate markers of plasma proteins: adrenomedullin (ADM), beta-2-microglobulim (B2M), cystatin C (Cystatin C), GDF-15, leptin (Leptin), PAI-1, and tissue inhibitor metalloproteinases 1 (TIMP-1) [21]. Prior history of diabetes was used as the covariate of GrimAge, and it is also found to be significantly related to incident type 2 diabetes in a follow-up study [27]. AAGrim was found to be related to T2D in a cross-sectional study and to have a positive correlation with glucose [21].

DunedinPACE (Pace of Aging Calculated from the Epigenome) performed mixed-effects growth modeling of longitudinal changes in 19 biomarkers measuring the integrity of the cardiovascular, metabolic, renal, hepatic, pulmonary, periodontal,

and immune systems at the age of 26, 32, 38, 45 in 1,037 people born in the same year, and used the personal rate to obtain the personal pace of aging. The CpGs selected for the methylation pattern obtained in the study are common CpGs of 450K and EPIC probes and a total of 173 CpGs. The 19 biomarkers consist of body mass index (BMI), waist-hip ratio, glycated hemoglobin, leptin, blood pressure (mean arterial pressure), cardiorespiratory fitness (VO2Max), forced vital capacity ratio (FEV1/FVC), forced expiratory volume in one second (FEV1), total cholesterol, triglycerides, high-density lipoprotein (HDL), lipoprotein(a), apolipoprotein B100/A1 ratio, estimated glomerular filtration rate (eGFR), blood urea nitrogen (BUN), high sensitivity C-reactive Protein (hs-CRP), white blood cell count, mean periodontal attachment loss (AL), and the number of dental-caries-affected tooth surfaces (tooth decay) [22].

4. DNA methylation profiling and calculation of mAge

After extracting genomic DNA from the peripheral leukocyte by the Gentra Puregene Blood kit (Qiagen, Hilden, Germany), the samples were bisulfite modified using the Zymo EZ-96 DNA methylation kit (Zymo Research, Irvine, California, USA), and each SNUH sample was hybridized to Illumina Infinium Methylation EPIC BeadChip (EPIC). The AS sample was hybridized to Illumina Infinium HumanMethylation450K BeadChip (450K) (EPIC and 450K both, Illumina, San Diego, California, USA) according to the manufacturer's protocols.

About the differentiation of the methylation data analysis platform between

the two cohorts, it has been reported that even if the methylation data profiled by the EPIC chip is applied to the clock made based on 450K, it operates without compromising accuracy [39]. In addition, there is a study that the accuracy was similar to when only EPIC data was used when 450K/EPIC chip common CpG was applied to the clock based on EPIC [40].

Overall analysis was performed with program R 4.1.2 (2021-11-01) [41]. First, the beta values were calculated from the raw methylation results for each cohort, and mAges/EAAs were calculated from this. The beta value measures the intensity of methylation of a CpG ranging from 0 to 1.

$$\mathrm{Beta_i} = \frac{\max(y_{i,methy}, 0)}{\max(y_{i,unmethy}, 0) + \max(y_{i,methy}, 0) + a}$$

The beta value of a specific CpG is the intensity of the methylated probe over the total amount of the intensity of the methylated probe and the intensity of the unmethylated probe plus a constant offset α. α is put in for regulation when both methylated and unmethylated probe intensities are low, Illumina recommends 100. If the intensity goes through background adjustment, it can produce negative values, in which case it is set to 0 [42].

When calculating the beta value, the preprocessing and normalization processes were also performed simultaneously, and this overall analysis was performed through the RnBeads package [43].

The initially extracted SNPs were 866895 and 485577 for SNUH and AS, respectively. Moreover, in the preprocessing process, SNP-enriched sites were removed using the SNP criterion "3", and cross-reactive probes were removed using a p-value threshold of 0.01. And after Beta-mixture quantile normalization (BMIQ) method normalization was performed, keep removed probes with not acceptable context and sites in sex chromosomes, and performed missing value removal using a sample quantile threshold of 0.5. As a result, SNUH was 778130 sites, and AS was 431074 sites left. The specific process of filtering CpG is shown below (**Figure 3**). A graph comparing the density of beta values before and after each performance is presented in the following (**Figure 4**).

For the SNUH cohort, additional batch effect removal was performed because the year of data analysis was different. Of the total cohort (N=429), 147 were analyzed in 2016, 137 in 2017, and 145 in 2018. To remove the batch effect from confounders, used parametric or non-parametric empirical Bayes frameworks to adjust data performed by sva package [44]. As major batches, the collection year (2016, 2017, 2018, total of 3 batches) was used. Principal component analysis (PCA) was performed to verify the results before and after the batch effect removal. The plots below (**Figure 5**) show that the batch effect removal was well performed, which were drawn by using ggfortify package [45].

Finally, Horvath online age calculator [18] and the DunedinPACE package [22] were used to calculate mAge and EAA from the beta values thus calculated. When using Horvath online age calculator, Normalize Data, and Advanced Analysis options are checked.

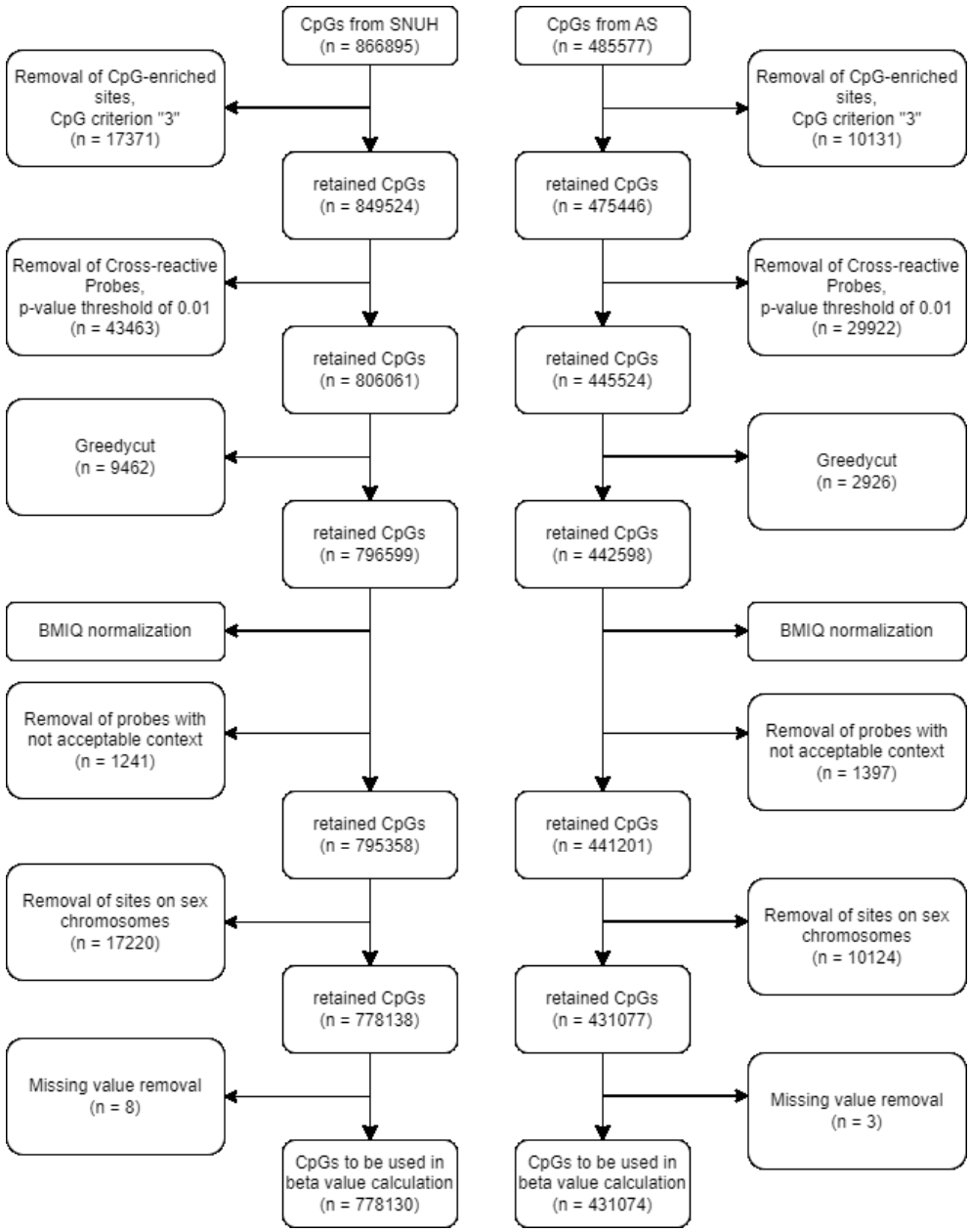

Figure 3. CpGs preprocessing diagram.

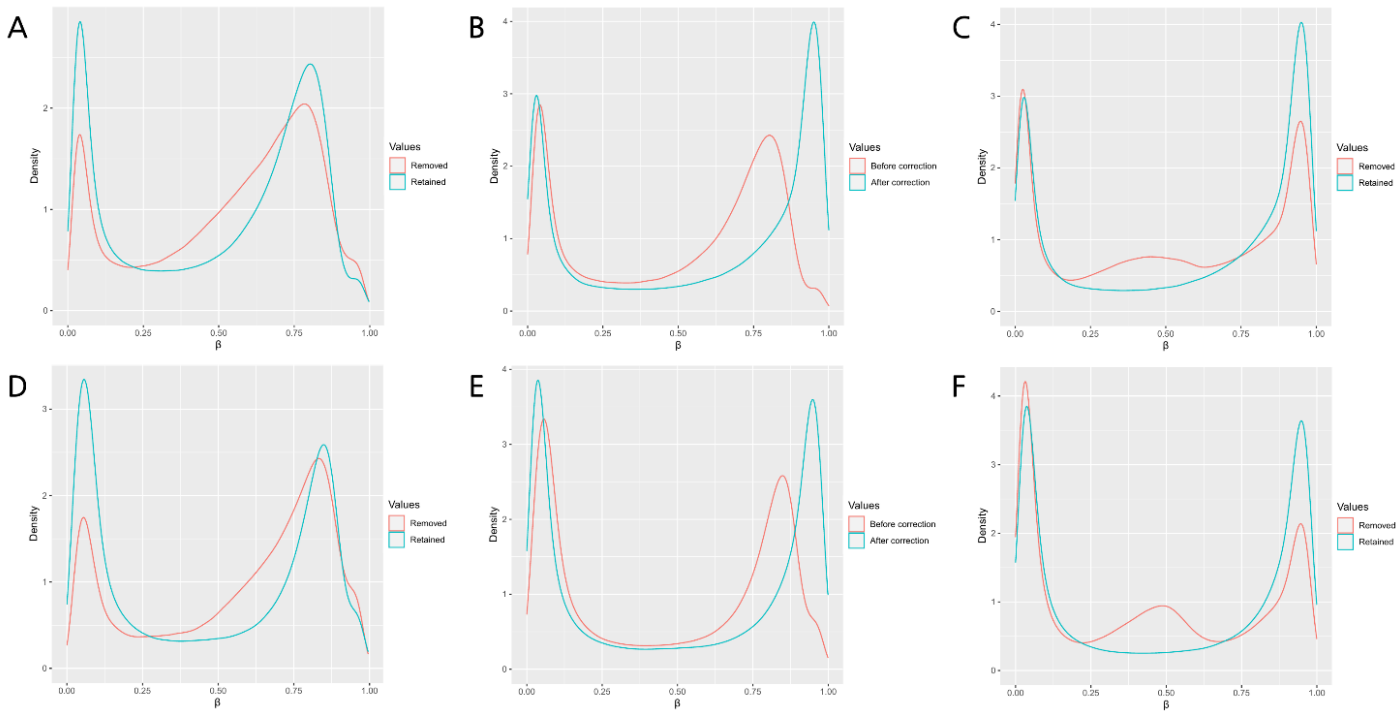

Figure 4. The density of beta value before and after preprocessing process. **A,** Before and after removal of SNP-enriched sites and cross-reactive probes in SNUH. **B,** Before and after BMIQ normalization in SNUH. **C,** Before and after removal probes with not acceptable context, sites in sex chromosomes, and SNPs with missing values in SNUH. **D,** Before and after removal SNP-enriched sites and cross-reactive probes in AS. **E,** Before and after BMIQ normalization in AS. **F,** Before and after removal of probes with not acceptable context, sites in sex chromosomes, and SNPs with missing values in AS.

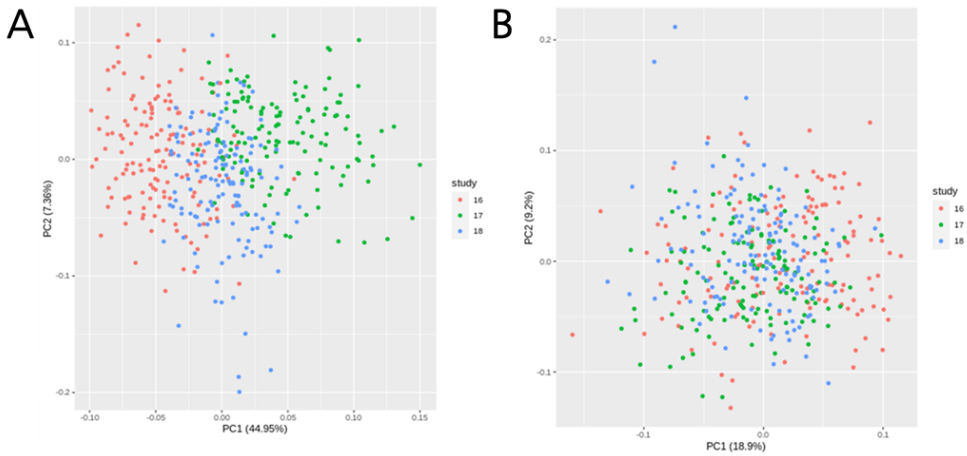

Figure 5. Before (A) and after (B) batch effect removal in SNUH. Studies 16 to 18 refer to batches from 2016 to 2018, respectively.

5. Statistical Analysis

Mean and standard deviation are shown for all variables, mAges and EAAs in SNUH and AS cohorts and the subgroups, respectively. The subgroups were grouped to test whether the mean of the variables was equal. At this time, Student's t-test or one-way ANOVA was used for continuous variables among 2 groups or 3 groups, respectively, and Chi-squared test for categorical variables. Also, F-test was used to compare the variance among the subgroups. The ggplot2 package was used to draw a plot to confirm the tendency of mAges/EAAs according to age for each subgroup and to draw a Bland-Altman plot to confirm the tendency of underestimation of mAge according to age [46]. The significant effect of glucose was confirmed through linear regression onto mAge and EAA by the cohort and the subgroups. In a list of

recently reviewed T2D-related DNA methylation CpGs [27], CpGs with significant differences in beta value by the subgroup were identified. After the equality of variance was tested through Levene's test, Welch's t-test was performed if the equal variance could not be assumed. The independent t-test was performed if an equal variance could be assumed. The Kruskal-Wallis test was performed when comparing the three groups with long duration, short group, and control group or the three groups divided according to CKD stage. It was also checked how many CpGs in the clocks corresponded to the CpGs in the list. The entire process so far is briefly expressed below (**Figure 6**).

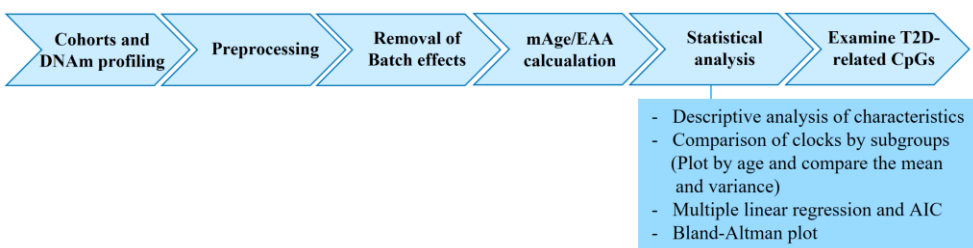

Figure 6. The overall flow of study.

III. Results

1. Descriptive analysis

To analyze, the subgroups were divided into DM/control (two groups, hereinafter abbreviated as DM/CTL), control/DM duration less than 10 years/10 years (three duration groups, hereinafter abbreviated as CTL/short/long), and CKD stage 3 or less/4 stage/5 (three CKD groups, hereinafter abbreviated as CKD 3/CKD 4/CKD 5) within the DM group. In the AS cohort, there were no samples of stage 4 or higher CKD, so the CKD groups were not analyzed.

First, the means of variables in the entire cohort and in each subgroup were compared. In SNUH, the DM group had significantly higher HbA1c and glucose and significantly lower eGFR, although their chronological age was significantly younger than the control group (**Table 2**). When the variances of the DM and CTL groups were compared, the DM group was significantly larger in Age, DM duration, HbA1c, glucose, and eGFR.

The DM group was divided into a short group if the duration of T2D was 10 years or less, and a long group if the duration of T2D was more than 10 years. When the means of the three groups---control, short, and long- were compared, the short group was significantly the youngest and had the highest glucose (**Table 3**). The long group had the highest mean of chronological age and HbA1c and the lowest eGFR. The variance of the CTL, short, and long groups was larger in the group with longer DM duration for each significant variable. In age, DM duration, HbA1c, glucose, and eGFR, the variance of the short group was greater than that of the CTL group, and the variance of the long group was significantly greater than that of the

CTL group. DM duration and eGFR had a significantly greater variance in the long group than in the short group.

The DM group was divided again into three groups, CKD 3 group for CKD stage 3 or less, CKD 4 group for stage 4, and CKD 5 group for stage 5. When the means were compared, the CKD 5 group had the shortest chronological age, the most prolonged DM duration, the highest HbA1c and glucose, and the lowest eGFR (**Table 4**). When the variances of the three groups were compared, the CKD 3 group had significantly larger variances of HbA1c, glucose, and eGFR than the CKD 4 group. The CKD 5 group had a significantly larger variance of glucose than the CKD 3 and CKD 4 groups, and a significantly larger variance of HbA1c than the CKD 4 group. The variance of eGFR was significantly greater in the CKD 3 group than in the CKD 5 group.

In AS cohort, the DM group had no significant difference in chronological age compared to the control group, but HbA1c and glucose were significantly higher, and eGFR was significantly lower (**Table 5**). Moreover, the variance of HbA1c and glucose was significantly greater in the DM group than in the CTL group.

As in SNUH, the AS DM group was divided according to the DM duration, and the mean of the three groups, including the control group, was compared. The short group was significantly the youngest (**Table 6**). The long group had the highest age, DM duration, HbA1c, and glucose, and the lowest eGFR.

Group (n)	SNUH (429)	DM (232)	CTL (197)	p	F	p
sex (male %)	202 (47.1)	104 (44.8)	98 (49.7)	0.358		
T2D patient (T2D %)	232 (54.1)	232 (100.0)	0 (0.0)	**<0.001**		
CKD stage				**<0.001**		
1, 2, 3 (%)	398 (92.8)	202 (87.1)	196 (99.5)			
4 (%)	15 (3.5)	14 (6.0)	1 (0.5)			
5 (%)	16 (3.7)	16 (6.9)	0 (0.0)			
Age (years)	62.61 (7.80)	61.49 (10.05)	63.93 (3.23)	**0.001**	9.679	**<0.001**
DM duration (years)	7.26 (9.37)	13.42 (8.93)	0.00 (0.00)	**<0.001**		
HbA1c (%)	6.74 (1.73)	7.93 (1.56)	5.35 (0.26)	**<0.001**	36.972	**<0.001**
glucose (mg/dl)	123.29 (49.48)	149.62 (54.47)	92.28 (7.86)	**<0.001**	48.011	**<0.001**
eGFR (ml/min)	77.58 (25.60)	70.95 (30.54)	85.38 (14.80)	**<0.001**	4.257	**<0.001**

Table 2. General characteristics of the whole cohort and the DM/control groups in SNUH. Chi-square test (categorical variable), Student's t-test (numerical variable), and F-test (numerical variable) were done between the DM/CTL groups. Bold numbers indicate p-value < 0.05.

Group (n)	CTL (197)	Short (93)	Long (139)	p*	p†	p‡	F*	p*	F†	p†	F‡	p‡
sex (male, %)	98 (49.7)	42 (45.2)	62 (44.6)	0.546	0.413	1						
CKD stage				**0.034**	**<0.001**	**0.005**						
1, 2, 3 (%)	196 (99.5)	89 (95.7)	113 (81.3)									
4 (%)	1 (0.5)	1 (1.1)	13 (9.4)									
5 (%)	0 (0.0)	3 (3.2)	13 (9.4)									
Age (years)	63.93 (3.23)	57.28 (10.27)	64.30 (8.88)	**<0.001**	0.594	**<0.001**	0.099	**<0.001**	0.132	**<0.001**	1.339	0.120
DM duration (years)	0.00 (0.00)	5.13 (3.61)	18.96 (6.89)								0.275	**<0.001**
HbA1c (%)	5.35 (0.26)	7.92 (1.69)	7.94 (1.47)	**<0.001**	**<0.001**	0.96	**0.023**	**<0.001**	**0.030**	**<0.001**	1.326	0.133
glucose (mg/dl)	92.28 (7.86)	152.26 (50.10)	147.86 (57.31)	**<0.001**	**<0.001**	0.547	**0.025**	**<0.001**	**0.019**	**<0.001**	0.764	0.167
eGFR (ml/min)	85.38 (14.80)	79.51 (22.74)	65.23 (33.67)	**0.009**	**<0.001**	**<0.001**	0.424	**<0.001**	0.193	**<0.001**	0.456	**<0.001**

Table 3. General characteristics of the CTL/short/long groups in SNUH. Chi-square test (categorical variable), Student's t-test (numerical variable), and F-test (numerical variable) were done between the *CTL/Short, †CTL/Long, ‡Short/Long groups. Bold numbers indicate p-value < 0.05.

Group (n)	CKD 3 (202)	CKD 4 (14)	CKD 5 (16)	p*	p†	p‡	F*	p*	F†	p†	F‡	p‡
sex (male, %)	87 (43.1)	6 (42.9)	11 (68.8)	1	0.084	0.29						
Age (years)	61.76 (10.07)	61.71 (9.43)	57.81 (10.19)	0.986	0.133	0.288	1.142	0.840	0.978	0.867	0.856	0.786
DM duration (years)	12.64 (8.93)	18.50 (5.81)	18.81 (8.17)	**0.016**	**0.008**	0.906	2.357	0.080	1.192	0.733	0.506	0.224
HbA1c (%)	8.00 (1.58)	7.44 (0.84)	7.49 (1.71)	0.187	0.22	0.909	3.482	**0.014**	0.845	0.574	0.243	**0.014**
glucose (mg/dl)	149.64 (51.91)	125.79 (28.19)	170.19 (88.96)	0.091	0.154	0.085	3.390	**0.016**	0.340	**0.001**	0.100	**0.001**
eGFR (ml/min)	79.37 (22.68)	20.49 (3.68)	8.81 (2.73)	**<0.001**	**<0.001**	**<0.001**	38.056	**<0.001**	68.880	**<0.001**	1.810	0.271

Table 4. General characteristics of the CKD 3/CKD 4/CKD 5 groups in SNUH. Chi-square test (categorical variable), Student's t-test (numerical variable), and F-test (numerical variable) were done between the *CKD 3/CKD 4, †CKD 3/CKD 5, ‡CKD 4/CKD 5 groups. Bold numbers indicate p-value < 0.05.

Group (n)	AS (400)	DM (200)	CTL (200)	p	F*	p*
sex (male, %)	200 (50.0)	100 (50.0)	100 (50.0)	1		
T2D patient (T2D, %)	200 (50.0)	200 (100.0)	0 (0.0)	**<0.001**		
CKD stage (under 4, %)	400 (100.0)	200 (100.0)	200 (100.0)			
Age (years)	60.96 (8.31)	61.10 (7.99)	60.81 (8.65)	0.728	0.853	0.263
DM duration (years)	3.52 (6.34)	7.04 (7.46)	0.00 (0.00)			
HbA1c (%)	6.46 (1.97)	8.05 (1.61)	4.87 (0.26)	**<0.001**	38.962	**<0.001**
glucose (mg/dl)	124.69 (56.99)	160.40 (62.38)	88.98 (7.61)	**<0.001**	67.122	**<0.001**
eGFR (ml/min)	73.66 (12.01)	71.90 (12.57)	75.43 (11.17)	**0.003**	1.267	0.096

Table 5. General characteristics of the whole cohort and the DM/CTL groups in AS. Chi-square test (categorical variable), Student's t-test (numerical variable), and F-test (numerical variable) were done between DM/CTL groups. Bold numbers indicate p-value < 0.05.

Group (n)	CTL (200)	Short (171)	Long (29)	p*	p†	p‡	F*	p*	F†	p†	F‡	p‡
sex (male %)	100 (50.0)	84 (49.1)	16 (55.2)	0.949	0.748	0.688						
Age (years)	60.81 (8.65)	60.44 (8.03)	65.03 (6.59)	0.666	**0.012**	**0.004**	1.161	0.317	1.720	0.090	1.482	0.220
DM duration (years)	0.00 (0.00)	4.58 (3.11)	21.48 (9.17)	**<0.001**	**<0.001**	**<0.001**					0.115	**<0.001**
HbA1c (%)	4.87 (0.26)	7.75 (1.50)	9.85 (0.94)	**<0.001**	**<0.001**	**<0.001**	**0.030**	**<0.001**	0.075	**<0.001**	2.526	**0.005**
glucose (mg/dl)	88.98 (7.61)	158.44 (60.93)	172.00 (70.38)	**<0.001**	**<0.001**	0.280	**0.016**	**<0.001**	**0.012**	**<0.001**	0.749	0.271
eGFR (ml/min)	75.43 (11.17)	72.66 (11.40)	67.41 (17.60)	**0.019**	**0.001**	**0.037**	0.960	0.780	0.403	**<0.001**	0.419	**<0.001**

Table 6. General characteristics of the CTL/short/long groups in AS. Chi-square test (categorical variable), Student's t-test (numerical variable), and F-test (numerical variable) were done between the *CTL/Short, †CTL/Long, ‡Short/Long groups. Bold numbers indicate p-value < 0.05.

2. Comparison of the clocks by the subgroups

First, mAges and EAAs according to the age of each subgroup were plotted to identify the tendency according to the subgroups.

When comparing four mAge clocks in SNUH, first, all four mAges increased with age in the entire cohort (**Figure 7**). Looking at the DM group and the CTL group (**Figure 8**), in the three mAges, Horvath, Hannum, and PhenoAge, the CM group had a larger slope of mAge according to age than the CTL group. There was no clock in which the age-dependent slope of mAge increased as the duration of diabetes was longer; rather, the long group slope for all four mAge clocks tended to be slightly lower than that of the short group (**Figure 9**). As CKD stage increases, the clocks whose slopes increase are Horvath and Hannum. In PhenoAge and GrimAge, the slope of stage 4 and the stage 5 diabetes group was greater than that of stage 3 or lower diabetes group and the control group (**Figure 10**).

When comparing the 5 EAA clocks in SNUH, only DunedinPACE showed an increase in EAA with age in the entire cohort, and there was no significant difference with age in the rest clocks (**Figure 11**). The clock with a greater age-dependent slope of EAA in the diabetic group was AAHorvath (**Figure 12**). In AAHannum, AAPheno, and AAGrim, there was little difference in the slopes of the two groups, and in DunedinPACE, the slope of the control group was rather large. The clock that had a larger age-dependent slope of EAA in the long group than in the short group was also AAHorvath (**Figure 13**). In AAPheno, AAGrim, and DunedinPACE, the slope in the long group was smaller than in the short and control groups. For AAHannum, the control group had the most prominent slope, followed by the long and short groups. As the CKD stage increased, the clocks in which the

slope of EAA according to age increased were AAHannum and DunedinPACE (**Figure 14**). AAHorvath and AAGrim differed in slope between the CKD stage 4 or higher diabetes groups and the CKD stage 3 or lower diabetes group and control group. AAPheno had a high slope in the CKD 4 group, but no significant change in slope among the control group, the CKD 3 group, and the CKD 5 group.

Comparing four mAge clocks in AS, mAges increased with age in the entire cohort (**Figure 15**). The slope of mAge according to age increased in all four clocks as the prevalence of diabetes and the duration of diabetes increased (**Figure 16-17**).

Comparing four EAA clocks in AS, as in SNUH, only DunedinPACE showed an increase in the 'slope of EAA with age' in the entire cohort (**Figure 18**). The diabetic group had a greater slope of all five EAAs by age than the control group (**Figure 19**). The longer the duration of diabetes, the higher the age-dependent slope of EAA was for AAHorvath and AAHannum, while the other three clocks tended to decrease (**Figure 20**).

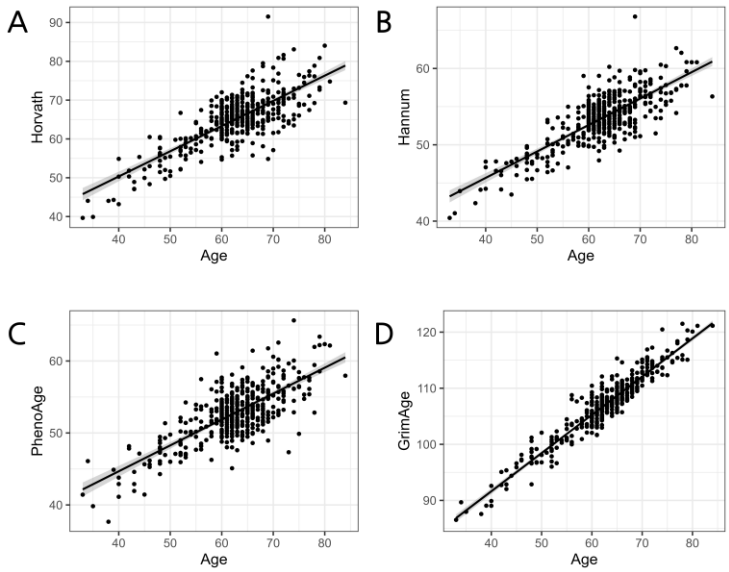

Figure 7. mAge by age in overall SNUH cohort. **A,** Horvath. **B,** Hannum. **C,** PhenoAge. **D,** GrimAge.

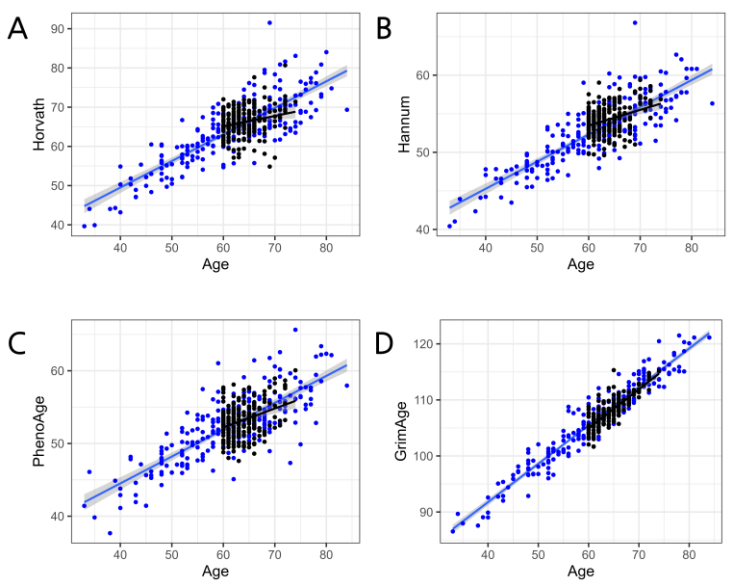

Figure 8. mAge by age in the DM (blue) and CTL (black) groups in SNUH. **A,** Horvath. **B,** Hannum. **C,** PhenoAge. **D,** GrimAge.

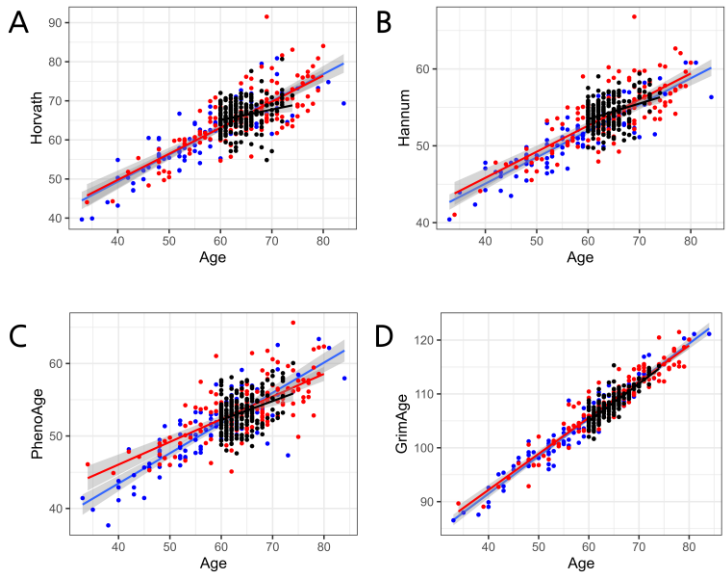

Figure 9. mAge by age in the long (red), short (blue), and CTL (black) groups in SNUH. **A,** Horvath. **B,** Hannum. **C,** PhenoAge. **D,** GrimAge.

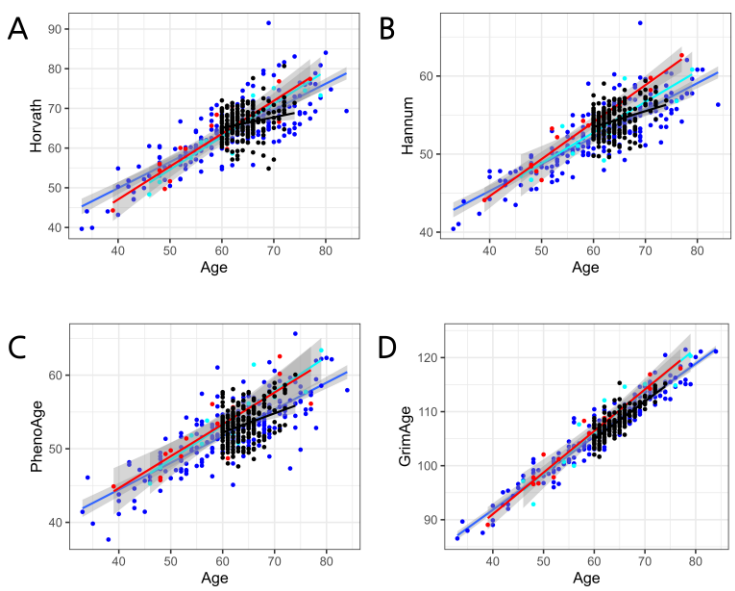

Figure 10. mAge by age in the CKD 5 (red), CKD 4 (cyan), CKD 3 (blue), and CTL (black) groups in SNUH. **A,** Horvath. **B,** Hannum. **C,** PhenoAge. **D,** GrimAge.

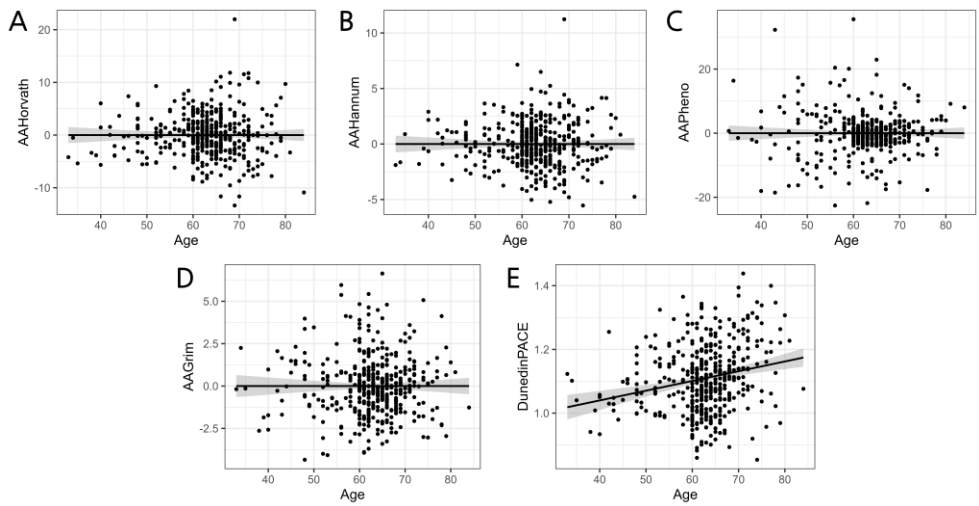

Figure 11. EAA by age in overall SNUH cohort. **A,** AAHorvath. **B,** AAHannum. **C,** AAPheno. **D,** AAGrim. **E,** DunedinPACE.

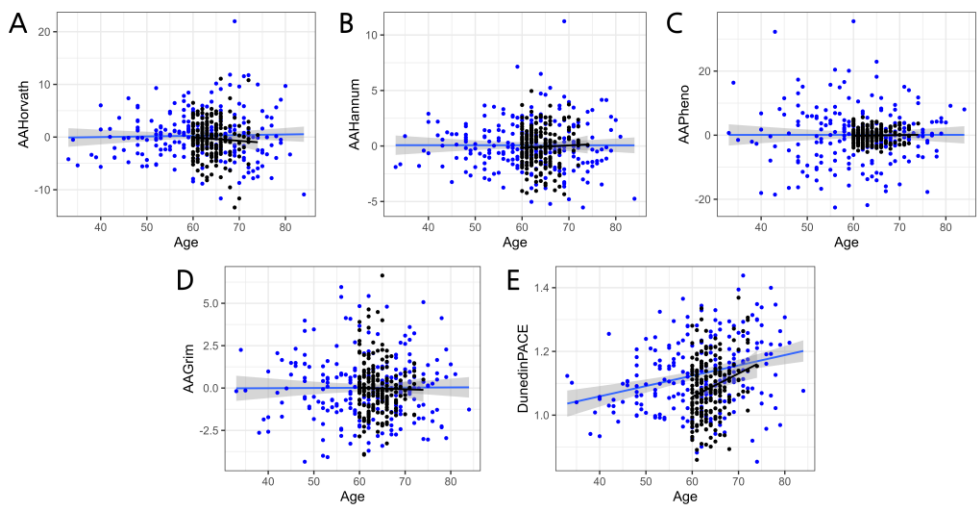

Figure 12. EAA by age in the DM (blue) and CTL (black) groups in SNUH. **A,** AAHorvath. **B,** AAHannum. **C,** AAPheno. **D,** AAGrim. **E,** DunedinPACE.

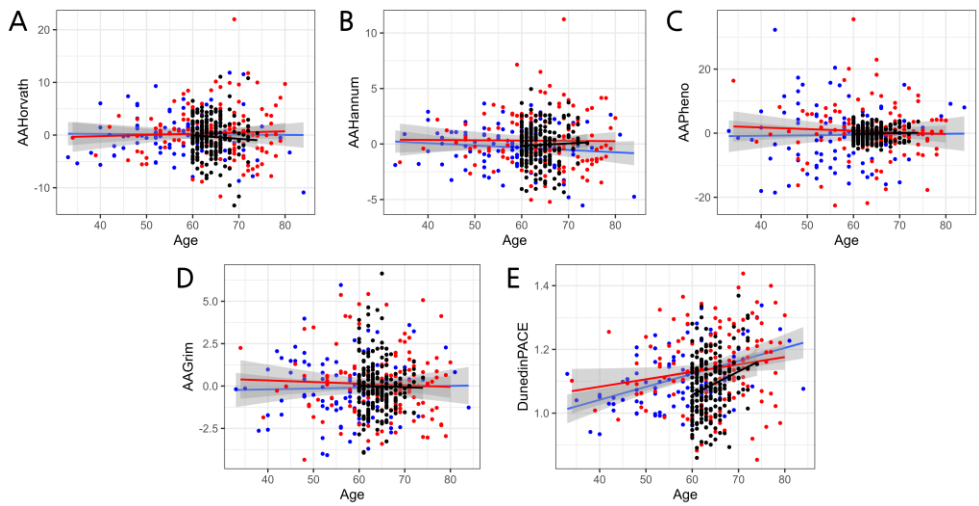

Figure 13. EAA by age in the long (red), short (blue), and CTL (black) groups in SNUH. **A,** AAHorvath. **B,** AAHannum. **C,** AAPheno. **D,** AAGrim. **E,** DunedinPACE.

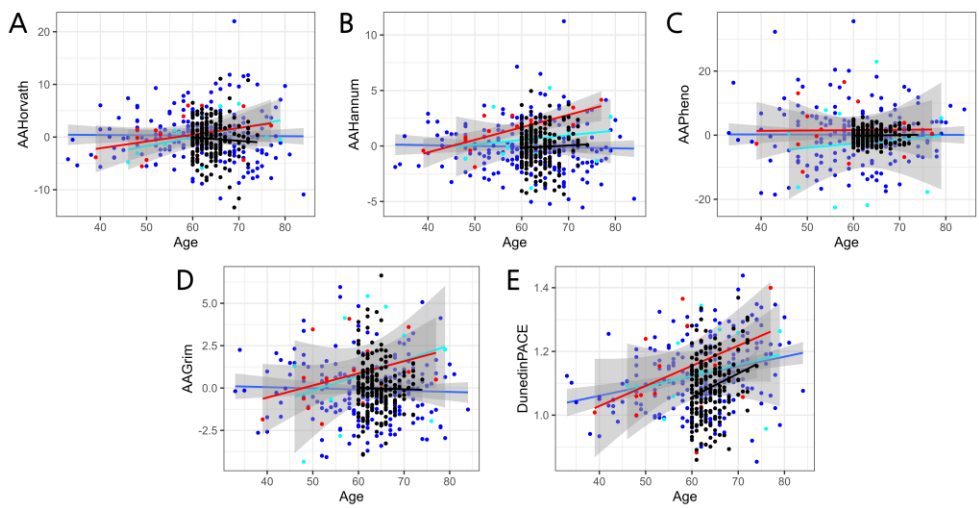

Figure 14. EAA by age in the CKD 5 (red), CKD 4 (cyan), CKD 3 (blue), and CTL (black) groups in SNUH. **A,** AAHorvath. **B,** AAHannum. **C,** AAPheno. **D,** AAGrim. **E,** DunedinPACE.

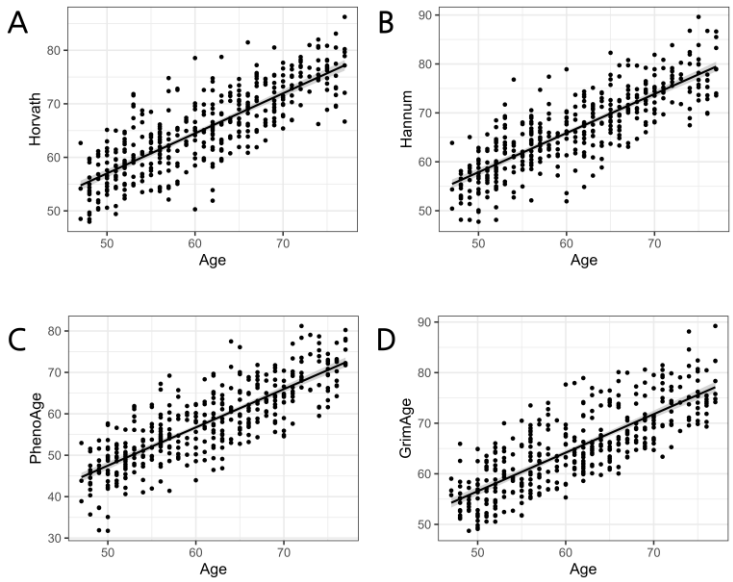

Figure 15. mAge by age in overall AS cohort. **A,** Horvath. **B,** Hannum. **C,** PhenoAge. **D,** GrimAge.

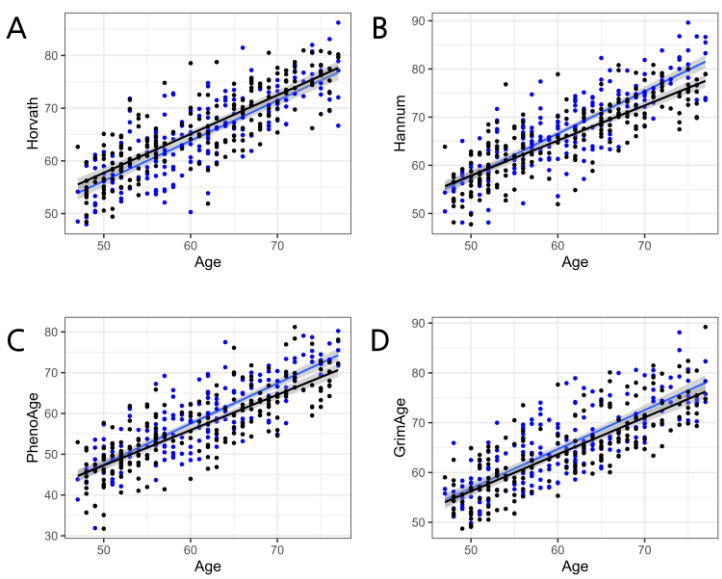

Figure 16. mAge by age in the DM (blue) and CTL (black) groups in AS. **A,** Horvath. **B,** Hannum. **C,** PhenoAge. **D,** GrimAge.

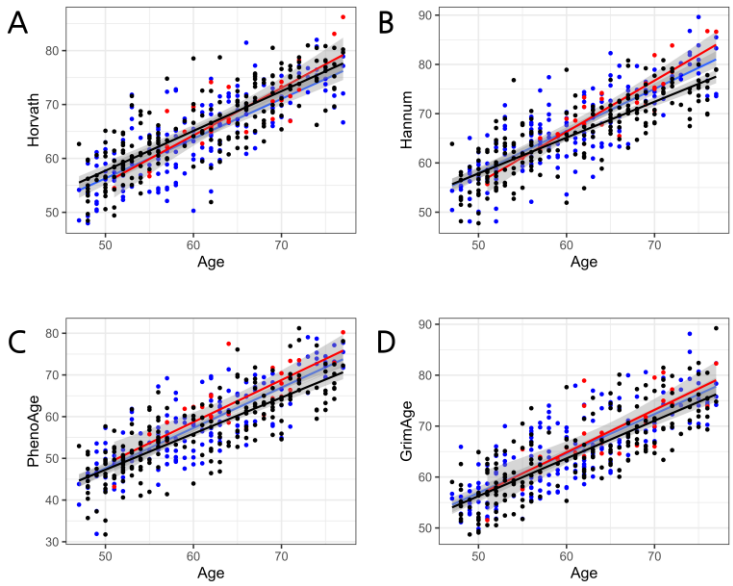

Figure 17. mAge by age in the long (red), short (blue), and CTL (black) groups in AS. **A,** Horvath. **B,** Hannum. **C,** PhenoAge. **D,** GrimAge.

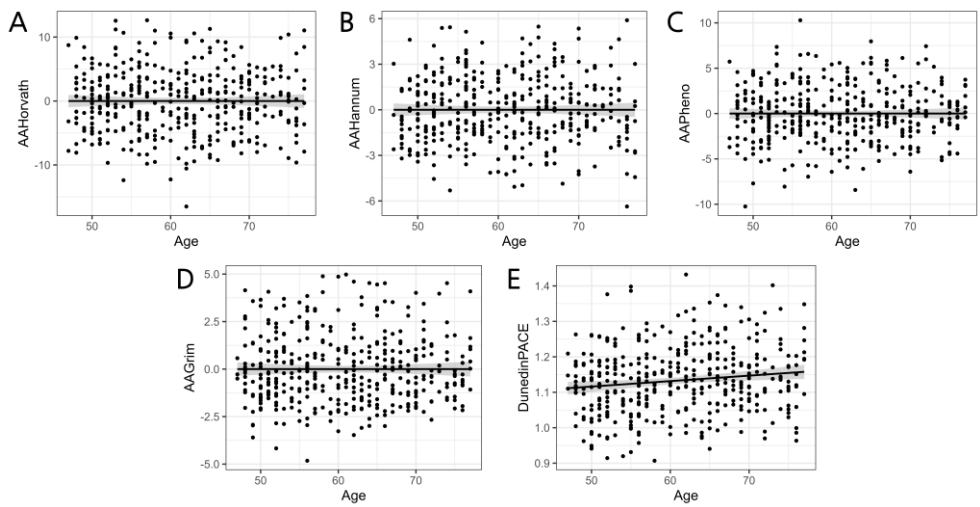

Figure 18. EAA by age in overall AS cohort. **A,** AAHorvath. **B,** AAHannum. **C,** AAPheno. **D,** AAGrim. **E,** DunedinPACE.

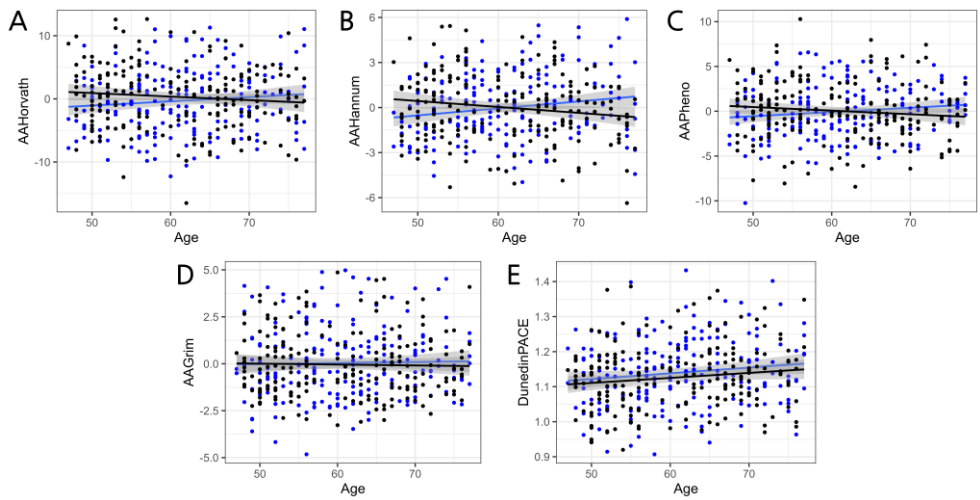

Figure 19. EAA by age in the DM (blue) and CTL (black) groups in AS. **A,** AAHorvath. **B,** AAHannum. **C,** AAPheno. **D,** AAGrim. **E,** DunedinPACE.

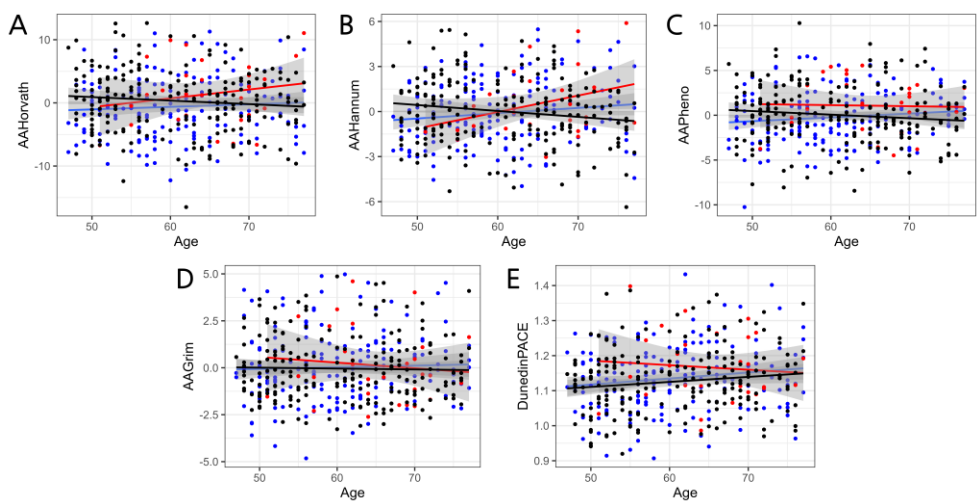

Figure 20. EAA by age in the long (red), short (blue), and CTL (black) groups in AS. **A,** AAHorvath. **B,** AAHannum. **C,** AAPheno. **D,** AAGrim. **E,** DunedinPACE.

Next, the mean of mAges/EAAs for the entire cohort and each subgroup was compared. Comparing the clocks of the DM and CTL groups in the SNUH cohort (**Table 7**), the CTL group had significantly higher means of Horvath, Hannum, PhenoAge, and GrimAge and smaller variance than the DM group, which is consistent with the trend of chronological age (**Table 2**). Seeing EAAs, the DM group had a significantly larger mean of DunedinPACE and significantly larger variances of AAHorvath, AAHannum, AAPheno, AAGrim, and DunedinPACE than the CTL group.

When compared by DM duration (**Table 8**), the short group had the lowest mean of Horvath, Hannum, PhenoAge, and GrimAge, and the highest variance. This tendency is consistent with the trend of chronological age. Meanwhile, the mean of DunedinPACE was significantly lowest in the CTL group, and the variance was significantly highest in the long group.

When the subgroups were divided by CKD stage (**Table 9**), the mean of AAHannum was significantly higher in the CKD 5 group than in the CKD 3 group, the mean of AAPheno was significantly higher in the CKD 3 group than in the CKD 4 group. The mean of AAGrim was The CKD 4 group was significantly higher than in the CKD 3 group.

Comparing the DM group and the CTL group in the AS cohort (**Table 10**), the DM group had significantly higher means of Hannum, PhenoAge, GrimAge, AAHannum, AAPheno, AAGrim, and DunedinPACE than the CTL group, and the CTL group had significantly higher means of AAHorvath than the DM group. The difference in chronological age between the two groups was insignificant (**Table 5**).

When divided by DM duration (**Table 11**), the CTL group had significantly

higher means of Horvath and AAHorvath than the short group, and the short group had significantly higher means of AAHannum, AAPheno, and DunedinPACE than the CTL group.

The long group had significantly higher means of Hannum, PhenoAge, and GrimAge than the CTL group, and significantly higher Horvath, Hannum, PhenoAge, and GrimAge than the short group, which was consistent with the trend of chronological age (**Table 6**). Also, the long group had significantly higher means of AAHannum, AAPheno, and DunedinPACE than the CTL group.

Group (n)	SNUH (429)	DM (232)	CTL (197)	p*	F*	p*
Horvath	65.01 (6.59)	64.06 (8.19)	66.13 (3.68)	**0.001**	4.951	**<0.001**
Hannum	53.50 (3.42)	52.85 (4.21)	54.26 (1.90)	**<0.001**	4.892	**<0.001**
PhenoAge	52.81 (3.81)	52.45 (4.65)	53.23 (2.43)	**0.034**	3.673	**<0.001**
GrimAge	107.03 (5.60)	106.48 (7.15)	107.67 (2.74)	**0.029**	6.809	**<0.001**
AAHorvath	0.00 (4.09)	0.27 (4.52)	-0.31 (3.50)	0.143	1.674	**<0.001**
AAHannum	0.00 (2.02)	0.07 (2.23)	-0.08 (1.74)	0.453	1.651	**<0.001**
AAPheno	0.00 (6.40)	0.10 (8.46)	-0.12 (2.24)	0.731	14.264	**<0.001**
AAGrim	0.00 (1.74)	0.02 (1.90)	-0.02 (1.55)	0.833	1.494	**0.004**
DunedinPACE	1.11 (0.11)	1.13 (0.11)	1.09 (0.10)	**<0.001**	1.331	**0.039**

Table 7. mAges/EAAs by the whole cohort and the DM/CTL groups in SNUH. Student's t-test and F-test were done between the DM/CTL groups. Bold numbers indicate p-value < 0.05.

Group (n)	CTL (197)	Short (93)	Long (139)	p*	p†	p‡	F*	p*	F†	p†	F‡	p‡
Horvath	66.13 (3.68)	61.20 (8.18)	65.98 (7.64)	**<0.001**	0.806	**<0.001**	0.202	**<0.001**	0.232	**<0.001**	1.146	0.465
Hannum	54.26 (1.90)	51.00 (3.98)	54.08 (3.90)	**<0.001**	0.573	**<0.001**	0.228	**<0.001**	0.238	**<0.001**	1.044	0.811
PhenoAge	53.23 (2.43)	50.65 (5.02)	53.66 (3.97)	**<0.001**	0.228	**<0.001**	0.233	**<0.001**	0.374	**<0.001**	1.604	**0.012**
GrimAge	107.67 (2.74)	103.52 (7.40)	108.46 (6.25)	**<0.001**	0.113	**<0.001**	0.137	**<0.001**	0.192	**<0.001**	1.404	0.071
AAHorvath	-0.31 (3.50)	0.14 (4.16)	0.35 (4.76)	0.336	0.14	0.724	0.705	**0.044**	0.539	**<0.001**	0.765	0.169
AAHannum	-0.08 (1.74)	-0.28 (1.89)	0.30 (2.41)	0.38	0.096	0.054	0.842	0.322	0.519	**<0.001**	0.616	**0.013**
AAPheno	-0.12 (2.24)	-0.55 (9.31)	0.53 (7.85)	0.54	0.276	0.344	0.058	**<0.001**	0.081	**<0.001**	1.406	0.070
AAGrim	-0.02 (1.55)	-0.11 (1.90)	0.10 (1.90)	0.671	0.528	0.413	0.670	**0.021**	0.667	**0.009**	0.995	0.990
DunedinPACE	1.09 (0.10)	1.11 (0.09)	1.14 (0.12)	**0.03**	**<0.001**	0.063	1.015	0.950	0.650	**0.006**	0.640	**0.023**

Table 8. mAges/EAAs by the CTL/short/long groups in SNUH. Student's t-test and F-test were done between the *CTL/Short, †CTL/Long, ‡Short/Long groups. Bold numbers indicate p-value < 0.05.

Group (n)	CKD 3 (202)	CKD 4 (14)	CKD 5 (16)	p*	p†	p‡	F*	p*	F†	p†	F‡	p‡
Horvath	64.22 (8.10)	64.28 (8.48)	61.82 (9.28)	0.981	0.259	0.458	0.912	0.727	0.761	0.392	0.835	0.751
Hannum	52.79 (4.15)	53.43 (4.32)	53.06 (5.01)	0.577	0.804	0.832	0.924	0.753	0.686	0.248	0.743	0.596
PhenoAge	52.35 (4.59)	53.98 (4.96)	52.37 (5.14)	0.203	0.987	0.393	0.856	0.610	0.797	0.469	0.931	0.906
GrimAge	106.55 (7.05)	107.57 (7.76)	104.73 (8.02)	0.603	0.328	0.335	0.825	0.544	0.772	0.415	0.936	0.914
AAHorvath	0.27 (4.64)	0.27 (3.56)	0.16 (3.95)	0.996	0.925	0.94	1.701	0.275	1.383	0.481	0.813	0.715
AAHannum	-0.08 (2.24)	0.62 (2.27)	1.43 (1.59)	0.263	**0.009**	0.259	0.970	0.846	1.976	0.129	2.037	0.189
AAPheno	0.16 (8.17)	-2.45 (12.87)	1.55 (7.57)	0.269	0.511	0.301	0.403	**0.007**	1.166	0.775	2.892	0.052
AAGrim	-0.10 (1.80)	0.89 (2.88)	0.71 (1.87)	0.059	0.086	0.841	0.388	**0.005**	0.922	0.744	2.373	0.112
DunedinPACE	1.13 (0.11)	1.13 (0.13)	1.14 (0.14)	0.949	0.633	0.818	0.682	0.266	0.539	0.059	0.790	0.676

Table 9. mAge/EAA by the CKD 3/CKD 4/CKD 5 groups in SNUH. Student's t-test and F-test were done between the *CKD 3/CKD 4, †CKD 3/CKD 5, ‡CKD 4/CKD 5 groups. Bold numbers indicate p-value < 0.05.

Group (n)	AS (400)	DM (200)	CTL (200)	p*	F*	p*
Horvath	65.19 (7.74)	64.68 (7.69)	65.70 (7.78)	0.189	0.975	0.860
Hannum	66.64 (8.12)	67.55 (8.39)	65.74 (7.75)	**0.025**	1.171	0.267
PhenoAge	57.61 (9.43)	58.59 (9.44)	56.63 (9.34)	**0.037**	1.023	0.875
GrimAge	64.91 (8.02)	65.56 (7.96)	64.26 (8.05)	0.107	0.978	0.875
AAHorvath	0.00 (4.62)	-0.62 (4.69)	0.62 (4.47)	**0.007**	1.103	0.491
AAHannum	0.00 (4.66)	0.79 (4.63)	-0.79 (4.56)	**0.001**	1.028	0.847
AAPheno	0.00 (5.47)	0.85 (5.21)	-0.85 (5.60)	**0.002**	0.866	0.312
AAGrim	0.00 (4.92)	0.54 (4.91)	-0.54 (4.89)	**0.029**	1.011	0.940
DunedinPACE	1.13 (0.11)	1.16 (0.11)	1.09 (0.10)	**<0.001**	1.080	0.586

Table 10. mAge/EAA by the whole cohort and the DM/CTL groups in AS. Student's t-test and F-test were done between the DM/CTL groups. Bold numbers indicate p-value < 0.05.

Group (n)	CTL (200)	Short (171)	Long (29)	p*	p†	p‡	F*	p*	F†	p†	F‡	p‡
Horvath	65.70 (7.78)	64.00 (7.61)	68.70 (6.99)	**0.035**	0.051	**0.002**	1.046	0.763	1.24	0.509	1.185	0.612
Hannum	65.74 (7.75)	66.87 (8.34)	71.55 (7.59)	0.176	**<0.001**	**0.005**	0.863	0.315	1.043	0.941	1.209	0.567
PhenoAge	56.63 (9.34)	57.71 (9.38)	63.77 (8.15)	0.266	**<0.001**	**0.001**	0.99	0.943	1.314	0.396	1.327	0.382
GrimAge	64.26 (8.05)	64.96 (7.97)	69.05 (7.07)	0.4	**0.003**	**0.01**	1.02	0.896	1.298	0.418	1.273	0.459
AAHorvath	0.62 (4.47)	-0.80 (4.78)	0.46 (4.02)	**0.003**	0.856	0.182	0.872	0.354	1.238	0.513	1.419	0.276
AAHannum	-0.79 (4.56)	0.64 (4.77)	1.66 (3.62)	**0.003**	**0.006**	0.277	0.916	0.548	1.588	0.146	1.735	0.087
AAPheno	-0.85 (5.60)	0.59 (5.26)	2.40 (4.74)	**0.012**	**0.003**	0.083	1.135	0.394	1.398	0.294	1.231	0.527
AAGrim	-0.54 (4.89)	0.45 (4.99)	1.03 (4.46)	0.055	0.104	0.557	0.958	0.769	1.2	0.582	1.253	0.491
DunedinPACE	1.09 (0.10)	1.15 (0.11)	1.19 (0.10)	**<0.001**	**<0.001**	0.078	0.93	0.622	0.968	0.852	1.040	0.947

Table 11. mAge/EAA by the CTL/short/long groups in AS. Student's t-test and F-test were done between the *CTL/Short, †CTL/Long, ‡Short/Long groups. Bold numbers indicate p-value < 0.05.

3. Multiple linear regression

To see the association between mAge/EAA and glucose, as a T2D-related factor, in the whole cohort and the subgroups, multiple linear regression was performed for mAge and EAA, respectively, through the following 2 models. Age and sex were considered when calculating mAge, so they were adjusted, but EAA considered that the effect of age was removed and did not adjust age. HbA1c was adjusted because it could act as a mediator between glucose and mAge/EAA. The reason glucose was set as an important variable is that insulin dysfunction is immediately reflected in glucose, and glucose is also the most basic test standard for diabetes.

model 1: mAge ~ Age + sex + HbA1c + glucose

model 2: EAA ~ sex + HbA1c + glucose

The subgroups and clocks in which glucose was significant in SNUH are as follows (**Table 12-13**): SNUH/Horvath, DM/Horvath, CKD 3/Horvath, CKD 4/Hannum, SNUH/AAHorvath, DM/AAHorvath, CKD 3/AAHorvath, CKD 4/AAHannum, CKD 3/DunedinPACE.

In the multiple linear regression model for mAge/EAA in AS (**Table 14-15**), glucose variables were significant across the five clocks (PhenoAge, GrimAge, AAPheno, AAGrim, and DunedinPACE), all in the CTL group.

When AIC was calculated for the 9-clock multiple linear regression model in the two whole cohorts (**Table 16**), DunedinPACE was the best model by AIC for both cohorts.

mAge	group	age		sex		HbA1c		glucose		(model)
		Estimate (S.E)	p	Estimate (S.E)	p	Estimate (S.E)	p	Estimate (S.E)	p	p
Horvath	**SNUH**	0.637 (0.027)	<0.001	1.078 (0.407)	0.008	0.188 (0.179)	0.296	-0.015 (0.006)	**0.015**	**<0.001**
Hannum		0.338 (0.013)	<0.001	0.991 (0.196)	<0.001	-0.243 (0.086)	0.005	0.001 (0.003)	0.864	**<0.001**
PhenoAge		0.368 (0.016)	<0.001	1.019 (0.248)	<0.001	0.084 (0.109)	0.441	-0.001 (0.004)	0.835	**<0.001**
GrimAge		0.687 (0.011)	<0.001	-0.048 (0.172)	0.78	0.152 (0.076)	0.046	-0.001 (0.003)	0.637	**<0.001**
Horvath	**DM**	0.67 (0.031)	<0.001	0.871 (0.604)	0.151	0.213 (0.242)	0.379	-0.014 (0.007)	**0.049**	**<0.001**
Hannum		0.348 (0.015)	<0.001	0.807 (0.295)	0.007	-0.263 (0.118)	0.027	0.0004 (0.003)	0.905	**<0.001**
PhenoAge		0.376 (0.019)	<0.001	0.833 (0.371)	0.026	0.106 (0.148)	0.475	-0.001 (0.004)	0.783	**<0.001**
GrimAge		0.686 (0.013)	<0.001	0.216 (0.256)	0.399	0.07 (0.102)	0.493	-0.002 (0.003)	0.592	**<0.001**
Horvath	**CTL**	0.267 (0.078)	0.001	1.433 (0.524)	0.007	1.218 (1.058)	0.251	-0.034 (0.035)	0.336	**0.001**
Hannum		0.211 (0.037)	<0.001	1.157 (0.249)	<0.001	-0.063 (0.503)	0.901	0.023 (0.017)	0.168	**<0.001**
PhenoAge		0.268 (0.049)	<0.001	1.138 (0.326)	0.001	-0.177 (0.658)	0.788	0.028 (0.022)	0.201	**<0.001**
GrimAge		0.695 (0.035)	<0.001	-0.347 (0.231)	0.135	0.177 (0.467)	0.704	0.002 (0.015)	0.898	**<0.001**
Horvath	**short**	0.684 (0.043)	<0.001	1.358 (0.901)	0.136	0.007 (0.326)	0.982	-0.015 (0.011)	0.197	**<0.001**
Hannum		0.347 (0.019)	<0.001	0.7 (0.398)	0.082	-0.297 (0.144)	0.042	0.003 (0.005)	0.495	**<0.001**
PhenoAge		0.432 (0.026)	<0.001	1.879 (0.549)	0.001	0.079 (0.198)	0.693	-0.002 (0.007)	0.792	**<0.001**
GrimAge		0.704 (0.02)	<0.001	0.612 (0.414)	0.143	-0.015 (0.15)	0.922	0.003 (0.005)	0.609	**<0.001**
Horvath	**long**	0.666 (0.05)	<0.001	0.67 (0.832)	0.422	0.423 (0.362)	0.245	-0.015 (0.009)	0.103	**<0.001**
Hannum		0.323 (0.025)	<0.001	0.79 (0.418)	0.061	-0.306 (0.182)	0.095	-0.001 (0.005)	0.867	**<0.001**
PhenoAge		0.311 (0.03)	<0.001	0.186 (0.493)	0.706	0.031 (0.214)	0.886	-0.002 (0.005)	0.646	**<0.001**

GrimAge		0.667 (0.02)	<0.001	-0.096 (0.333)	0.773	0.092 (0.145)	0.525	-0.004 (0.004)	0.243	**<0.001**
Horvath	**CKD 3**	0.649 (0.034)	<0.001	0.952 (0.664)	0.153	0.247 (0.262)	0.348	-0.017 (0.008)	**0.035**	**<0.001**
Hannum		0.338 (0.016)	<0.001	0.842 (0.316)	0.008	-0.193 (0.125)	0.124	-0.003 (0.004)	0.39	**<0.001**
PhenoAge		0.37 (0.02)	<0.001	0.804 (0.397)	0.044	0.159 (0.157)	0.312	-0.002 (0.005)	0.682	**<0.001**
GrimAge		0.678 (0.013)	<0.001	0.193 (0.26)	0.46	0.107 (0.103)	0.299	-0.003 (0.003)	0.414	**<0.001**
Horvath	**CKD 4**	0.74 (0.129)	<0.001	-0.935 (2.32)	0.696	0.694 (1.303)	0.607	0.054 (0.042)	0.233	**<0.001**
Hannum		0.295 (0.071)	0.003	-1.612 (1.279)	0.239	0.223 (0.718)	0.763	0.061 (0.023)	**0.027**	**0.001**
PhenoAge		0.366 (0.078)	0.001	-1.876 (1.394)	0.211	1.141 (0.783)	0.179	0.048 (0.025)	0.088	**0.001**
GrimAge		0.679 (0.1)	<0.001	-1.422 (1.798)	0.449	0.577 (1.009)	0.581	0.054 (0.033)	0.129	**<0.001**
Horvath	**CKD 5**	0.811 (0.117)	<0.001	-0.048 (2.549)	0.985	0.001 (1.169)	1	-0.008 (0.023)	0.726	**<0.001**
Hannum		0.481 (0.039)	<0.001	0.154 (0.863)	0.861	-0.312 (0.396)	0.448	0.007 (0.008)	0.395	**<0.001**
PhenoAge		0.425 (0.078)	<0.001	1.285 (1.716)	0.47	-0.032 (0.787)	0.969	-0.002 (0.016)	0.897	**0.002**
GrimAge		0.761 (0.053)	<0.001	-0.109 (1.149)	0.926	0.157 (0.527)	0.772	-0.005 (0.01)	0.611	**<0.001**

Table 12. Multiple linear regression results of mAge in SNUH. Bold numbers indicate p-value < 0.05 (applied only to the p-value of glucose and the model).

EAA	group	sex		HbA1c		glucose		(model)
		Estimate (S.E)	p	Estimate (S.E)	p	Estimate (S.E)	p	p
AAHorvath	**SNUH**	1.002 (0.394)	0.011	0.403 (0.173)	0.02	-0.014 (0.006)	**0.02**	**0.01**
AAHannum		0.927 (0.191)	<0.001	-0.085 (0.084)	0.316	0.001 (0.003)	0.714	**<0.001**
AAPheno		-3.168 (0.606)	<0.001	-0.114 (0.267)	0.669	0.004 (0.009)	0.688	**<0.001**
AAGrim		-0.042 (0.17)	0.804	0.047 (0.075)	0.531	-0.001 (0.003)	0.617	0.92
DunedinPACE		0.053 (0.01)	<0.001	0.017 (0.004)	<0.001	-0.0003 (0.0002)	0.055	**<0.001**
AAHorvath	**DM**	0.81 (0.596)	0.175	0.186 (0.239)	0.436	-0.014 (0.007)	**0.048**	0.132
AAHannum		0.751 (0.289)	0.01	-0.25 (0.116)	0.032	0.001 (0.003)	0.864	**0.004**
AAPheno		-6.852 (1.034)	<0.001	-0.204 (0.414)	0.623	0.005 (0.012)	0.681	**<0.001**
AAGrim		0.223 (0.252)	0.378	0.051 (0.101)	0.617	-0.001 (0.003)	0.609	0.796
DunedinPACE		0.056 (0.014)	<0.001	0.009 (0.006)	0.103	-0.0003 (0.0002)	0.061	**<0.001**
AAHorvath	**CTL**	1.381 (0.511)	0.007	1.081 (1.03)	0.295	-0.043 (0.034)	0.212	0.053
AAHannum		1.116 (0.243)	<0.001	-0.044 (0.49)	0.929	0.018 (0.016)	0.265	**<0.001**
AAPheno		1.064 (0.322)	0.001	-0.062 (0.649)	0.924	0.025 (0.021)	0.249	**0.002**
AAGrim		-0.358 (0.229)	0.12	0.238 (0.463)	0.608	0.005 (0.015)	0.76	0.373
DunedinPACE		0.051 (0.014)	<0.001	0.03 (0.028)	0.271	-0.00002 (0.0009)	0.979	**0.002**
AAHorvath	**short**	1.235 (0.899)	0.173	-0.024 (0.328)	0.943	-0.014 (0.011)	0.223	0.308
AAHannum		0.631 (0.402)	0.12	-0.284 (0.147)	0.056	0.004 (0.005)	0.407	0.081
AAPheno		-10.13 (1.727)	<0.001	-1.007 (0.629)	0.113	0.028 (0.022)	0.202	**<0.001**
AAGrim		0.571 (0.411)	0.169	-0.038 (0.15)	0.801	0.002 (0.005)	0.641	0.444
DunedinPACE		0.058 (0.02)	0.004	0.013 (0.007)	0.067	-0.0002 (0.0002)	0.387	**0.008**

AAHorvath	**long**	0.611 (0.814)	0.454	0.395 (0.346)	0.255	-0.015 (0.009)	0.093	0.334
AAHannum		0.772 (0.406)	0.059	-0.255 (0.173)	0.141	-0.0004 (0.004)	0.932	0.052
AAPheno		-4.797 (1.289)	<0.001	0.418 (0.548)	0.447	-0.002 (0.014)	0.871	**0.002**
AAGrim		-0.061 (0.327)	0.852	0.106 (0.139)	0.448	-0.004 (0.004)	0.28	0.745
DunedinPACE		0.052 (0.02)	0.01	0.004 (0.008)	0.636	-0.0003 (0.0002)	0.162	**0.03**
AAHorvath	**CKD 5**	-0.06 (2.475)	0.981	0.125 (1.132)	0.914	-0.012 (0.022)	0.599	0.887
AAHannum		0.266 (1.014)	0.798	-0.217 (0.464)	0.648	0.003 (0.009)	0.776	0.965
AAPheno		-9.044 (3.565)	0.026	-1.672 (1.63)	0.325	0.016 (0.031)	0.624	0.051
AAGrim		-0.185 (1.17)	0.877	0.227 (0.535)	0.679	-0.008 (0.01)	0.456	0.866
DunedinPACE		0.037 (0.091)	0.694	0.002 (0.042)	0.958	-0.0002 (0.0008)	0.779	0.932
AAHorvath	**CKD 4**	-1.958 (1.945)	0.338	1.155 (1.166)	0.345	0.061 (0.034)	0.105	0.258
AAHannum		-1.258 (1.185)	0.314	0.205 (0.711)	0.779	0.05 (0.021)	**0.039**	0.17
AAPheno		-12.147 (7.607)	0.141	2.52 (4.56)	0.593	0.03 (0.134)	0.826	0.495
AAGrim		-1.571 (1.585)	0.345	0.629 (0.95)	0.523	0.053 (0.028)	0.086	0.27
DunedinPACE		-0.086 (0.067)	0.232	0.068 (0.04)	0.125	0.002 (0.001)	0.157	0.179
AAHorvath	**CKD 3**	0.976 (0.658)	0.139	0.218 (0.259)	0.4	-0.016 (0.008)	**0.043**	0.111
AAHannum		0.802 (0.313)	0.011	-0.178 (0.123)	0.149	-0.003 (0.004)	0.493	**0.007**
AAPheno		-6.6 (1.077)	<0.001	-0.046 (0.424)	0.914	0.0003 (0.013)	0.979	**<0.001**
AAGrim		0.24 (0.258)	0.352	0.09 (0.101)	0.376	-0.002 (0.003)	0.46	0.665
DunedinPACE		0.063 (0.014)	<0.001	0.01 (0.006)	0.077	-0.0004 (0.0002)	**0.038**	**<0.001**

Table 13. Multiple linear regression results of EAA in SNUH. Bold numbers indicate p-value < 0.05 (applied only to the p-value of glucose and the model).

mAge	group	age		sex		HbA1c		glucose		(model)
		Estimate (S.E)	p	Estimate (S.E)	p	Estimate (S.E)	p	Estimate (S.E)	p	p
Horvath	**AS**	0.761 (0.028)	<0.001	1.967 (0.458)	<0.001	-0.044 (0.196)	0.822	-0.005 (0.007)	0.485	**<0.001**
Hannum		0.829 (0.026)	<0.001	3.579 (0.432)	<0.001	0.311 (0.185)	0.093	0.002 (0.006)	0.709	**<0.001**
PhenoAge		0.944 (0.032)	<0.001	2.039 (0.537)	<0.001	0.269 (0.23)	0.243	0.009 (0.008)	0.261	**<0.001**
GrimAge		0.82 (0.021)	<0.001	6.939 (0.351)	<0.001	0.154 (0.15)	0.304	0.007 (0.005)	0.177	**<0.001**
Horvath	**DM**	0.777 (0.042)	<0.001	1.758 (0.659)	0.008	0.48 (0.273)	0.08	-0.008 (0.007)	0.29	**<0.001**
Hannum		0.909 (0.039)	<0.001	3.048 (0.621)	<0.001	0.09 (0.257)	0.725	0.003 (0.007)	0.639	**<0.001**
PhenoAge		1.005 (0.047)	<0.001	0.578 (0.738)	0.434	0.289 (0.305)	0.344	0.006 (0.008)	0.422	**<0.001**
GrimAge		0.846 (0.033)	<0.001	6.623 (0.516)	<0.001	0.186 (0.213)	0.383	0.005 (0.006)	0.332	**<0.001**
Horvath	**CTL**	0.736 (0.039)	<0.001	2.061 (0.669)	0.002	1.23 (1.378)	0.373	0.072 (0.042)	0.087	**<0.001**
Hannum		0.744 (0.036)	<0.001	3.987 (0.625)	<0.001	1.16 (1.286)	0.368	0.065 (0.039)	0.098	**<0.001**
PhenoAge		0.884 (0.047)	<0.001	2.954 (0.808)	<0.001	0.056 (1.664)	0.973	0.153 (0.051)	**0.003**	**<0.001**
GrimAge		0.797 (0.029)	<0.001	6.913 (0.505)	<0.001	-0.272 (1.04)	0.794	0.086 (0.032)	**0.007**	**<0.001**
Horvath	**short**	0.767 (0.047)	<0.001	1.87 (0.738)	0.012	0.17 (0.42)	0.686	0.001 (0.01)	0.897	**<0.001**
Hannum		0.899 (0.045)	<0.001	3.168 (0.704)	<0.001	-0.179 (0.401)	0.656	0.01 (0.01)	0.323	**<0.001**
PhenoAge		0.997 (0.052)	<0.001	0.847 (0.816)	0.301	-0.142 (0.465)	0.76	0.015 (0.011)	0.197	**<0.001**
GrimAge		0.87 (0.036)	<0.001	6.816 (0.569)	<0.001	0.222 (0.324)	0.493	0.008 (0.008)	0.341	**<0.001**
Horvath	**long**	0.904 (0.122)	<0.001	1.124 (1.85)	0.549	0.39 (1.035)	0.71	-0.021 (0.011)	0.061	**<0.001**
Hannum		1.048 (0.101)	<0.001	2.987 (1.535)	0.063	0.758 (0.859)	0.386	-0.005 (0.009)	0.588	**<0.001**
PhenoAge		1.042 (0.155)	<0.001	-0.724 (2.352)	0.761	0.585 (1.316)	0.661	0.006 (0.014)	0.671	**<0.001**
GrimAge		0.755 (0.108)	<0.001	5.895 (1.646)	0.002	-0.206 (0.921)	0.825	-0.002 (0.01)	0.849	**<0.001**

Table 14. Multiple linear regression results of mAge in AS. Bold numbers indicate p-value < 0.05 (applied only to the p-value of glucose and the model).

EAA	group	sex		HbA1c		glucose		(model)
		Estimate (S.E)	p	Estimate (S.E)	p	Estimate (S.E)	p	p
AAHorvath	**AS**	1.939 (0.454)	<0.001	-0.036 (0.195)	0.855	-0.005 (0.007)	0.45	**<0.001**
AAHannum		3.518 (0.428)	<0.001	0.329 (0.184)	0.075	0.002 (0.006)	0.798	**<0.001**
AAPheno		1.998 (0.533)	<0.001	0.281 (0.229)	0.22	0.008 (0.008)	0.286	**<0.001**
AAGrim		6.818 (0.351)	<0.001	0.19 (0.151)	0.208	0.006 (0.005)	0.289	**<0.001**
DunedinPACE		0.063 (0.01)	<0.001	0.017 (0.004)	<0.001	0.000005 (0.0002)	0.974	**<0.001**
AAHorvath	**DM**	1.704 (0.654)	0.01	0.48 (0.272)	0.079	-0.008 (0.007)	0.253	**0.023**
AAHannum		2.845 (0.627)	<0.001	0.091 (0.261)	0.728	0.001 (0.007)	0.867	**<0.001**
AAPheno		0.426 (0.737)	0.564	0.29 (0.307)	0.346	0.005 (0.008)	0.538	0.255
AAGrim		6.465 (0.52)	<0.001	0.187 (0.217)	0.39	0.004 (0.006)	0.493	**<0.001**
DunedinPACE		0.049 (0.015)	0.001	0.011 (0.006)	0.065	0.0001 (0.0001)	0.682	**0.002**
AAHorvath	**CTL**	2.065 (0.668)	0.002	1.077 (1.267)	0.397	0.072 (0.042)	0.088	**0.001**
AAHannum		4.005 (0.627)	<0.001	0.404 (1.189)	0.734	0.064 (0.039)	0.104	**<0.001**
AAPheno		2.966 (0.807)	<0.001	-0.488 (1.533)	0.75	0.152 (0.051)	**0.003**	**<0.001**
AAGrim		6.901 (0.505)	<0.001	0.215 (0.959)	0.823	0.087 (0.032)	**0.007**	**<0.001**
DunedinPACE		0.061 (0.014)	<0.001	0.005 (0.027)	0.859	0.004 (0.001)	**<0.001**	**<0.001**
AAHorvath	**short**	1.819 (0.725)	0.013	0.161 (0.418)	0.701	0.001 (0.01)	0.922	0.069
AAHannum		2.902 (0.702)	<0.001	-0.226 (0.405)	0.578	0.008 (0.01)	0.421	**0.001**
AAPheno		0.651 (0.807)	0.421	-0.176 (0.465)	0.705	0.013 (0.011)	0.238	0.381
AAGrim		6.525 (0.574)	<0.001	0.171 (0.331)	0.606	0.006 (0.008)	0.485	**<0.001**
DunedinPACE		0.052 (0.016)	0.001	0.01 (0.009)	0.267	-0.00005 (0.0002)	0.818	**0.004**
AAHorvath	**long**	0.906 (1.866)	0.631	-0.072 (0.984)	0.942	-0.02 (0.011)	0.081	0.346
AAHannum		2.641 (1.676)	0.128	0.025 (0.883)	0.978	-0.003 (0.01)	0.786	0.32
AAPheno		-0.889 (2.322)	0.705	0.236 (1.224)	0.848	0.007 (0.014)	0.611	0.898
AAGrim		5.904 (1.606)	0.001	-0.187 (0.847)	0.827	-0.002 (0.009)	0.84	**0.001**
DunedinPACE		-0.007 (0.051)	0.885	-0.021 (0.027)	0.437	0.00002 (0.0003)	0.96	0.855

Table 15. Multiple linear regression results of EAA in AS. Bold numbers indicate p-value < 0.05 (applied only to the p-value of glucose and the model).

AIC	SNUH	AS
Horvath	2425.279	2349.705
Hannum	1806.197	2302.342
PhenoAge	2794.437	2477.607
GrimAge	1705.518	2135.752
AAPheno	2792.578	2475.996
AAHorvath	2423.283	2347.957
AAHannum	1804.211	2301.642
AAGrim	1703.518	2141.498
DunedinPACE	-746.366	-697.937

Table 16. AIC for the multiple linear regression models in SNUH and AS.

4. Bland-Altman plot

Bland-Altman plots (Difference plots) between four mAges and two cohorts were drawn to check the underestimation tendency of mAges (**Figure 21-22**). A tendency for mAge to be underestimated as age increased in the SNUH cohort was clear, but was weak in the AS cohort. As age increased, there was a tendency for PhenoAge to be even overestimated in the AS cohort, but this was also not clear.

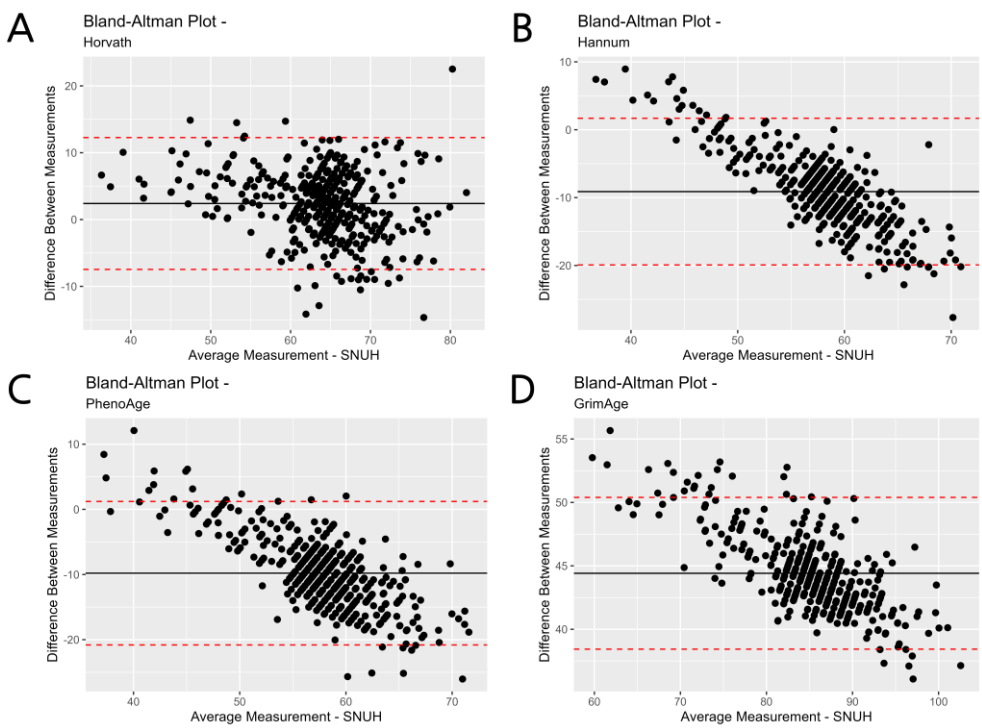

Figure 21. Bland-Altman plot between four mAges and SNUH cohort. **A,** Horvath and SNUH age. **B,** Hannum and SNUH age. **C,** PhenoAge and SNUH age **D,** GrimAge and SNUH age. The horizontal lines in each plot show the mean difference ± 1.96 * sd.

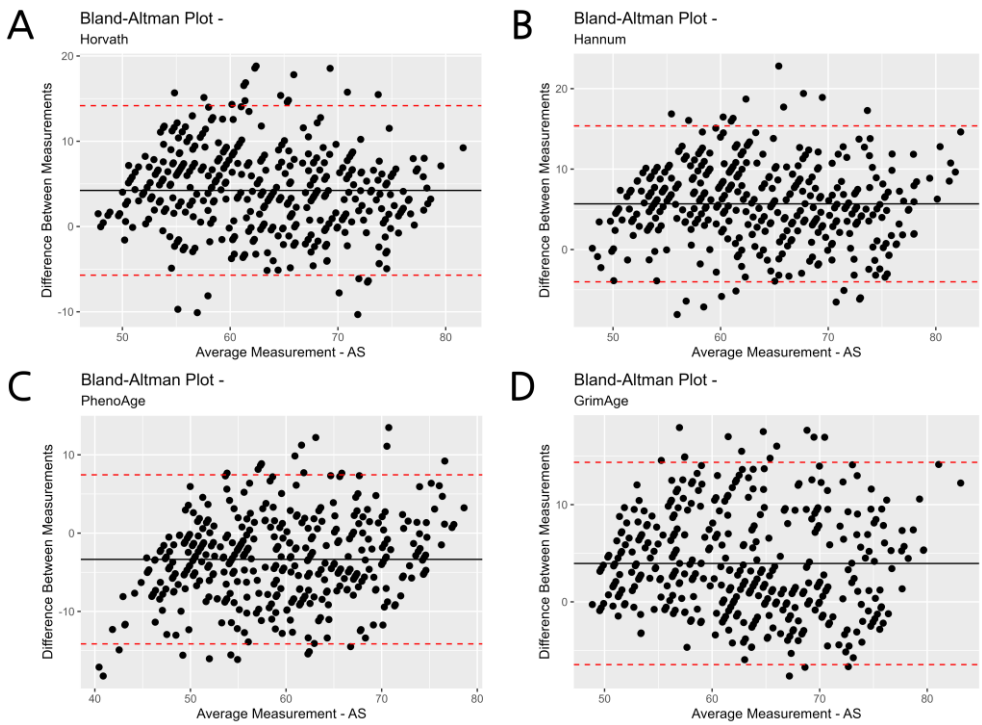

Figure 22. Bland-Altman plot between four mAges and AS cohort. **A,** Horvath and AS age. **B,** Hannum and AS age. **C,** PhenoAge and AS age **D,** GrimAge and AS age. The horizontal lines in each plot show the mean difference ± 1.96 * sd.

5. T2D-related CpGs in the clocks and the cohorts

As a result of arranging overlapping CpGs in the T2D-related CpGs list with CpGs disclosed in the clock studies, 10 CpGs were found only in DunedinPACE and none from other clocks (**Table 17**). However, GrimAge did not disclose all 1030 CpGs, so they were not collated.

In addition, the methylation data of SNUH and AS were compared with the corresponding list [27] to collect overlapping CpGs. Among the CpGs, CpGs with significant differences in the subgroup means of methylation beta were listed: In SNUH, 83 CpGs with significant differences between the DM/CTL groups (**Table 18**), 91 CpGs with significant differences among the CTL/short/long groups (**Table 19**), and 70 CpGs with significant differences among the CKD 3/4/5 groups (**Table 20**), and in AS, 70 CpGs with significant differences between the DM/CTL groups in AS (**Table 21**), 67 CpGs with significant differences among the CTL/short/long groups (**Table 22**). A total of 73 CpGs were identified with significant differences between the subgroups in AS. In addition, a total of 107 CpGs with significant differences were found in the entire cohort.

Marker name	Distance	Nearest gene symbol	Illumina gene name	Effect	p
cg06500161	0	ABCG1	ABCG1	0.0111	3.68E-21
cg00574958	0	CPT1A	CPT1A	-0.0052	3.17E-13
cg11202345	0	LGALS3BP	LGALS3BP	0.0077	3.47E-09
cg01101459	4086	LINC01132		0.0077	3.57E-08
cg04927537	29	LGALS3BP	LGALS3BP	0.0104	6.04E-08
cg17901584	784	DHCR24	DHCR24	-0.0094	9.45E-08
cg18181703	0	SOCS3	SOCS3	0.95	2.1x10-7
cg02650017	0	PHOSPHO1	PHOSPHO1	0.94	2.1x10-9
cg10919522	0	ELMSAN1	C14orf43	0.46	5x10-10
cg24531955	0	LOXL2	LOXL2	0.37	9.29x10-9

Table 17. Overlapping CpGs between DunedinPACE and recently reviewed T2D-related CpGs. The whole list is originally from Fraszczyk E. et al. (2022) [27].

SNUH CpG ID	DM mean (SD)	CTL mean (SD)	p
cg00076653	0.376 (0.019)	0.365 (0.016)	<0.001
cg00574958	0.236 (0.013)	0.242 (0.01)	<0.001
cg01332882	0.444 (0.042)	0.46 (0.041)	<0.001
cg01373896	0.567 (0.021)	0.56 (0.02)	<0.001
cg01657995	0.249 (0.02)	0.255 (0.017)	<0.001
cg02050917	0.581 (0.023)	0.591 (0.019)	<0.001
cg02879453	0.549 (0.025)	0.535 (0.025)	<0.001
cg02976843	0.439 (0.027)	0.429 (0.022)	<0.001
cg03106207	0.354 (0.015)	0.344 (0.013)	<0.001
cg03497652	0.548 (0.03)	0.559 (0.031)	<0.001
cg03691549	0.396 (0.02)	0.383 (0.017)	<0.001
cg04344749	0.426 (0.018)	0.413 (0.015)	<0.001
cg04682775	0.442 (0.02)	0.43 (0.019)	<0.001
cg04727071	0.342 (0.036)	0.362 (0.035)	<0.001
cg05400498	0.495 (0.021)	0.482 (0.02)	<0.001
cg05478824	0.446 (0.024)	0.438 (0.024)	<0.001
cg05778424	0.399 (0.026)	0.391 (0.023)	<0.001
cg06178887	0.488 (0.023)	0.48 (0.023)	<0.001
cg06378491	0.42 (0.016)	0.407 (0.014)	<0.001
cg06500161	0.477 (0.019)	0.46 (0.016)	<0.001

cg06721411	0.477 (0.025)	0.465 (0.025)	<0.001
cg06940720	0.545 (0.025)	0.536 (0.026)	<0.001
cg07021906	0.582 (0.024)	0.59 (0.021)	<0.001
cg07504977	0.366 (0.023)	0.357 (0.019)	<0.001
cg07719604	0.462 (0.026)	0.452 (0.02)	<0.001
cg08788930	0.52 (0.022)	0.508 (0.021)	<0.001
cg08994060	0.357 (0.039)	0.378 (0.033)	<0.001
cg09294084	0.355 (0.043)	0.374 (0.041)	<0.001
cg09664445	0.468 (0.019)	0.454 (0.018)	<0.001
cg10639435	0.398 (0.029)	0.388 (0.022)	<0.001
cg10717869	0.546 (0.021)	0.538 (0.021)	<0.001
cg11024682	0.414 (0.019)	0.397 (0.016)	<0.001
cg11269166	0.348 (0.018)	0.339 (0.015)	<0.001
cg12257439	0.426 (0.019)	0.413 (0.017)	<0.001
cg14597545	0.46 (0.03)	0.45 (0.027)	<0.001
cg14870271	0.36 (0.022)	0.352 (0.017)	<0.001
cg14956201	0.525 (0.022)	0.536 (0.02)	<0.001
cg16097041	0.5 (0.023)	0.485 (0.023)	<0.001
cg16809457	0.497 (0.026)	0.483 (0.025)	<0.001
cg16861241	0.463 (0.023)	0.475 (0.022)	<0.001
cg17058475	0.248 (0.021)	0.255 (0.015)	<0.001
cg17540192	0.662 (0.018)	0.672 (0.019)	<0.001
cg18568872	0.439 (0.017)	0.424 (0.016)	<0.001
cg19693031	0.539 (0.035)	0.562 (0.027)	<0.001
cg19750657	0.541 (0.024)	0.528 (0.023)	<0.001
cg20784591	0.394 (0.018)	0.381 (0.015)	<0.001
cg21480264	0.431 (0.018)	0.417 (0.015)	<0.001
cg22650271	0.551 (0.02)	0.543 (0.02)	<0.001
cg22909677	0.618 (0.019)	0.61 (0.018)	<0.001
cg23021329	0.347 (0.014)	0.336 (0.011)	<0.001
cg24145109	0.349 (0.04)	0.362 (0.035)	<0.001
cg24259291	0.397 (0.023)	0.388 (0.02)	<0.001
cg25217710	0.487 (0.019)	0.474 (0.019)	<0.001
cg26546155	0.439 (0.029)	0.428 (0.026)	<0.001
cg26608667	0.505 (0.027)	0.493 (0.024)	<0.001
cg26804423	0.523 (0.025)	0.514 (0.026)	<0.001
cg26846781	0.427 (0.02)	0.414 (0.015)	<0.001
cg27243685	0.609 (0.017)	0.6 (0.018)	<0.001
cg09247619	0.341 (0.019)	0.337 (0.018)	0.033
cg01676795	0.441 (0.039)	0.433 (0.034)	0.031
cg18181703	0.394 (0.022)	0.389 (0.02)	0.021
cg04816311	0.521 (0.04)	0.53 (0.034)	0.013
cg13640297	0.458 (0.033)	0.45 (0.029)	0.011
cg21766592	0.231 (0.015)	0.234 (0.012)	0.006

cg13059136	0.43 (0.035)	0.422 (0.027)	0.005
cg13199639	0.222 (0.007)	0.22 (0.004)	0.005
cg04645070	0.273 (0.024)	0.279 (0.02)	0.004
cg04927537	0.403 (0.031)	0.395 (0.026)	0.004
cg17666418	0.257 (0.021)	0.262 (0.019)	0.004
cg21699330	0.251 (0.018)	0.256 (0.015)	0.004
cg25130381	0.633 (0.019)	0.638 (0.021)	0.004
cg26663590	0.444 (0.029)	0.435 (0.029)	0.003
cg01101459	0.636 (0.021)	0.642 (0.019)	0.002
cg03403093	0.549 (0.023)	0.542 (0.02)	0.002
cg08309687	0.392 (0.033)	0.401 (0.026)	0.002
cg14020176	0.492 (0.026)	0.5 (0.026)	0.002
cg20507228	0.404 (0.03)	0.396 (0.027)	0.002
cg00994936	0.672 (0.021)	0.679 (0.02)	0.001
cg06192883	0.332 (0.023)	0.326 (0.018)	0.001
cg11202345	0.282 (0.019)	0.277 (0.014)	0.001
cg17901584	0.398 (0.032)	0.408 (0.027)	0.001
cg21703988	0.554 (0.023)	0.547 (0.022)	0.001
cg24531955	0.284 (0.014)	0.279 (0.012)	0.001

Table 18. CpGs with significant differences in the mean of methylation beta between the DM and CTL groups in SNUH.

SNUH CpG ID	CTL mean (SD)	Short mean (SD)	Long mean (SD)	p
cg00076653	0.365 (0.016)	0.368 (0.017)	0.376 (0.021)	<0.001
cg00574958	0.242 (0.01)	0.24 (0.012)	0.235 (0.012)	<0.001
cg01332882	0.46 (0.041)	0.455 (0.04)	0.442 (0.045)	<0.001
cg01373896	0.56 (0.02)	0.563 (0.02)	0.565 (0.02)	<0.001
cg01657995	0.255 (0.017)	0.254 (0.018)	0.247 (0.02)	<0.001
cg02050917	0.591 (0.019)	0.588 (0.02)	0.581 (0.025)	<0.001
cg02879453	0.535 (0.025)	0.539 (0.026)	0.549 (0.023)	<0.001
cg02976843	0.429 (0.022)	0.433 (0.024)	0.437 (0.027)	<0.001
cg03106207	0.344 (0.013)	0.347 (0.015)	0.354 (0.014)	<0.001
cg03497652	0.559 (0.031)	0.556 (0.03)	0.548 (0.032)	<0.001
cg03691549	0.383 (0.017)	0.388 (0.019)	0.393 (0.02)	<0.001
cg04344749	0.413 (0.015)	0.416 (0.016)	0.427 (0.017)	<0.001
cg04682775	0.43 (0.019)	0.434 (0.021)	0.442 (0.019)	<0.001
cg04727071	0.362 (0.035)	0.357 (0.035)	0.338 (0.037)	<0.001
cg05400498	0.482 (0.02)	0.487 (0.022)	0.492 (0.021)	<0.001
cg05778424	0.391 (0.023)	0.395 (0.025)	0.397 (0.027)	<0.001
cg06178887	0.48 (0.023)	0.483 (0.023)	0.487 (0.023)	<0.001
cg06378491	0.407 (0.014)	0.412 (0.016)	0.419 (0.016)	<0.001

cg06500161	0.46 (0.016)	0.465 (0.018)	0.478 (0.02)	<0.001
cg06721411	0.465 (0.025)	0.47 (0.026)	0.476 (0.026)	<0.001
cg07504977	0.357 (0.019)	0.36 (0.021)	0.366 (0.023)	<0.001
cg07719604	0.452 (0.02)	0.456 (0.023)	0.462 (0.026)	<0.001
cg08788930	0.508 (0.021)	0.511 (0.022)	0.52 (0.022)	<0.001
cg08994060	0.378 (0.033)	0.371 (0.035)	0.358 (0.043)	<0.001
cg09294084	0.374 (0.041)	0.368 (0.04)	0.355 (0.047)	<0.001
cg09664445	0.454 (0.018)	0.458 (0.019)	0.468 (0.019)	<0.001
cg10639435	0.388 (0.022)	0.389 (0.024)	0.403 (0.029)	<0.001
cg10717869	0.538 (0.021)	0.54 (0.021)	0.547 (0.021)	<0.001
cg11024682	0.397 (0.016)	0.402 (0.018)	0.414 (0.019)	<0.001
cg11269166	0.339 (0.015)	0.342 (0.016)	0.348 (0.018)	<0.001
cg12257439	0.413 (0.017)	0.418 (0.019)	0.425 (0.019)	<0.001
cg14597545	0.45 (0.027)	0.455 (0.029)	0.455 (0.029)	<0.001
cg14870271	0.352 (0.017)	0.353 (0.018)	0.363 (0.024)	<0.001
cg14956201	0.536 (0.02)	0.533 (0.021)	0.524 (0.023)	<0.001
cg16097041	0.485 (0.023)	0.49 (0.024)	0.498 (0.023)	<0.001
cg16809457	0.483 (0.025)	0.49 (0.026)	0.493 (0.027)	<0.001
cg16861241	0.475 (0.022)	0.472 (0.022)	0.461 (0.024)	<0.001
cg17058475	0.255 (0.015)	0.254 (0.018)	0.246 (0.019)	<0.001
cg17540192	0.672 (0.019)	0.67 (0.018)	0.66 (0.019)	<0.001
cg18568872	0.424 (0.016)	0.429 (0.018)	0.437 (0.018)	<0.001
cg19693031	0.562 (0.027)	0.555 (0.031)	0.538 (0.036)	<0.001
cg19750657	0.528 (0.023)	0.534 (0.024)	0.538 (0.024)	<0.001
cg20784591	0.381 (0.015)	0.386 (0.017)	0.394 (0.019)	<0.001
cg21480264	0.417 (0.015)	0.423 (0.018)	0.429 (0.019)	<0.001
cg22650271	0.543 (0.02)	0.546 (0.021)	0.55 (0.02)	<0.001
cg22909677	0.61 (0.018)	0.613 (0.019)	0.615 (0.019)	<0.001
cg23021329	0.336 (0.011)	0.339 (0.013)	0.348 (0.013)	<0.001
cg24259291	0.388 (0.02)	0.392 (0.021)	0.396 (0.023)	<0.001
cg25217710	0.474 (0.019)	0.478 (0.02)	0.486 (0.019)	<0.001
cg26546155	0.428 (0.026)	0.433 (0.027)	0.436 (0.031)	<0.001
cg26608667	0.493 (0.024)	0.498 (0.025)	0.502 (0.029)	<0.001
cg26846781	0.414 (0.015)	0.419 (0.017)	0.426 (0.021)	<0.001
cg27243685	0.6 (0.018)	0.603 (0.018)	0.609 (0.018)	<0.001
cg08309687	0.401 (0.026)	0.396 (0.029)	0.398 (0.033)	<0.001
cg06192883	0.326 (0.018)	0.327 (0.02)	0.334 (0.023)	<0.001
cg17901584	0.408 (0.027)	0.405 (0.029)	0.396 (0.032)	<0.001
cg21703988	0.547 (0.022)	0.547 (0.022)	0.559 (0.023)	<0.001
cg24531955	0.279 (0.012)	0.281 (0.013)	0.282 (0.014)	<0.001
cg03725309	0.26 (0.012)	0.261 (0.014)	0.254 (0.015)	<0.001
cg04816311	0.53 (0.034)	0.529 (0.034)	0.519 (0.044)	0.048
cg02711608	0.274 (0.011)	0.275 (0.012)	0.274 (0.013)	0.030
cg04992150	0.306 (0.016)	0.308 (0.018)	0.305 (0.021)	0.030

cg13199639	0.22 (0.004)	0.221 (0.005)	0.222 (0.007)	0.029
cg04973995	0.269 (0.015)	0.27 (0.016)	0.266 (0.017)	0.024
cg09247619	0.337 (0.018)	0.339 (0.018)	0.34 (0.02)	0.019
cg10508317	0.245 (0.008)	0.245 (0.009)	0.248 (0.009)	0.018
cg01676795	0.433 (0.034)	0.437 (0.034)	0.437 (0.041)	0.016
cg26836479	0.238 (0.01)	0.239 (0.011)	0.235 (0.012)	0.011
cg25130381	0.638 (0.021)	0.637 (0.02)	0.632 (0.019)	0.009
cg26712428	0.33 (0.037)	0.328 (0.038)	0.322 (0.042)	0.008
cg05478824	0.438 (0.024)	0.44 (0.024)	0.447 (0.025)	0.006
cg01101459	0.642 (0.019)	0.64 (0.019)	0.636 (0.022)	0.006
cg21699330	0.256 (0.015)	0.255 (0.016)	0.25 (0.018)	0.005
cg20507228	0.396 (0.027)	0.399 (0.027)	0.403 (0.031)	0.004
cg17836612	0.521 (0.025)	0.521 (0.025)	0.529 (0.028)	0.004
cg04927537	0.395 (0.026)	0.396 (0.027)	0.406 (0.033)	0.003
cg13640297	0.45 (0.029)	0.454 (0.029)	0.454 (0.036)	0.002
cg13059136	0.422 (0.027)	0.426 (0.03)	0.427 (0.036)	0.002
cg17666418	0.262 (0.019)	0.262 (0.019)	0.255 (0.021)	0.002
cg06940720	0.536 (0.026)	0.54 (0.025)	0.543 (0.026)	0.001
cg07021906	0.59 (0.021)	0.588 (0.021)	0.583 (0.027)	0.001
cg24145109	0.362 (0.035)	0.358 (0.038)	0.349 (0.039)	0.001
cg26804423	0.514 (0.026)	0.517 (0.026)	0.521 (0.026)	0.001
cg21766592	0.234 (0.012)	0.234 (0.013)	0.229 (0.015)	0.001
cg04645070	0.279 (0.02)	0.279 (0.022)	0.27 (0.024)	0.001
cg26663590	0.435 (0.029)	0.439 (0.029)	0.441 (0.029)	0.001
cg03403093	0.542 (0.02)	0.545 (0.022)	0.547 (0.023)	0.001
cg14020176	0.5 (0.026)	0.498 (0.025)	0.49 (0.028)	0.001
cg00994936	0.679 (0.02)	0.677 (0.019)	0.67 (0.023)	0.001
cg11202345	0.277 (0.014)	0.278 (0.015)	0.284 (0.02)	0.001
cg07092212	0.25 (0.012)	0.251 (0.013)	0.247 (0.013)	0.001

Table 19. CpGs with significant differences in the mean of methylation beta among the CTL, short, and long groups in SNUH.

SNUH CpG ID	**CKD 3**	**CKD 4**	**CKD 5**	**p**
	mean (SD)	**mean (SD)**	**mean (SD)**	
cg04816311	0.529 (0.035)	0.494 (0.043)	0.471 (0.03)	<0.001
cg08309687	0.394 (0.03)	0.423 (0.025)	0.428 (0.02)	<0.001
cg02050917	0.587 (0.021)	0.567 (0.026)	0.562 (0.012)	<0.001
cg00994936	0.677 (0.02)	0.657 (0.019)	0.653 (0.018)	<0.001
cg14956201	0.532 (0.021)	0.516 (0.019)	0.503 (0.019)	<0.001
cg16861241	0.47 (0.023)	0.449 (0.024)	0.442 (0.02)	<0.001
cg17836612	0.522 (0.026)	0.539 (0.02)	0.552 (0.015)	<0.001

cg10639435	0.392 (0.025)	0.417 (0.031)	0.425 (0.028)	<0.001
cg17540192	0.668 (0.018)	0.654 (0.023)	0.644 (0.014)	<0.001
cg01332882	0.454 (0.041)	0.42 (0.051)	0.406 (0.028)	<0.001
cg21703988	0.55 (0.023)	0.563 (0.018)	0.575 (0.013)	<0.001
cg14020176	0.497 (0.025)	0.483 (0.033)	0.463 (0.02)	<0.001
cg04645070	0.277 (0.022)	0.268 (0.024)	0.247 (0.019)	<0.001
cg04727071	0.354 (0.035)	0.329 (0.041)	0.306 (0.038)	<0.001
cg19266329	0.465 (0.023)	0.484 (0.016)	0.486 (0.02)	<0.001
cg14870271	0.355 (0.02)	0.367 (0.022)	0.377 (0.016)	<0.001
cg03106207	0.349 (0.015)	0.364 (0.012)	0.359 (0.016)	<0.001
cg10717869	0.541 (0.021)	0.557 (0.018)	0.561 (0.017)	<0.001
cg01676795	0.439 (0.036)	0.421 (0.039)	0.4 (0.025)	<0.001
cg07021906	0.588 (0.023)	0.568 (0.025)	0.567 (0.019)	<0.001
cg03699074	0.261 (0.013)	0.273 (0.016)	0.274 (0.017)	<0.001
cg11202345	0.279 (0.017)	0.291 (0.016)	0.295 (0.016)	<0.001
cg23021329	0.341 (0.014)	0.353 (0.009)	0.35 (0.011)	<0.001
cg08994060	0.369 (0.037)	0.345 (0.047)	0.33 (0.035)	<0.001
cg03725309	0.259 (0.014)	0.255 (0.016)	0.244 (0.013)	<0.001
cg04992150	0.308 (0.019)	0.301 (0.026)	0.289 (0.017)	<0.001
cg07092212	0.25 (0.013)	0.249 (0.018)	0.236 (0.012)	<0.001
cg02711608	0.275 (0.012)	0.28 (0.013)	0.264 (0.008)	<0.001
cg21766592	0.233 (0.013)	0.23 (0.016)	0.219 (0.012)	<0.001
cg24145109	0.357 (0.037)	0.33 (0.047)	0.323 (0.046)	0.001
cg17666418	0.26 (0.02)	0.256 (0.023)	0.241 (0.019)	0.001
cg04927537	0.398 (0.029)	0.407 (0.029)	0.424 (0.027)	0.001
cg03497652	0.555 (0.031)	0.535 (0.027)	0.534 (0.026)	0.001
cg07504977	0.361 (0.022)	0.379 (0.02)	0.371 (0.016)	0.001
cg26608667	0.5 (0.026)	0.495 (0.026)	0.478 (0.021)	0.001
cg11269166	0.343 (0.017)	0.355 (0.012)	0.354 (0.014)	0.001
cg15962267	0.468 (0.025)	0.487 (0.029)	0.487 (0.026)	0.001
cg13059136	0.428 (0.031)	0.417 (0.034)	0.4 (0.026)	0.001
cg17315426	0.241 (0.012)	0.239 (0.013)	0.23 (0.011)	0.002
cg24259291	0.394 (0.022)	0.394 (0.025)	0.377 (0.009)	0.002
cg13199639	0.221 (0.006)	0.225 (0.004)	0.222 (0.006)	0.002
cg18568872	0.431 (0.018)	0.447 (0.018)	0.437 (0.016)	0.002
cg26836479	0.238 (0.011)	0.24 (0.014)	0.228 (0.011)	0.003
cg20784591	0.388 (0.018)	0.403 (0.019)	0.395 (0.016)	0.003
cg09294084	0.365 (0.043)	0.339 (0.038)	0.341 (0.036)	0.003
cg21699330	0.254 (0.016)	0.247 (0.024)	0.239 (0.015)	0.004
cg07719604	0.458 (0.024)	0.464 (0.025)	0.439 (0.017)	0.004
cg17058475	0.252 (0.019)	0.247 (0.02)	0.237 (0.015)	0.004
cg13640297	0.455 (0.031)	0.452 (0.031)	0.431 (0.021)	0.006
cg05778424	0.396 (0.025)	0.393 (0.031)	0.375 (0.027)	0.006
cg06192883	0.328 (0.021)	0.335 (0.019)	0.344 (0.021)	0.007

cg14597545	0.456 (0.029)	0.454 (0.026)	0.434 (0.023)	0.008
cg02650017	0.227 (0.007)	0.231 (0.008)	0.23 (0.008)	0.008
cg26546155	0.435 (0.028)	0.432 (0.03)	0.414 (0.022)	0.009
cg00076653	0.37 (0.018)	0.381 (0.015)	0.365 (0.029)	0.013
cg06940720	0.542 (0.026)	0.542 (0.024)	0.524 (0.019)	0.017
cg01101459	0.64 (0.02)	0.627 (0.026)	0.629 (0.02)	0.018
cg11024682	0.405 (0.02)	0.417 (0.013)	0.408 (0.014)	0.019
cg26804423	0.519 (0.026)	0.516 (0.024)	0.504 (0.014)	0.021
cg25130381	0.636 (0.02)	0.629 (0.014)	0.624 (0.018)	0.023
cg25217710	0.481 (0.02)	0.486 (0.021)	0.469 (0.012)	0.024
cg16097041	0.493 (0.024)	0.498 (0.024)	0.479 (0.017)	0.027
cg15020801	0.36 (0.018)	0.357 (0.02)	0.349 (0.014)	0.028
cg06500161	0.469 (0.02)	0.481 (0.014)	0.472 (0.021)	0.029
cg06178887	0.485 (0.024)	0.481 (0.017)	0.471 (0.017)	0.031
cg26262157	0.334 (0.026)	0.331 (0.036)	0.315 (0.027)	0.033
cg14476101	0.485 (0.032)	0.471 (0.034)	0.469 (0.03)	0.036
cg01657995	0.252 (0.019)	0.244 (0.018)	0.241 (0.019)	0.037
cg26846781	0.421 (0.019)	0.43 (0.019)	0.414 (0.014)	0.039
cg02879453	0.542 (0.026)	0.555 (0.025)	0.535 (0.024)	0.050

Table 20. CpGs with significant differences in the mean of methylation beta among the CKD 3, CKD 4, and CKD 5 groups in SNUH.

AS	**CTL**	**DM**	
CpG ID	**mean (SD)**	**mean (SD)**	**p**
cg00076653	0.407 (0.04)	0.419 (0.038)	0.002
cg06397161	0.536 (0.064)	0.55 (0.063)	0.031
cg00574958	0.112 (0.034)	0.096 (0.029)	<0.001
cg00994936	0.821 (0.033)	0.84 (0.041)	<0.001
cg01101459	0.719 (0.037)	0.74 (0.035)	<0.001
cg01332882	0.581 (0.058)	0.601 (0.054)	<0.001
cg01676795	0.553 (0.075)	0.582 (0.074)	<0.001
cg02650017	0.067 (0.015)	0.062 (0.014)	<0.001
cg17901584	0.508 (0.065)	0.494 (0.071)	0.038
cg02879453	0.741 (0.046)	0.756 (0.04)	<0.001
cg02976843	0.551 (0.048)	0.567 (0.046)	0.001
cg03497652	0.663 (0.049)	0.693 (0.055)	<0.001
cg03691549	0.444 (0.044)	0.456 (0.04)	0.004
cg03699074	0.169 (0.034)	0.158 (0.032)	0.001
cg04682775	0.551 (0.041)	0.574 (0.037)	<0.001
cg04816311	0.526 (0.065)	0.56 (0.055)	<0.001
cg07021906	0.661 (0.036)	0.67 (0.04)	0.02

cg05400498	0.688 (0.044)	0.706 (0.04)	<0.001
cg05478824	0.562 (0.049)	0.581 (0.051)	<0.001
cg06178887	0.675 (0.041)	0.692 (0.041)	<0.001
cg06192883	0.317 (0.051)	0.335 (0.048)	<0.001
cg06500161	0.589 (0.03)	0.616 (0.032)	<0.001
cg06721411	0.596 (0.051)	0.636 (0.048)	<0.001
cg06940720	0.757 (0.048)	0.774 (0.044)	<0.001
cg07504977	0.377 (0.05)	0.415 (0.048)	<0.001
cg03403093	0.748 (0.043)	0.759 (0.042)	0.012
cg08309687	0.527 (0.066)	0.508 (0.067)	0.005
cg08788930	0.681 (0.041)	0.699 (0.04)	<0.001
cg08994060	0.394 (0.064)	0.376 (0.054)	0.002
cg09294084	0.477 (0.072)	0.494 (0.064)	0.009
cg09664445	0.535 (0.035)	0.549 (0.033)	<0.001
cg10508317	0.074 (0.022)	0.084 (0.022)	<0.001
cg10639435	0.424 (0.05)	0.441 (0.054)	0.001
cg10717869	0.78 (0.036)	0.791 (0.032)	0.003
cg10919522	0.239 (0.038)	0.228 (0.038)	0.002
cg11024682	0.472 (0.038)	0.489 (0.032)	<0.001
cg11202345	0.192 (0.04)	0.206 (0.042)	0.001
cg11269166	0.362 (0.041)	0.377 (0.042)	<0.001
cg13059136	0.523 (0.065)	0.545 (0.06)	0.001
cg13199639	0.059 (0.013)	0.052 (0.012)	<0.001
cg13640297	0.582 (0.063)	0.6 (0.06)	0.004
cg14020176	0.618 (0.036)	0.628 (0.035)	0.005
cg14204586	0.207 (0.045)	0.221 (0.042)	0.002
cg14597545	0.604 (0.065)	0.624 (0.061)	0.002
cg15020801	0.373 (0.031)	0.389 (0.029)	<0.001
cg15585213	0.956 (0.01)	0.96 (0.009)	<0.001
cg16097041	0.666 (0.046)	0.697 (0.044)	<0.001
cg16809457	0.65 (0.054)	0.667 (0.056)	0.002
cg17058475	0.152 (0.045)	0.137 (0.043)	0.001
cg18568872	0.568 (0.036)	0.578 (0.032)	0.002
cg19266329	0.635 (0.045)	0.62 (0.044)	0.001
cg19693031	0.809 (0.049)	0.765 (0.067)	<0.001
cg19750657	0.774 (0.049)	0.815 (0.045)	<0.001
cg20507228	0.457 (0.066)	0.481 (0.064)	<0.001
cg20784591	0.378 (0.031)	0.388 (0.03)	0.001
cg21480264	0.546 (0.039)	0.568 (0.036)	<0.001
cg12257439	0.537 (0.042)	0.546 (0.037)	0.018
cg22909677	0.868 (0.033)	0.885 (0.03)	<0.001
cg23021329	0.349 (0.033)	0.358 (0.028)	0.003
cg25130381	0.578 (0.027)	0.612 (0.032)	<0.001
cg25217710	0.639 (0.038)	0.654 (0.033)	<0.001

cg26546155	0.546 (0.063)	0.581 (0.055)	<0.001
cg26608667	0.704 (0.047)	0.718 (0.043)	0.002
cg26663590	0.563 (0.07)	0.588 (0.064)	<0.001
cg26712428	0.312 (0.091)	0.343 (0.103)	0.001
cg26804423	0.697 (0.052)	0.715 (0.045)	<0.001
cg27243685	0.89 (0.026)	0.899 (0.024)	<0.001
cg07960624	0.39 (0.066)	0.376 (0.063)	0.039
cg21766592	0.093 (0.038)	0.085 (0.035)	0.049
cg26262157	0.348 (0.066)	0.336 (0.055)	0.048

Table 21. CpGs with significant differences in the mean of methylation beta between the DM and CTL groups in AS.

AS	CTL	Short	Long	p
CpG ID	mean (SD)	mean (SD)	mean (SD)	
cg00076653	0.407 (0.04)	0.413 (0.039)	0.414 (0.044)	0.019
cg00277397	0.781 (0.049)	0.786 (0.049)	0.769 (0.043)	0.019
cg00574958	0.112 (0.034)	0.105 (0.033)	0.094 (0.025)	<0.001
cg00994936	0.821 (0.033)	0.829 (0.039)	0.849 (0.03)	<0.001
cg01101459	0.719 (0.037)	0.728 (0.037)	0.746 (0.042)	<0.001
cg01332882	0.581 (0.058)	0.59 (0.056)	0.608 (0.062)	0.005
cg01676795	0.553 (0.075)	0.566 (0.075)	0.588 (0.076)	0.001
cg02650017	0.067 (0.015)	0.065 (0.015)	0.059 (0.01)	0.001
cg02711608	0.186 (0.032)	0.185 (0.032)	0.17 (0.024)	0.02
cg02879453	0.741 (0.046)	0.747 (0.044)	0.765 (0.043)	0.003
cg02976843	0.551 (0.048)	0.559 (0.047)	0.565 (0.054)	0.007
cg03497652	0.663 (0.049)	0.676 (0.053)	0.704 (0.062)	<0.001
cg03691549	0.444 (0.044)	0.45 (0.042)	0.454 (0.039)	0.03
cg03699074	0.169 (0.034)	0.165 (0.034)	0.151 (0.027)	0.003
cg04682775	0.551 (0.041)	0.561 (0.04)	0.582 (0.039)	<0.001
cg04816311	0.526 (0.065)	0.541 (0.062)	0.565 (0.062)	<0.001
cg04973995	0.198 (0.051)	0.199 (0.05)	0.172 (0.039)	0.018
cg05400498	0.688 (0.044)	0.696 (0.043)	0.707 (0.04)	0.001
cg05478824	0.562 (0.049)	0.572 (0.051)	0.567 (0.049)	<0.001
cg06178887	0.675 (0.041)	0.682 (0.041)	0.696 (0.047)	<0.001
cg06192883	0.317 (0.051)	0.325 (0.05)	0.348 (0.043)	0.001
cg06500161	0.589 (0.03)	0.602 (0.034)	0.614 (0.03)	<0.001
cg06721411	0.596 (0.051)	0.614 (0.054)	0.639 (0.045)	<0.001
cg06940720	0.757 (0.048)	0.766 (0.047)	0.769 (0.048)	<0.001
cg07504977	0.377 (0.05)	0.395 (0.053)	0.414 (0.047)	<0.001
cg07960624	0.39 (0.066)	0.386 (0.065)	0.349 (0.054)	0.001
cg08309687	0.527 (0.066)	0.519 (0.067)	0.5 (0.067)	0.006

cg08788930	0.681 (0.041)	0.689 (0.041)	0.703 (0.046)	<0.001
cg08994060	0.394 (0.064)	0.387 (0.059)	0.365 (0.059)	0.001
cg09294084	0.477 (0.072)	0.485 (0.068)	0.487 (0.072)	0.029
cg09664445	0.535 (0.035)	0.542 (0.035)	0.551 (0.03)	<0.001
cg10508317	0.074 (0.022)	0.079 (0.023)	0.077 (0.015)	<0.001
cg10639435	0.424 (0.05)	0.431 (0.052)	0.451 (0.056)	0.003
cg10717869	0.78 (0.036)	0.785 (0.035)	0.797 (0.035)	0.008
cg10919522	0.239 (0.038)	0.235 (0.038)	0.218 (0.039)	0.002
cg11024682	0.472 (0.038)	0.48 (0.036)	0.492 (0.042)	<0.001
cg11202345	0.192 (0.04)	0.198 (0.042)	0.201 (0.035)	0.003
cg11269166	0.362 (0.041)	0.37 (0.043)	0.363 (0.036)	<0.001
cg13059136	0.523 (0.065)	0.533 (0.063)	0.55 (0.069)	0.003
cg13199639	0.059 (0.013)	0.056 (0.013)	0.048 (0.011)	<0.001
cg13640297	0.582 (0.063)	0.591 (0.062)	0.601 (0.063)	0.02
cg14020176	0.618 (0.036)	0.622 (0.035)	0.63 (0.047)	0.024
cg14204586	0.207 (0.045)	0.214 (0.044)	0.212 (0.043)	0.003
cg14597545	0.604 (0.065)	0.613 (0.063)	0.627 (0.069)	0.011
cg15020801	0.373 (0.031)	0.38 (0.031)	0.391 (0.03)	<0.001
cg15585213	0.956 (0.01)	0.958 (0.009)	0.961 (0.008)	<0.001
cg16097041	0.666 (0.046)	0.68 (0.047)	0.701 (0.049)	<0.001
cg16809457	0.65 (0.054)	0.657 (0.054)	0.672 (0.068)	0.01
cg17058475	0.152 (0.045)	0.146 (0.045)	0.132 (0.046)	0.003
cg18568872	0.568 (0.036)	0.573 (0.034)	0.573 (0.034)	0.014
cg19266329	0.635 (0.045)	0.629 (0.045)	0.605 (0.045)	0.001
cg19693031	0.809 (0.049)	0.79 (0.062)	0.751 (0.058)	<0.001
cg19750657	0.774 (0.049)	0.794 (0.051)	0.804 (0.049)	<0.001
cg20507228	0.457 (0.066)	0.468 (0.066)	0.491 (0.067)	0.001
cg20784591	0.378 (0.031)	0.383 (0.03)	0.389 (0.033)	0.013
cg21480264	0.546 (0.039)	0.557 (0.039)	0.569 (0.039)	<0.001
cg21766592	0.093 (0.038)	0.09 (0.037)	0.07 (0.027)	0.003
cg22909677	0.868 (0.033)	0.875 (0.032)	0.888 (0.031)	<0.001
cg23021329	0.349 (0.033)	0.353 (0.031)	0.357 (0.028)	0.01
cg25130381	0.578 (0.027)	0.593 (0.034)	0.617 (0.032)	<0.001
cg25217710	0.639 (0.038)	0.646 (0.036)	0.66 (0.036)	0.002
cg26546155	0.546 (0.063)	0.562 (0.062)	0.591 (0.057)	<0.001
cg26608667	0.704 (0.047)	0.711 (0.046)	0.719 (0.048)	0.026
cg26663590	0.563 (0.07)	0.573 (0.068)	0.599 (0.065)	0.001
cg26712428	0.312 (0.091)	0.327 (0.099)	0.328 (0.078)	<0.001
cg26804423	0.697 (0.052)	0.705 (0.05)	0.719 (0.051)	0.001
cg27243685	0.89 (0.026)	0.894 (0.026)	0.902 (0.023)	0.001

Table 22. CpGs with significant differences in the mean of methylation beta among the CTL, short, and long groups in AS.

IV. Discussion

Looking at the plot, in SNUH, if there was diabetes, the longer the duration of diabetes and the more progressive the CKD stage, the clock reflecting aging was AAHorvath. Horvath, Hannum, and PhenoAge were the clocks that reflected only diabetes status and CKD progression in aging. The clocks reflecting only the duration of diabetes and CKD progression as aging were AAHannum, and the clocks reflecting only CKD progression were DunedinPACE and AAGrim. Only diabetes mellitus status and CKD progression were reflected in aging in the clocks of Horvath, Hannum, and PhenoAge. The clocks reflecting only the duration of diabetes and CKD progression as aging were AAHannum, and the clocks reflecting only CKD progression were DunedinPACE and AAGrim. In AS, Horvath, Hannum, PhenoAge, GrimAge, AAHorvath, and AAHannum were the clocks that reflected aging as diabetes status and the duration of diabetes. The clocks that only diabetes status was reflected in aging were PhenoAge, AAGrim, and DunedinPACE.

Excluding the results consistent with the trend of chronological age, in SNUH, the clocks with increased mean and variance of EAA in the diabetic group was DunedinPACE, and the clocks with increased variance were AAHorvath, AAHannum, AAPheno, and AAGrim. With the duration of diabetes, the mean and the variance of DunedinPACE increased. As the CKD stage progressed, AAPheno was the clock that significantly increased the mean of EAA. In AS, except for the results consistent with the trend of chronological age as in SNUH, the clocks with increased mean mAge/EAA in the diabetic group were Hannum, PhenoAge, GrimAge, AAHannum, AAPheno, AAGrim, and DunedinPACE. The clocks whose mAge/EAA increased as the duration of diabetes was prolonged were AAHannum,

AAPheno, and DunedinPACE.

In the multiple linear regression models, the clocks with significant fasting blood glucose coefficients regardless of the subgroup were Horvath, Hannum, AAHorvath, AAHannum, and DunedinPACE in SNUH. In AS, they were PhenoAge, GrimAge, AAPheno, AAGrim, and DunedinPACE.

When the AIC of multiple linear regression models was calculated in the whole cohorts, the clock with the lowest AIC was DunedinPACE in both cohorts.

As a result of checking the underestimation tendency of mAge according to age through the Bland-Altman plot, the underestimation tendency of all four clocks was evident in the SNUH cohort. However, the tendency did not appear well in the AS cohort.

CpGs with significant differences between the diabetes group and the control group, among groups with more than 10 years of diabetes and less than 10 years and the control group, and among diabetes groups with CKD stage 3 or less, stage 4, and stage 5 were identified from the list of T2D-related genes provided in the recent review [27].

Taken together, the clocks that showed significant differences according to diabetes status in the SNUH cohort were Horvath and AA Horvath, as concluded through the plots and the multiple linear regression models. The clocks that showed significant differences depending on whether the duration of diabetes prevalence was more than 10 years or less were, in the plots, were AAHorvath and AAHannum. The clocks that showed a significant increase as the CKD stage progressed were Horvath, Hannum, AA Horvath, AA Hannum, and DunedinPACE in the plot and the multiple

regression models. For the clocks that showed significant differences according to diabetes status in the AS cohort, PhenoAge, GrimAge, AAPheno, AAGrim, and DunedinPACE are derived from the plot and multiple regression models. The clock that showed significant differences depending on whether the duration of diabetes was more than 10 years or less was concluded to be AAHannum from the plot and mean/dispersion comparison. The lowest AIC of the multiple linear regression model was DunedinPACE for both cohorts.

As a result, the clocks that reflect the tendency of Korean diabetic patients to aging well were Horvath mAge and EAA, Hannum mAge and EAA, and DunedinPACE in the SNUH cohort. However, in the AS cohort, no clock reflected the long duration of diabetes or the status of diabetes in aging. This difference seems to be due to the difference between the two cohorts. The two cohorts have different severity levels of the patients being composed. There are many cases of long-term diabetes and aftereffects in the SNUH cohort, which has 31 people with a CKD stage of 4 or higher, and 139 people with DM duration of more than 10 years.

On the other hand, in the AS cohort, no one with a CKD stage of 4 or higher, and patients with DM duration exceeding 10 years are only 29 people. In other words, it may mean that the severity of diabetes must be high for glucose to be significantly expressed in mAge or EAA in diabetic patients. The difference between cohorts also explains the result that, in the multiple regression model, unlike the SNUH cohort, there was no subgroup with a significant fasting blood glucose coefficient among the subgroups with DM in AS.

The Bland-Altman plot found that the previously known age-related underestimation tendency also appeared in the Korean diabetic cohort in all four

mAges (Horvath, Hannum, PhenoAge, and GrimAge). However, the tendency of underestimation according to age was more evident in the cohort with more T2D patients with a longer duration or a higher CKD stage. This result suggests the possibility that a further underestimation tendency may appear in a group with a large number of patients with accelerated aging due to aggravated disease progression.

There are also several hypotheses as to the reason for this underestimation tendency. First, there is a survival bias hypothesis that people who participated in cohort studies as they got older measured their aging low because they had managed their health well for a long time, and this was reflected in their mAges and EAAs. Alternatively, there is a hypothesis that it is influenced by statistical methods [47]. Third, there is a hypothesis that the problem is that the clocks rely on linear models. The saturation of the methylation marker according to age must consider both linear and nonlinear relationships. Therefore, if we recruit more diverse people to the cohort and re-select or re-model CpGs in consideration of the nonlinearity of the marker's saturation with age, we can develop a future clock that complements them.

Meanwhile, in the multiple regression model, unlike the SNUH cohort, there was no subgroup with a significant fasting blood glucose coefficient among the subgroups with DM in AS. In both cohorts, the coefficient of fasting blood glucose was negative or very small overall, which seems to be a problem caused by the large proportion of age and sex variables in the multiple linear regression model and the small number of cohorts and subgroups used. This phenomenon was similar to Hutchinson‑Gilford Progeria Syndrome (HGPS) [18] or syndrome X, Multifocal developmental functions in children [48], which is estimated to have been caused by a small number of rare diseases [47]. Therefore, the small sample size is believed to

be a problem with the Korean diabetic cohort.

This study is the first to examine mAge in Korean patients with type 2 diabetes and contributes to the field of DNA methylation clocks in that it is the second study to look at mAge in a Korean cohort [28]. In addition, while most studies examining the association between mAge and disease focus on disease prediction, it is original in that it focuses on the association between type 2 diabetes and aging and finds a clock that reflects the characteristics and diseases of the cohort.

From above, as a result of applying nine epigenetic clocks to Korean with type 2 diabetes and examining them in various ways, it was found that Horvath mAge/EAA, Hannum mAge/EAA, and DunedinPACE relatively well reflect aging due to type 2 diabetes in Koreans. In addition, there was a tendency that aging was better captured when diabetes was more severe or progressed for longer. This tendency suggests that even though the existing clocks do not contain many diabetes-related CpGs, biological aging progresses in various ways if the degree of diabetes progresses severely.

Since each clock looks at different aspects of aging and defines aging differently, the clocks showing significant differences between groups have changed depending on how you compare mAge/EAA — plotting, comparing means and variances, finding a significant coefficient of a variable in multiple regression models, and calculating AIC — and depending on which nature of the cohort and which subgroup. It may be natural that the results vary depending on the examination method result since few clocks consider diabetes-related CpGs in their calculations. Therefore, unlike the design of this study, if the age of the diabetic group and the

control group are fixed equally, the difference in absolute value, as well as the difference in slope in the plot of mAges/EAAs, will be seen. Based on the results of this study, it is also worth considering creating a next-generation clock that measures aging caused by type 2 diabetes in Koreans, including CpGs related to type 2 diabetes in Koreans.

In addition, the association between Korean with T2D and mAge/EAA in this study suggests that mAge can be used to reveal epigenetic mechanisms of complications and lifespan of Korean T2D patients because mAge is related to the prognosis of life and life. Therefore, it can serve as a basis to prove the usefulness of using epigenetic age in future studies on the pathological mechanism of T2D in Koreans. Furthermore, it will broaden our understanding of the use of mAge for early diagnosis, prevention, and treatment of T2D in Koreans.

However, there are limitations to this study. The first is that the results of the two cohorts were partially different. Only 64 people said they had been diagnosed in the AS cohort, so the remaining 136 were patients with glucose or HbA1c that met the criteria for type 2 diabetes but were unaware of their disease. Therefore, this seems to have been reflected in the results because there was a difference in the state of medication from the SNUH cohort for T2D patients attending the hospital. In addition, there were 31 people in the SNUH cohort with more than 4 CKD stages, while there was no one in the AS cohort, and those with DM duration of more than 10 years were about 100 less than the SNUH cohort, which is believed to have made a big difference in the cohort composition. The second is that the cohort is small. The total number of both cohorts is less than 1,000, but in this study, meta-analysis was not performed, so only one cohort was up to 429. Therefore, a follow-up study is needed to verify the results of this study. In addition, if the sample size is larger, it

will be possible to design a study that looks at the difference in absolute value and the difference in slope in the mAge plot by fixing the age of the diabetic group and the control group the same.

V. Conclusion

As a result of applying nine epigenetic clocks in Korean diabetic cohort and examining them in various ways, the clocks in which diabetes status and progression of CKD stage in diabetic patients were reflected in aging were Horvath mAge/EAA, Hannum mAge/EAA, and DunedinPACE. In addition, in the group with more patients with accelerated aging due to aggravated progression of T2D, the glucose variable in the multiple linear regression model appeared significantly, and the underestimation tendency was prominent. The above indicated an association between the Korean diabetic cohorts and mAges/EAAs. Therefore, this study provides a basis for using mAge/EAA to study aging and complications in Korean diabetic patients. On the other hand, it was found that T2D-related CpGs with significant differences between the subgroups in the Korean diabetic cohort were not included more than 10 in the clocks. Therefore, to study aging and complications in Korean with T2D, we propose to create a next-generation clock that includes them. However, since the two cohorts differ in the composition of diabetes severity and the whole sample size is small, follow-up studies are needed to support this result. If a large sample size is obtained, it is proposed to conduct a study to see the difference in absolute values and the difference in slope in the plot of mAges/EAAs by fixing the age of the diabetic group and the control group equally.

VI. Acknowledgement

The biospecimens for this study were provided by the Seoul National University Hospital Human Biobank, a member of the National Biobank of Korea, which the Ministry of Health and Welfare supports. All samples from the National Biobank of Korea were obtained with informed consent under institutional review board-approved protocols. Also, the bioresources from the National Biobank of Korea, the Center for Disease Control and Prevention, Republic of Korea (KBN-2019-000) were used. The Institutional Review Board (IRB) of Seoul National University reviewed the study protocol and participants and approved it (IRB No. E2211/004-004).

VII. Reference

1. Centers for Disease Control and Prevention, National Diabetes Statistics Report, September 30, 2022.

2. Bae JH, Han KD, Ko SH, Yang YS, Choi JH, Choi KM, Kwon HS, Won KC. Diabetes Fact Sheet in Korea 2021. Diabetes Metab J. 2022 May;46(3):417-426.

3. Yang Y, Luan Y, Feng Q, Chen X, et al. Epigenetics and Beyond: Targeting Histone Methylation to Treat Type 2 Diabetes Mellitus. Front Pharmacol. 2022 Jan 11;12:807413.

4. Palmer AK, Gustafson B, Kirkland JL, Smith U. Cellular senescence: at the nexus between ageing and diabetes. Diabetologia. 2019 Oct;62(10):1835-1841.

5. Narasimhan A, Flores RR, Robbins PD, Niedernhofer LJ. Role of Cellular Senescence in Type II Diabetes. Endocrinology. 2021 Oct 1;162(10):bqab136.

6. Juvinao-Quintero DL, Marioni RE, Ochoa-Rosales C, Russ TC, et al. DNA methylation of blood cells is associated with prevalent type 2 diabetes in a meta-analysis of four European cohorts. Clin Epigenetics. 2021 Feb 23;13(1):40.

7. Rope-Autin C, Blasco MA, Partridge L, Serrano M, Chromer G. Aging Features Cell. 2013 Jun 6;153(6):1194-217.

8. Parrillo L, Spinelli R, Nicolò A, Longo M, et al. Nutritional Factors, DNA Methylation, and Risk of Type 2 Diabetes and Obesity: Perspectives and Challenges. Int J Mol Sci. 2019 Jun 19;20(12):2983.

9. Berdasco M, Esteller M. Clinical epigenetics: seizing opportunities for translation. Nat Rev Genet. 2019 Feb;20(2):109-127.

10. Arguelles AO, Meruvu S, Bowman JD, Choudhury M. Are epigenetic drugs for diabetes and obesity at our door step? Drug Discov Today. 2016 Mar;21(3):499-509.

11. Rosen ED, Kaestner KH, Natarajan R, Patti ME, et al. Epigenetics and Epigenomics: Implications for Diabetes and Obesity. Diabetes. 2018 Oct;67(10):1923-1931.

12. Ward-Caviness CK. Accelerated Epigenetic Aging and Incident Atrial Fibrillation: New Outlook on an Immutable Risk Factor? Circulation. 2021 Dec 14;144(24):1912-1914.

13. Kuroda A, Rauch TA, Todorov I, Ku HT, Al-Abdullah IH, Kandeel F, Mullen Y, Pfeifer GP, Ferreri K. Insulin gene expression is regulated by DNA methylation. PLoS One. 2009 Sep 9;4(9):e6953.

14. Hay CW, Docherty K. Comparative analysis of insulin gene promoters: implications for diabetes research. Diabetes. 2006;55:3201–13.

15. Salameh Y, Bejaoui Y, El Hajj N. DNA Methylation Biomarkers in Aging and Age-Related Diseases. Front Genet. 2020 Mar 10;11:171.

16. Jylhävä J, Pedersen NL, Hägg S. Biological Age Predictors. EBioMedicine.

2017 Jul;21:29-36.

17. El Khoury, L.Y., Gorrie-Stone, T., Smart, M. et al. Systematic underestimation of the epigenetic clock and age acceleration in older subjects. Genome Biol, 2019; 20:283.

18. Horvath S. DNA methylation age of human tissues and cell types. Genome Biol. 2013;14(10):R115. https://dnamage.genetics.ucla.edu/new

19. Hannum G, Guinney J, Zhao L, Zhang L, Hughes G, Sadda S, Klotzle B, Bibikova M, Fan JB, Gao Y, Deconde R, Chen M, Rajapakse I, Friend S, Ideker T, Zhang K. Genome-wide methylation profiles reveal quantitative views of human aging rates. Mol Cell. 2013 Jan 24;49(2):359-367.

20. Levine ME, Lu AT, Quach A, Chen BH, Assimes TL, Bandinelli S, Hou L, Baccarelli AA, Stewart JD, Li Y, Whitsel EA, Wilson JG, Reiner AP, Aviv A, Lohman K, Liu Y, Ferrucci L, Horvath S. An epigenetic biomarker of aging for lifespan and healthspan. Aging (Albany NY). 2018 Apr 18;10(4):573-591.

21. Lu AT, Quach A, Wilson JG, Reiner AP, Aviv A, Raj K, Hou L, Baccarelli AA, Li Y, Stewart JD, Whitsel EA, Assimes TL, Ferrucci L, Horvath S. DNA methylation GrimAge strongly predicts lifespan and healthspan. Aging (Albany NY). 2019 Jan 21;11(2):303-327.

22. Daniel W Belsky, Avshalom Caspi, David L Corcoran, Karen Sugden, Richie Poulton, Louise Arseneault, Andrea Baccarelli, Kartik Chamarti, Xu Gao, Eilis Hannon, Hona Lee Harrington, Renate Houts, Meeraj Kothari, Dayoon Kwon, Jonathan Mill, Joel Schwartz, Pantel Vokonas, Cuicui Wang,

Benjamin S Williams, Terrie E Moffitt. DunedinPACE, a DNA methylation biomarker of the pace of aging. eLife 2022;11:e73420

23. Quach A, Levine ME, Tanaka T, Lu AT, Chen BH, Ferrucci L, Ritz B, Bandinelli S, Neuhouser ML, Beasley JM, Snetselaar L, Wallace RB, Tsao PS, Absher D, Assimes TL, Stewart JD, Li Y, Hou L, Baccarelli AA, Whitsel EA, Horvath S. Epigenetic clock analysis of diet, exercise, education, and lifestyle factors. Aging (Albany NY). 2017 Feb 14;9(2):419-446.

24. Hillary RF, Stevenson AJ, McCartney DL, Campbell A, Walker RM, Howard DM, Ritchie CW, Horvath S, Hayward C, McIntosh AM, Porteous DJ, Deary IJ, Evans KL, Marioni RE. Epigenetic measures of ageing predict the prevalence and incidence of leading causes of death and disease burden. Clin Epigenetics. 2020 Jul 31;12(1):115.

25. Smith BH, Campbell A, Linksted P, Fitzpatrick B, Jackson C, Kerr SM, Deary IJ, Macintyre DJ, Campbell H, McGilchrist M, Hocking LJ, Wisely L, Ford I, Lindsay RS, Morton R, Palmer CN, Dominiczak AF, Porteous DJ, Morris AD. Cohort Profile: Generation Scotland: Scottish Family Health Study (GS:SFHS). The study, its participants and their potential for genetic research on health and illness. Int J Epidemiol. 2013 Jun;42(3):689-700.

26. Fraszczyk E, Luijten M, Spijkerman AMW, Snieder H, Wackers PFK, Bloks VW, Nicoletti CF, Nonino CB, Crujeiras AB, Buurman WA, Greve JW, Rensen SS, Wolffenbuttel BHR, van Vliet-Ostaptchouk JV. The effects of bariatric surgery on clinical profile, DNA methylation, and ageing in

severely obese patients. Clin Epigenetics. 2020 Jan 20;12(1):14.

27. Fraszczyk E, Thio CHL, Wackers P, Dollé MET, Bloks VW, Hodemaekers H, Picavet HS, Stynenbosch M, Verschuren WMM, Snieder H, Spijkerman AMW, Luijten M. DNA methylation trajectories and accelerated epigenetic aging in incident type 2 diabetes. Geroscience. 2022 Aug 10.

28. Lee HS, Park T. The influences of DNA methylation and epigenetic clocks, on metabolic disease, in middle-aged Koreans. Clin Epigenetics. 2020 Oct 15;12(1):148.

29. Golden SH, Yajnik C, Phatak S, Hanson RL, Knowler WC. Racial/ethnic differences in the burden of type 2 diabetes over the life course: a focus on the USA and India. Diabetologia. 2019 Oct;62(10):1751-1760.

30. Takeuchi M, Okamoto K, Takagi T, Ishii H. Ethnic difference in patients with type 2 diabetes mellitus in inter-East Asian populations: a systematic review and meta-analysis focusing on fasting serum insulin. Diabetes Res Clin Pract. 2008 Sep;81(3):370-6.

31. Kwak SH, Chae J, Lee S, Choi S, et al. Nonsynonymous Variants in PAX4 and GLP1R Are Associated With Type 2 Diabetes in an East Asian Population. Diabetes. 2018 Sep;67(9):1892-1902.

32. Kim Y, Han BG; KoGES group. Cohort Profile: The Korean Genome and Epidemiology Study (KoGES) Consortium. Int J Epidemiol. 2017 Apr 1;46(2):e20.

33. American Diabetes Association Professional Practice Committee; 2.

Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes—2022. Diabetes Care 1 January 2022; 45 (Supplement_1): S17–S38.

34. Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S, Kusek JW, Van Lente F; Chronic Kidney Disease Epidemiology Collaboration. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. Ann Intern Med. 2006 Aug 15;145(4):247-54.

35. Andrassy KM. Comments on 'KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease'. Kidney international. 2013 Sep 1;84(3):622-3.

36. Horvath, S., Raj, K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. Nat Rev Genet, 2018;19:371–384.

37. Franzago M, Pilenzi L, Di Rado S, Vitacolonna E, Stuppia L. The epigenetic aging, obesity, and lifestyle. Front Cell Dev Biol. 2022 Sep 13;10:985274.

38. Noroozi R, Ghafouri-Fard S, Pisarek A, Rudnicka J, Spólnicka M, Branicki W, Taheri M, Pośpiech E. DNA methylation-based age clocks: From age prediction to age reversion. Ageing Res Rev. 2021 Jul;68:101314.

39. McEwen LM, Jones MJ, Lin DTS, Edgar RD, Husquin LT, MacIsaac JL, Ramadori KE, Morin AM, Rider CF, Carlsten C, Quintana-Murci L, Horvath S, Kobor MS. Systematic evaluation of DNA methylation age estimation with common preprocessing methods and the Infinium

MethylationEPIC BeadChip array. Clin Epigenetics. 2018 Oct 16;10(1):123.

40. Lee Y, Haftorn KL, Denault WRP, Nustad HE, Page CM, Lyle R, Lee-Ødegård S, Moen GH, Prasad RB, Groop LC, Sletner L, Sommer C, Magnus MC, Gjessing HK, Harris JR, Magnus P, Håberg SE, Jugessur A, Bohlin J. Blood-based epigenetic estimators of chronological age in human adults using DNA methylation data from the Illumina MethylationEPIC array. BMC Genomics. 2020 Oct 27;21(1):747.

41. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, 2021.

42. Du P, Zhang X, Huang CC, Jafari N, Kibbe WA, Hou L, Lin SM. Comparison of Beta-value and M-value methods for quantifying methylation levels by microarray analysis. BMC Bioinformatics. 2010 Nov 30;11:587.

43. Mueller F, Scherer M, Assenov Y, Lutsik P, Walter J, Lengauer T, Bock C. RnBeads 2.0: comprehensive analysis of DNA methylation data. Genome Biology, 2019;20(55).

44. Leek JT, Johnson WE, Parker HS, Fertig EJ, Jaffe AE, Zhang Y, Storey JD, Torres LC. sva: Surrogate Variable Analysis. R package version 3.46.0, 2022.

45. Tang Y, Horikoshi M, Li W. ggfortify: Unified Interface to Visualize Statistical Result of Popular R Packages. The R Journal. 2016;8(2):474–485.

46. Wickham H. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016. ISBN 978-3-319-24277-4

47. Simpson DJ, Chandra T. Epigenetic age prediction. Aging Cell. 2021 Sep;20(9):e13452.

48. Walker RF, Liu JS, Peters BA, Ritz BR, Wu T, Ophoff RA, Horvath S. Epigenetic age analysis of children who seem to evade aging. Aging (Albany NY). 2015 May;7(5):334-9.

VIII. Abstract (국문 초록)

제2형 당뇨병을 가진 한국인에서

DNA 메틸레이션 시계 분석 및 비교

서울대학교 보건대학원

보건학과 유전체역학 전공

이승은

세계적으로 유병률이 증가하고 있는 만성질환인 당뇨병은 전신적이고 복합적인 합병증을 유발하므로 관리가 중요한 질환이다. 또 당뇨병은 나이가 들수록 유병률이 증가하는 경향성이 있고, 당뇨병 환자에서는 연령 관련 질환들이 일찍 나타나는 것으로 알려져 있다. 그중에서도 제2형 당뇨병은 전체 당뇨병 환자의 90%를 차지할 만큼 중요한 당뇨병 유형이다.

한편 후생유전학적 노화의 메커니즘을 연구하는 데에 있어 DNA

메틸레이션은 떠오르는 도구로, 개체의 환경 및 생활습관의 영향이 반영되고 유전자 발현에 변화를 일으키기 때문에 다양한 질환의 발병에 중요한 역할을 하는 것으로 알려져 있다. 또 안정적이고 침습성이 낮으며 가역적이라는 특성이 있어 임상적, 역학적으로 중요한 바이오마커로 기능할 수 있다.

DNA 메틸화가 된 CpG의 위치와 정도에 따라 연령을 추산하는 것을 DNA 메틸레이션 연령이라고 부르고, 이것은 후성유전학적 노화 바이오마커라고도 부른다. 기본 원리는 암, 비만, 심혈관 질환 등 노화와 관련된 다양한 건강 위험들을 기준으로 과메틸화 또는 과소메틸화된 CpG를 선정하고 이를 통해 선형적으로 노화를 측정하는 것이며, 이렇게 메틸레이션 연령을 산출하는 모델링을 메틸레이션 시계라고 부른다. 한편 메틸레이션 시계는 60-65세 이상에서 과소평가되는 경향성이 있고, 특히 아시아인에서 민족 다양성이 부족하여, 한국인 당뇨병 코호트에서 메틸레이션 연령의 과소평가 경향성이나 당뇨 관련 요인들과 메틸레이션 연령의 관련성은 확인되지 않았다.

따라서 메틸레이션 연령과 한국인 당뇨병 코호트와의 상관관계를 통해 제2형 당뇨병을 가진 한국인과 노화의 관계를 알아보기 위해, 본 연

구는 메틸레이션 연령 및 가속도를 계산하는 메틸레이션 시계 9가지 (Horvath, Hannum, PhenoAge, GrimAge, AAHorvath, AAHannum, AAPheno, AAGrim, and DunedinPACE)와 서울대학교병원 인체자원은행 당뇨병 클리닉 코호트의 일부(SNUH), 질병관리청 한국인유전체역학조사사업 KoGES의 안산/안성 지역사회 기반 코호트의 일부(AS)의 관계를 살펴보았다.

SNUH 코호트는 제2형 당뇨병 환자 232명, 대조군 197명 총 429명으로 구성되었으며, AS 코호트는 제2형 당뇨병 환자 200명, 대조군 200명 총 400명으로 구성되었다. 두 그룹의 DNA 메틸레이션 원시 데이터를 받아 메틸레이션 밀도를 베타 값으로 계산하고, 이로부터 메틸레이션 연령과 가속도를 구했다. 또 두 그룹의 당뇨 여부, 당뇨 유병 기간, CKD 기수에 따라 각각 당뇨군/대조군, 유병 기간 긴 군(10년 초과)/짧은 군(10년 이하)/비당뇨군, CKD 5기/CKD 4기/CKD 3기 이하의 당뇨군으로 하위군을 나누어 각 군의 메틸레이션 연령과 가속도를 연령에 따라 그래프를 그리고, 평균과 분산을 비교했다. 또 다중선형회귀식을 적용해 당뇨병의 결과가 반영되는 지표이자 당뇨병 진단의 1차적 기준이 되는 공복 혈당을 유의하게 반영하는 시계를 확인했다. 또 전체 코호트에서 각 시계별 다중선형회귀식

모델의 AIC를 비교하였다. 메틸레이션 연령이 고령자에서는 과소평가되는 경향성이 알려져 있는데, 이것이 한국인 당뇨병 코호트에서도 나타나는지를 블랜드-알트만 플랏을 통해 확인했다. 마지막으로 제2형 당뇨병과 유관한 것으로 알려진 CpG들이 본 연구에 사용된 시계들에도 포함되어 있는지 확인하고, 또 해당 CpG들 중 본 연구에서 그룹 간 차이를 유의하게 나타낸 CpG들을 정리했다.

한국인 당뇨병 코호트에서 9가지 후생유전학 시계들을 적용시키고 위와 같은 다양한 방법으로 살펴본 결과 당뇨병 여부와 당뇨병 환자의 CKD 단계의 심화가 노화에 반영되는 시계는 Horvath, Hannum, AAHorvath, AAHannum, DunedinPACE였다. 또 제2형 당뇨병의 진행 상태가 심화되어 노화가 가속화된 환자가 많은 군일수록 다중선형회귀모델 중 공복 혈당 변수가 유의미하게 나타나고, 과소평가 경향성이 두드러지게 나타났다. 이상에서 제2형 당뇨병을 가진 한국인 코호트와 mAge/EAA는 연관성이 있었다. 따라서 본 연구는 한국인 당뇨병 환자의 노화 및 합병증 연구에 mAge/EAA를 사용할 수 있는 근거가 된다. 한편 한국인 당뇨병 코호트에서 하위군 간 유의한 차이를 갖는 T2D 관련 CpG들은 시계에 많이 포함되지 않은 것으로 나타났다. 따라서 T2D를 가진 한국인 코호트에

서 노화 및 합병증을 연구하기 위해 이 CpG들을 포함하는 차세대 시계를 만들 것을 제안한다. 다만 두 코호트에는 당뇨병 중증도의 구성에 있어 차이가 있고 표본 크기가 작기 때문에 본 연구의 결과를 뒷받침할 후속 연구가 필요하다. 큰 샘플 사이즈가 확보된다면, 당뇨군과 컨트롤군의 나이를 동일하게 고정하여 mAge의 플랏에서의 기울기의 차이뿐만 아니라 절대적인 값의 차이를 보는 연구를 해볼 것을 제안한다.

주요어: 2형 당뇨병, 노화, DNA 메틸레이션 시계, 후생유전학 시계, 메틸레이션 연령, 한국인 코호트

학 번: 2021-23644