scientific reports

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Exploring DNA methylation age and the influence of physical performance, and hypertension on frailty in elderly women

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Epigenetic age provides a reliable biomarker for biological aging, reflecting the cumulative impact on health over time. Frailty is common among elderly individuals and is further compounded by hypertension, which increases the risk associated with aging. Therefore, we examined the relationship between epigenetic aging and frailty in a non-Western population and explored synergistic effects of frailty and hypertension on epigenetic age. Thai women (60–80 years) were assessed for physical, blood, and biochemical parameters. Age acceleration (AA) residuals were derived to explore deviations between chronological and epigenetic age. We classified 126 participants into robust, pre-frail, and frail groups based on the Fried phenotype and Kihon Checklist. GrimAge1 and GrimAge2 outperformed other epigenetic age estimators in terms of correlation with frailty status. Furthermore, these age models were significantly correlated with physical performance tests. AA varied significantly among groups, with robust individuals having lower Grim1AA and Grim2AA levels than pre-frail individuals. Furthermore, hypertensive participants with pre-frail had significantly different levels of Grim1AA and Grim2AA compared to robust without hypertension. Our findings reveal a complex relationship among frailty, epigenetic age, physical performances, and hypertension. Grim2Age exhibits a strong correlation with chronological age and shows accelerated AA in frail individuals, particularly those with hypertension.

Keywords Age acceleration, DNA methylation, Epigenetic age, Frailty syndrome, Hypertension

Epigenetic age, a biomarker based on DNA methylation (DNAm) patterns, has emerged as a reliable indicator of biological aging[1]. Unlike the chronological age, which simply measures the number of years lived, epigenetic age reflects the cumulative impact of environmental, genetic, disease and lifestyle factors on an individual's genome[2–4]. The difference between the predicted DNAm age and chronological age, referred to as age acceleration (AA), is characterized by an individual's biological age relative to chronological age. Furthermore, AA has been shown to predict mortality independently of chronological age[5,6] and is associated with a wide range of health and disease outcomes, such as obesity[7] hypertension[8] major depressive disorder[9]and low physical performance[10,11].

Frailty has emerged as a significant threat to public health, particularly with the aging global population. Defined by a decline in physiological reserve and increased vulnerability to adverse health outcomes, frailty is a complex and multifaceted condition rather than an inevitable outcome of aging. It results from an intricate combination of factors, such as physiological decline, genetic and epigenetic changes, nutritional deficiencies, psychological well-being, and social and environmental influences[12,13]. The most effective evidence-based method for detecting frailty and assessing its severity is a comprehensive geriatric assessment. Fried et al.. developed a standardized definition of frailty (Fried phenotype) that includes the simultaneous presence of three to five criteria, as follows: self-reported exhaustion, decreased physical activity, slow walking speed,

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insufficient hand-grip strength, and unintentional weight loss[14]. Recently, the Kihon Checklist has offered a broader approach to assess frailty, encompassing not only physical aspects such as mobility and strength but also cognitive, social, and nutritional factors. By combining a more comprehensive range of indications, the Kihon Checklist assists in recognizing older persons at risk of frailty in a holistic manner, enabling earlier and more targeted interventions[15].

Hypertension, commonly referred to as high blood pressure, is a prevalent condition among elderly individuals. It is characterized by sustained elevated blood pressure levels, which can lead to significant health complications, including cardiovascular disease, stroke, and cognitive decline[16]. The World Health Organization (WHO) has estimated that the global population of individuals aged 60 and over will grow from 1 billion in 2019 to 1.4 billion by 2050. In Thailand, more than 50% of individuals aged 30–79 years are affected by hypertension and poorly controlled blood pressure[17]. In the context of frailty, hypertension poses a considerable risk, as it can exacerbate the physiological decline associated with aging. Recently, studies have shown a strong link between hypertension and an increased risk of frailty, emphasizing how high blood pressure can negatively impact physical function and mobility[18–20].

This study contributes to the limited evidence on the relationship between epigenetic aging, frailty, and hypertension, particularly in non-Western populations. Moreover, the synergistic effects of frailty and hypertension on AA have yet to be explored in older adults. Elucidating the interactions between these assessments, health conditions, and epigenetic age could yield significant insights into the biological underpinnings of frailty, potentially informing the development of more targeted interventions to mitigate age-related health risks.

Materials and methods

Participants

All research protocols strictly adhered to the principles of the Declaration of Helsinki, and the reporting was conducted in accordance with the guidelines of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement. Prior to enrollment, all participants provided written informed consent for the collection of blood samples, clinical data, and genetic analysis. The Siriraj Institutional Review Board, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, granted ethical approval for this study (COA no. Si 213/2021). The study cohort consisted of 870 participants over the age of 60 who had lived in 26 communities in Bangkok Noi, Thailand, for at least a year. Exclusion criteria included: (1) a documented diagnosis of cognitive impairment or a score below 18/30 on the Thai mini-mental state examination[21] precluding participation in study activities; (2) physical disability preventing participation in the required physical assessments (gait speed, functional reach, sit-and-reach, chair stand, and hand-grip strength). Twenty-two participants were excluded based on these pre-defined criteria. The remaining participants were interviewed to gather demographic and lifestyle information, medication use, and health details. Additionally, trained medical staff followed standardized protocols to collect whole blood samples and measure height, weight, and resting blood pressure.

Frailty assessment

Frailty status among elderly participants aged 60–80 years was assessed and classified via the Fried phenotype[14] which is based on five criteria: (1) unintentional weight loss exceeding 5% in the past year, calculated from self-reported past weight and current weight (normal < 5%); (2) hand-grip strength, determined by the best of two measurements with the preferred hand using Takei Tkk5401 digital hand grip gauge (Takei, Japan) (normal for female > 18.5 kg); (3) slow gait speed, measured by the minimum of two trials over 4 m (normal < 4.17 s/4meters); (4) low physical activity levels, expressed as weekly energy expenditure via the Global Physical Activity Questionnaire (GPAQ) (normal for female > 367 scores)[22]; and (5) exhaustion, evaluated via two questions from the Center for Epidemiologic Studies Depression Scale (CES-D) (normal = 0/2 score)[23]. Participants meeting three or more criteria were classified as frail, those meeting one or two criteria as pre-frail, and those without any criteria as robust. We extended our evaluation by measuring physical performance through the sit and reach, functional reach, back scratch, and chair stand tests. We strengthened the criteria of frailty status using the Kihon Checklist, which includes seven subdomains: instrumental activities of daily living disability, physical inactivity, malnutrition, oral hypofunction, socialization, cognitive function, and depression. This checklist evaluates both physical and non-physical aspects, with scores ranging from 0 (indicating no frailty) to 25 (indicating high frailty). Participants were categorized as robust (0–3), vulnerable (≥ 4), further subdivided into pre-frail (4–8) and frail (9–25) groups[15].

Sample collection and laboratory data collection

This study is part of a community-based cohort of older adults in the Bangkok Noi district ($n = 870$). To ensure accurate identification of individuals with frailty, we applied stringent criteria by selecting participants who were consistently classified as frail according to both the Fried phenotype and the Kihon Checklist assessments. Only 371 participants met these criteria. Among the eligible participants, 24 were over 80 years old and were excluded, while 145 were excluded due to insufficient blood samples or inadequate DNA concentrations. From the remaining participants, we included all 29 individuals classified as frail. To allow for group comparisons, we randomly selected age-matched participants from the robust and pre-frail groups using stratified sampling to select robust ($n = 48$) and pre-frail ($n = 49$). The study flow chart is shown in Fig. S1. Blood samples from these participants were collected in BD Vacutainer tubes containing lithium heparin. The buffy coat and plasma were then isolated using density gradient centrifugation and stored at −80 °C until analysis.

DNA methylation and DNAm age calculation

Genomic DNA was extracted from the buffy coat using a QIAamp Blood Mini Kit (Qiagen, Germany). The concentration and quality of the DNA were accurately determined by a DeNovix fluorometer (DeNovix, USA), and the DNA was stored at −20 °C until analysis. Genome-wide DNAm was assessed using the Infinium Methylation EPIC v2.0 BeadChip Kit (Illumina, USA) through the University of Minnesota, Genomics Center, Minnesota, USA. The IDAT files were imported using the Minfi R package[24,25]. The data were subsequently normalized using the PreprocessNoob method within the Minfi package. Following the calculation of β values, seven different epigenetic ages and age accelerations were computed using the online Horvath calculator (https://dnamage.clockfoundation.org/)[1] except for GrimAge version 2 (GrimAge2). It was estimated using the algorithm and code provided by the authors[26]. Missing values for approximately 18% of the cytosine-phosphate-guanine dinucleotide (CpG) sites required for GrimAge2 calculation were imputed using median values from reference dataset[26]. The calculator generated epigenetic age estimates both first- and second-generation clocks. The first-generation epigenetic clocks, including HorvathAge[1] and HannumAge[27] are trained based on chronological age. Second-generation epigenetic clocks, including PhenoAge[28] SkinBloodAge[29] ZhangAge[30] FitAge[31] and GrimAge versions 1 (GrimAge1)[32] were developed to improve prediction of mortality and diverse health risks. GrimAge1 and GrimAge2 components have also been calculated including seven DNAm-based predictions of plasma protein levels (adrenomedullin (ADM), beta-2-microglobulin, cystatin C, growth differentiation factor-15, leptin, plasminogen activation inhibitor-1, and tissue inhibitor of metalloproteinases-1 (TIMP-1)). Additionally, DNAm-based estimates of $\log_2$ C-reactive protein, $\log_2$ hemoglobin A1c, and smoking pack-years (DNAmPACKYRS) were determined for the GrimAge2 calculation. While FitAge incorporated DNAm-based biomarkers of physical fitness, including gait speed (DNAmGaitspeed), hand grip strength (DNAmGripmax), forced expiratory volume in 1 s (DNAmFEV1), and maximal oxygen uptake (DNAmVO2max). Accelerations in each epigenetic age from chronological age and intrinsic epigenetic age acceleration (IEAA) were calculated as the residuals from linear regression. Positive residuals indicated that epigenetic age exceeded chronological age, while negative residuals denoted the converse. Additionally, we used a third-generation epigenetic clock–DunedinPACE scores–to estimate the pace of aging using the code provided by Belsky et al. 2022[33]. A DunedinPACE score of 1 reflects the expected rate of biological aging in midlife adults, corresponding to 1 year of biological aging per 1 year of chronological aging. The scores above 1 indicate a faster pace of aging, while scores below 1 indicate a slower pace of aging.

Statistical analysis

The Shapiro–Wilk test was employed to assess the normality of the data distribution. Data are presented as the means ± standard deviations (SDs) or medians with interquartile ranges (IQRs) for continuous variables. Categorical variables were compared using the chi-square or Fisher's exact test. Group comparisons were performed either between two groups (Student's t-test or Mann–Whitney U test) or multiple groups (One-way ANOVA followed by Tukey's post hoc test or Kruskal–Wallis rank sum test followed by Dunn's tests). The correlation between the predicted DNAm age and chronological age, as well as physical performance, AA, Fried phenotype scores, and the Kihon Checklist scores, was examined using Spearman's rank correlation test. A p value of less than 0.05 was considered statistically significant. All the statistical analyses and graphical presentations were conducted using R (version 4.4.2) and RStudio (version 2024.04.2 + 764). To strengthen the statistical validity of our pairwise comparisons, we conducted a post-hoc effect size estimation using Cohen's d. The achieved statistical power (1–β) was subsequently calculated using G*Power software (version 3.1)[34].

Results

Participant characteristics

In this research, a total of 126 elderly women aged 60 to 80 years were selected and categorized into three groups according to the Fried phenotype alongside the Kihon Checklist: robust ($n = 48$); pre-frail ($n = 49$); and frail ($n = 29$). There was no statistically significant difference in chronological age or body mass index among the participants. For the physical characteristics, gait speed over 4 m and hand-grip strength, which are key components of the Fried phenotype, as well as the results of the chair stand and functional reach tests, demonstrated a statistically significant difference among the three groups ($p < 0.01$), validating the appropriate categorization of these individuals into the robust, pre-frail, and frail groupings. Additionally, the number of elderly individuals with hypertension did not differ significantly among the robust, pre-frail, and frail groups. The demographic information of the participants is shown in Table 1.

Correlation between chronological age and DNA methylation age estimators

To date, studies of epigenetic aging and frailty have exclusively been performed in Western populations. We investigated the relationship between chronological age and eight DNAm age estimators (HorvathAge, HannumAge, PhenoAge, SkinBloodAge, ZhangAge, GrimAge1 and GrimAge2) in an elderly Thai population. GrimAge1 had the strongest correlation with chronological age (Spearman rank correlation test; $r = 0.733$, $p < 0.001$), even when analyzed by subgroups based on frailty status: robust, pre-frail, and frail (Fig. 1). FitAge followed, showing a correlation with chronological age of $r = 0.725$ ($p < 0.001$). Strong correlation was observed between GrimAge1 and FitAge. As expected, GrimAge1 showed a strong correlation with GrimAge2, even when analyzed by subgroups based on frailty status.

While all models demonstrated strong correlations with chronological age, these were weaker than previously reported in Western populations. The HorvathAge, HannumAge, and PhenoAge models each displayed correlations of $r = 0.518$–0.614 in our Thai population, in contrast to values of $r > 0.77$ often reported in elderly Western populations[35,36] although others have reported more moderate correlations of $r = 0.62$–0.71 for these same models[37].

	Robust ($n=48$)	Pre-frail ($n=49$)	Frail ($n=29$)	p-value *
Age (years), Mean ± SD	71.19 ± 3.09	71.80 ± 4.43	72.67 ± 5.03	0.314
BMI (kg/m^2), Median (IQR)	24.53 (5.35)	25.93 (5.26)	25.52 (5.65)	0.103
Blood pressure (mmHg), Mean ± SD				
DBP	79.25 ± 9.60	76.35 ± 10.45	76.41 ± 12.16	0.335
SBP	143.52 ± 15.80	139.90 ± 17.35	140.14 ± 22.87	0.572
Hypertension, n (%)	20 (41.67)	32 (65.31)	15 (51.72)	0.065
Smoking, n (%)				
Never	48 (100.00)	45 (91.84)	26 (89.66)	0.095
Current	0 (0.00)	3 (6.12)	1 (3.44)	
Former	0 (0.00)	1 (2.04)	2 (6.90)	
Physical characteristics, Median (IQR)				
Chair stand in 5 times (sec)	9.88 (3.33)	12.29 (5.36) [a]	13.84 (8.18) [a]	< 0.001
Functional reach (cm)	30.00 (6.00)	26.00 (10.00) [a]	24.00 (8.00) [a]	< 0.01
Gait speed in 4 m (sec)	3.32 (0.65)	4.47 (1.24) [a]	5.33 (1.62) [b, c]	< 0.001
Hand-grip strength (kg)	22.25 (4.78)	21.40 (3.90)	17.80 (2.40) [b, c]	< 0.001
Sit and reach (cm)	4.80 (13.23)	6.90 (14.40)	3.90 (14.20)	0.267

Table 1. Demographic and clinical and physical characteristics of the participants. IQR = interquartile range; DBP = diastolic blood pressure; SBP = systolic blood pressure. *) p values were calculated using one-way ANOVA of means or the Kruskal–Wallis rank sum test for continuous variables, while categorical variables were compared using the chi-square or Fisher's exact test. Lowercase letters a, b, and c indicate significant differences ($p < 0.05$) between pre-frail vs. robust, frail vs. robust, and frail vs. pre-frail groups, respectively, as determined by Tukey HSD or Dunn's test with Benjamini-Hochberg correction.

DNA methylation age estimators and their age acceleration among robust, pre-frail, and frail individuals

We further analyzed the differences in DNAm age estimators across each group. Among the DNAm age estimators, significant differences among robust, pre-frail, and frail participants were found in GrimAge1 and GrimAge2 (one-way ANOVA; $p = 0.024$ and $p < 0.01$, respectively) (Table 2).

Pairwise comparisons by Tukey HSD showed GrimAge1 and GrimAge2 of the robust group were significantly lower than the pre-frail group ($\Delta_{GrimAge1} = -2.07$ years, $p = 0.044$, Cohen's d = 0.51; $\Delta_{GrimAge2} = -2.67$ years, $p = 0.011$, Cohen's d = 0.61). Moreover, GrimAge2 was significantly lower in the robust group compared to the frail group ($\Delta_{GrimAge2} = -2.83$ years, $p = 0.023$, Cohen's d = 0.62). As expected, the age acceleration of GrimAge1 (Grim1AA) and GrimAge2 (Grim2AA) showed significant differences among the groups (one-way ANOVA; $p = 0.022$ and $p < 0.01$, respectively). Specifically, we observed that Grim1AA and Grim2AA were significantly greater in the pre-frail group compared to the robust group ($\Delta_{Grim1AA} = 1.60$ years, $p = 0.018$, Cohen's d = 0.54; $\Delta_{Grim2AA} = 2.21$ years, $p < 0.01$, Cohen's d = 0.64). However, no statistically significant differences were observed in other epigenetic clocks (Table 2), as well as DNAm-based predictions of plasma protein levels and smoking, and DNAm-based biomarkers of physical fitness, except for some DNAm-based predictions of GrimAge1 and GrimAge2 (Table S1). DNAm-based predictions of TIMP-1 of GrimAge1 showed significantly difference among robust, pre-frail, and frail participants (one-way ANOVA; $p = 0.026$). Pairwise comparisons revealed robust group was significantly lower than frail group ($\Delta_{DNAmTIMP-1} = -483.04$ pg/mL, $p = 0.038$, Cohen's d = 0.55). While DNAm-based predictions of plasma protein levels of GrimAge2 including DNAmADM, DNAmB2M, DNAmTIMP-1, and DNAmlog$_2$CRP were significant differences among robust, pre-frail, and frail participants (one-way ANOVA; $p = 0.040$, 0.036, 0.029, and 0.011, respectively). Pairwise comparisons showed robust group was significantly lower in DNAmADM ($\Delta_{DNAmADM} = -9.20$ pg/mL, $p = 0.037$, Cohen's d = 0.48), DNAmTIMP-1 ($\Delta_{DNAmTIMP-1} = -479.10$ pg/mL, $p = 0.041$; Cohen's d = 0.29), and DNAmlog$_2$CRP ($\Delta_{DNAmlog2CRP} = -0.26$ pg/mL, $p = 0.034$, Cohen's d = 0.60) compared to the frail group. Furthermore, we observed significantly lower in DNAmlog$_2$CRP between robust and pre-frail groups ($\Delta_{DNAmlog2CRP} = -0.24$ pg/mL, $p = 0.023$, Cohen's d = 0.55) (Table S1).

Age acceleration, frailty assessments, and physical performance levels

To explore the correlation between age accelerations and frailty, we expanded our analysis to examine AA in relation to physical performance and physical activity levels. Specifically, we examined five parameters from the Fried frailty phenotype: unintentional weight loss, hand-grip strength, gait speed, physical activity score (GPAQ), and exhaustion, as well as sit and reach, functional reach, and chair stand tests. Our findings revealed that Grim1AA and Grim2AA positively correlated with Fried phenotype scores, especially Grim2AA (Fig. 2A). Additionally, PhenoAA, Grim1AA, and Grim2AA showed positive correlations with Kihon Checklist scores. For five parameters, the strongest positive correlations were observed between unintentional weight loss and PhenoAA, gait speed and Grim2AA, and gait speed and DunedinPACE. Moreover, the chair stand test represented a positive correlation with Grim1AA, Grim2AA, and DunedinPACE. We further investigated the differences in AA and physical performance levels. Our results showed that higher Grim1AA, Grim2AA, and

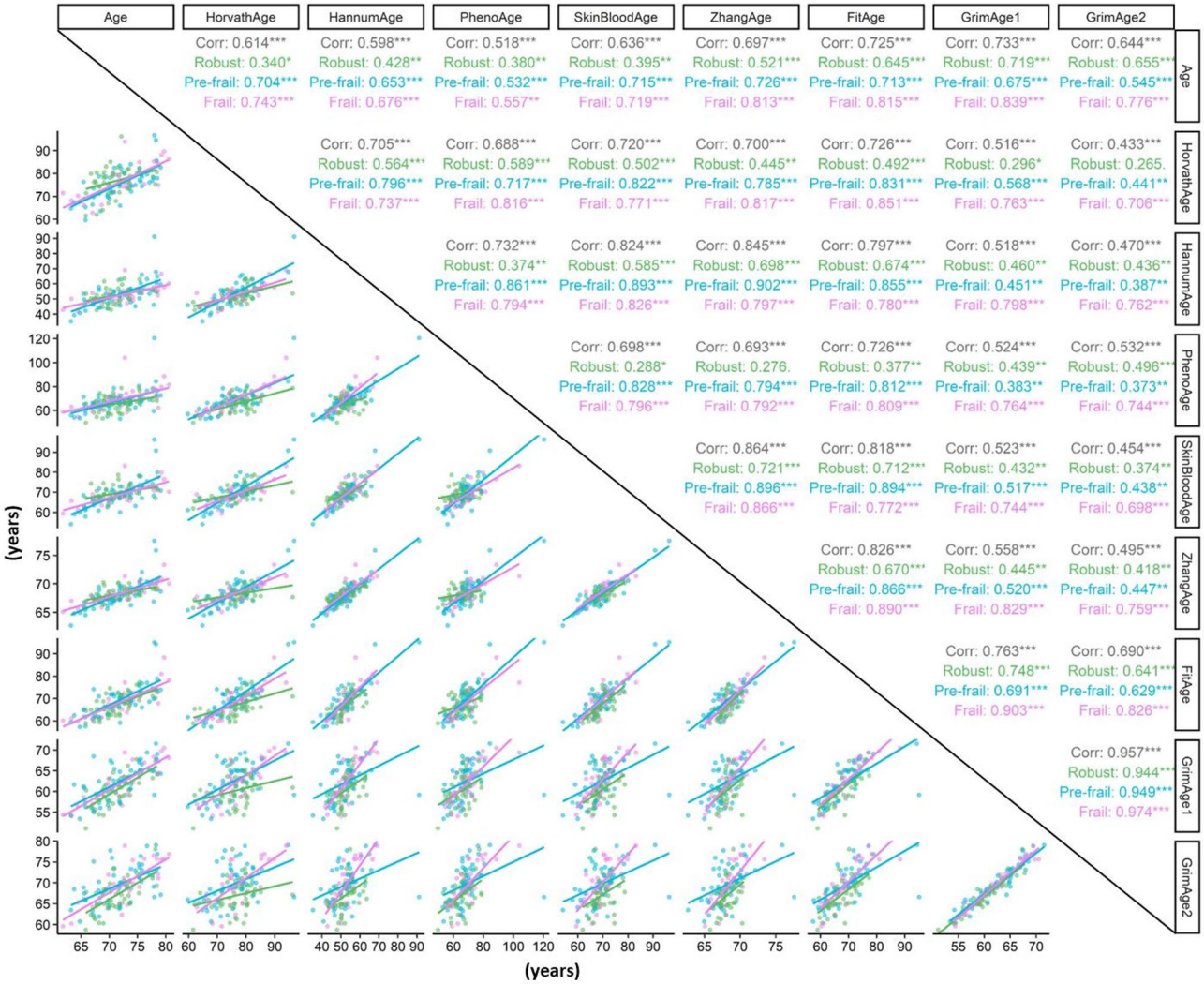

Fig. 1. Spearman's rank correlation coefficient between chronological age and DNA methylation age estimators of elderly women participants. The black, green, blue, and purple letters indicate correlations coefficient (Corr) of all participants ($n = 126$), robust ($n = 48$), pre-frailty ($n = 49$), and frailty ($n = 29$) groups, respectively. Statistical significance is denoted by asterisks, with * representing p-values < 0.05, ** indicating p-values < 0.01, and *** indicating p-values < 0.001.

DunedinPACE were significantly associated with lower levels of physical activity (GPAQ) ($\Delta_{Grim1AA} = 1.83$ years, $p < 0.01$, Cohen's d = 0.63; $\Delta_{Grim2AA} = 2.24$ years, $p < 0.01$, Cohen's d = 0.63; and $\Delta_{DunedinPACE} = 0.17$, $p = 0.015$, Cohen's d = 0.50) (Fig. 2B). Lower gait speed was significantly associated with higher Grim2AA ($\Delta_{Grim2AA} = 1.44$, $p = 0.023$, Cohen's d = 0.41). Moreover, higher %weight loss and lower hand grip strength was significantly associated with higher DunedinPACE ($\Delta_{DunedinPACE} = 0.03$, $p = 0.046$, Cohen's d = 0.37 and $\Delta_{DunedinPACE} = 0.03$, $p < 0.01$, Cohen's d = 0.51, respectively). No significant differences were observed in Horvath IEAA, Hannum IEAA, PhenoAA, and FitAA for five parameters of Fried phenotype compared to the normal group (Fig. S2).

GrimAA in robust, pre-frail, and frail individuals with hypertension

To investigate AA in relation to frailty and hypertension, we divided the participants into subgroups based on their frailty status. Each subgroup was further stratified by the presence or absence of hypertension, which was determined through medication history and self-reports. In our cohort, the prevalence of hypertension in elderly women was 67/126 (53.17%). We identified statistically significant lower differences between elderly individuals with normotension and those with hypertension in Grim1AA ($\Delta_{Grim1AA} = -1.04$ years, $p = 0.048$, Cohen's d = 0.36) and DunedinPACE ($\Delta_{DunedinPACE} = -0.03$, $p = 0.026$, Cohen's d = 0.42) (Fig. 3). When examined within different frailty statuses, the robust group without hypertension was significantly lower in Grim1AA ($\Delta_{Grim1AA} = -2.32$ years, $p = 0.026$, Cohen's d = 0.99) and Grim2AA ($\Delta_{Grim2AA} = -2.81$ years, $p = 0.024$, Cohen's d = 0.96) compared to the pre-frail group with hypertension. However, while Grim1AA and DunedinPACE were higher amongst hypertensive individuals compared to normotensive ones within each of the three frailty groups, these did not reach the level of statistical significance. Apart from those AA, Horvath IEAA, Hannum

	Robust ($n=48$)	Pre-frail ($n=49$)	Frail ($n=29$)	p-value *
Age (years), Mean ± SD	71.19 ± 3.09	71.80 ± 4.43	72.67 ± 5.03	0.314
Epigenetic age (years)				
HorvathAge, Mean ± SD	76.81 ± 6.01	75.43 ± 7.29	77.19 ± 7.54	0.469
HannumAge, Median (IQR)	52.07 (6.23)	53.05 (9.15)	52.37 (6.62)	0.920
PhenoAge, Median (IQR)	66.18 (8.45)	66.71 (6.56)	69.92 (9.67)	0.081
SkinBloodAge, Median (IQR)	68.91 (4.70)	68.50 (7.11)	68.98 (5.93)	0.830
ZhangAge, Median (IQR)	68.06 (1.70)	67.92 (2.68)	68.39 (1.98)	0.472
FitAge, Median (IQR)	66.43 (7.02)	69.10 (7.65)	68.65 (8.52)	0.343
GrimAge1, Mean ± SD	60.28 ± 3.49	62.35 ± 4.55 [a]	62.53 ± 4.68	0.024
GrimAge2, Mean ± SD	65.89 ± 3.76	68.56 ± 4.71 [a]	68.72 ± 5.15 [b]	< 0.01
Age acceleration (years)				
Horvath IEAA, Median (IQR)	1.46 (6.94)	−1.69 (5.49)	−0.06 (6.26)	0.130
Hannum IEAA, Mean ± SD	0.38 ± 4.83	−0.20 ± 4.55	−0.28 ± 4.01	0.767
PhenoAA, Median (IQR)	−2.43 (11.32)	−0.78 (7.49)	0.95 (7.74)	0.215
FitAA, Median (IQR)	−1.31 (5.68)	−0.32 (5.71)	−1.04 (4.40)	0.393
Grim1AA, Mean ± SD	−0.91 ± 2.43	0.69 ± 3.37 [a]	0.20 ± 2.55	0.022
Grim2AA, Mean ± SD	−1.30 ± 2.81	0.91 ± 3.98 [a]	0.43 ± 3.27	< 0.01
DunedinPACE, Median (IQR)	1.10 (0.10)	1.14 (0.09)	1.14 (0.07)	0.114

Table 2. Epigenetic age, age acceleration, and DunedinPACE of participants. IQR = interquartile range; AA = age acceleration; IEAA = intrinsic epigenetic age acceleration. *) p values were calculated using one-way ANOVA of means or the Kruskal–Wallis rank sum test. Lowercase letters a and b indicate significant differences ($p < 0.05$) between pre-frail vs. robust and frail vs. robust groups, respectively, as determined by Tukey HSD or Dunn's test with Benjamini-Hochberg correction.

IEAA, PhenoAA, and FitAA did not differ among both comparisons between normotension and hypertension participants and among frailty statuses with hypertension statuses, except for Horvath IEAA (Fig. S3).

Discussion

In this study, we present what is, to our knowledge, the first investigation of epigenetic age acceleration in relation to frailty and associated hypertension in an elderly non-Western population. Our data has revealed that frailty and hypertension are both significantly associated with increased GrimAge acceleration, with non-significant acceleration observed with hypertension within each frailty group.

Evidence is emerging for a role in physical activity in modulating DNAm age, suggesting that engaging in regular physical activity may help mitigate the biological effects of aging and improve overall health outcomes[38,39]. Epigenetic clocks trained on phenotypic markers and/or mortality (such as GrimAge1, GrimAge2, PhenoAge, and Zhang) have shown the strongest correlations with the prevalence of frailty[40]. GrimAge1 and GrimAge2 outperform other epigenetic clocks in predicting frailty, largely due to their incorporation of several plasma proteins—such as adrenomedullin, beta-2 microglobulin, and Cystatin-C—which are closely associated with age-related health conditions[26,32,35]. These biomarkers not only reflect physiological aging but also provide insight into the underlying biological mechanisms driving frailty, such as inflammation, hypertension, and cardiovascular health. Our study has similarly revealed an association of Grim1AA and Grim2AA and frailty in an elderly Thai cohort, consistent with the study by Seligman et al.. that demonstrated a correlation between the score of the Fried phenotypes and Grim1AA in a Western population[41]. Interestingly, despite a slight increase in Grim1AA and Grim2AA in the frail group, only the comparison of the robust and pre-frail groups reached statistical significance. Moreover, our results revealed DNAm-based prediction of plasma protein levels of TIMP-1, ADM, and CRP differed between robust and pre-frail groups. These proteins are known to be involved in anti-apoptotic function[42] senescence[43] and inflammatory[44]. Notably, DNAm-CRP has been previously highlighted as the strongest frailty-associated GrimAge component in a pilot study of adults with cirrhosis[45] suggesting potential biological associations between early frailty status and systemic physiological alterations. While this is likely the product of sample size ($n = 48$ vs. $n = 29$), it could also reflect the influence of other factors on age acceleration that potentially obscure the relationship with advancing age. In our study, both low gait speed and reduced physical activity were associated with increased Grim2AA. This finding is consistent with previous research showing that a faster walking pace[46] and regular physical activity[38] are linked to a slower epigenetic age.

The incidence of hypertension increases with age, affecting up to 75% of the elderly population[47]. Hypertension is a major risk factor for cardiovascular diseases, including coronary artery disease, heart attack, and stroke[48,49]. Moreover, associations between frailty and hypertension are well-documented[50–52]. To the best of our knowledge, no previous studies have examined the influence of DNAm age on frail individuals with hypertension. Studies to date have examined hypertension and accelerated biological aging[8,53,54] including both in middle-aged[53] and elderly[8] populations. Kresovich et al.. reported that hypertensive women had higher PhenoAA and GrimAA than normotensive women did at baseline[53]. Our results revealed that older women with hypertension only exhibited higher Grim1AA and DunedinPACE compared to those without hypertension.

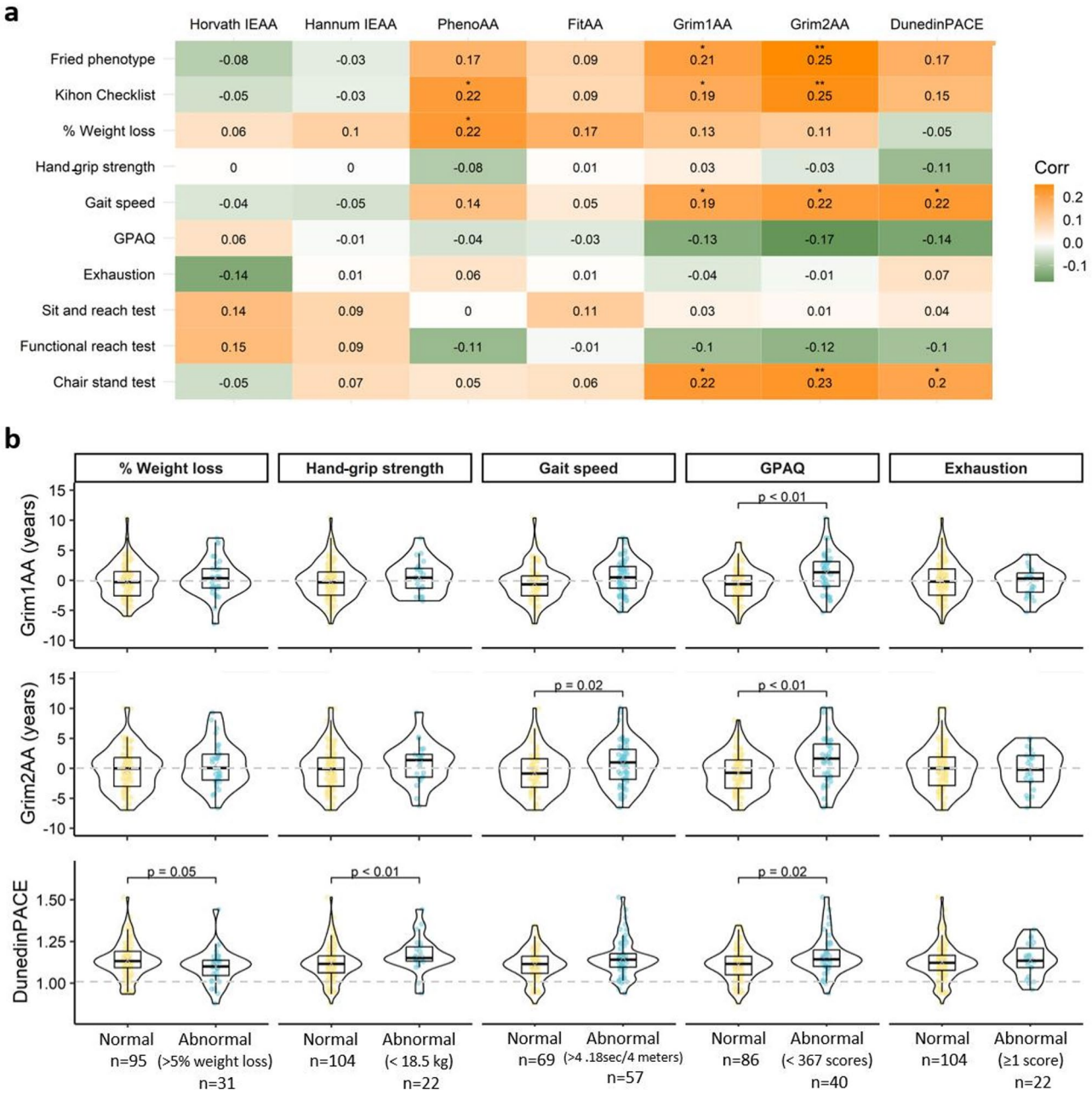

Fig. 2. Association among intrinsic epigenetic age acceleration (IEAA), age acceleration (AA), DunedinPACE, frailty assessments score, five parameters of Fried phenotype, and physical performances. **a** Spearman's rank correlation coefficient (Corr) (dark orange and dark green indicate positive and negative correlation, respectively; * and ** asterisks indicate $p < 0.05$, 0.01, and 0.001, respectively). **b** Differences in Grim1AA, Grim2AA, and DunedinPACE across five parameters were analyzed by comparing normal and abnormal groups.

Together, our findings provide further evidence that hypertension is associated with accelerated biological aging. This is potentially due to chronic inflammation and vascular damage are commonly linked to elevated blood pressure, but further studies will be required to establish the causal mechanism associating epigenetic ageing and hypertension. Furthermore, we found that hypertensive individuals, particularly those who are pre-frail, exhibit significantly accelerated Grim1AA and Grim2AA compared with robust individuals. However, no significant differences in Grim1AA and Grim2AA were observed between normotensive and hypertensive women within the frail, pre-frail, or robust groups. It is possible that the relationship between hypertension and frailty is mediated or confounded by other factors such as medication use, comorbidities, or lifestyle behaviors. A longitudinal study by Tang et al.. supports this possibility, demonstrating that antihypertensive medication use in elderly participants was associated with a reduction in GrimAge[34]. Furthermore, some antihypertensive drugs have been reported to alter DNA methylation patterns, including those of the aminoacylase 3 gene[55]

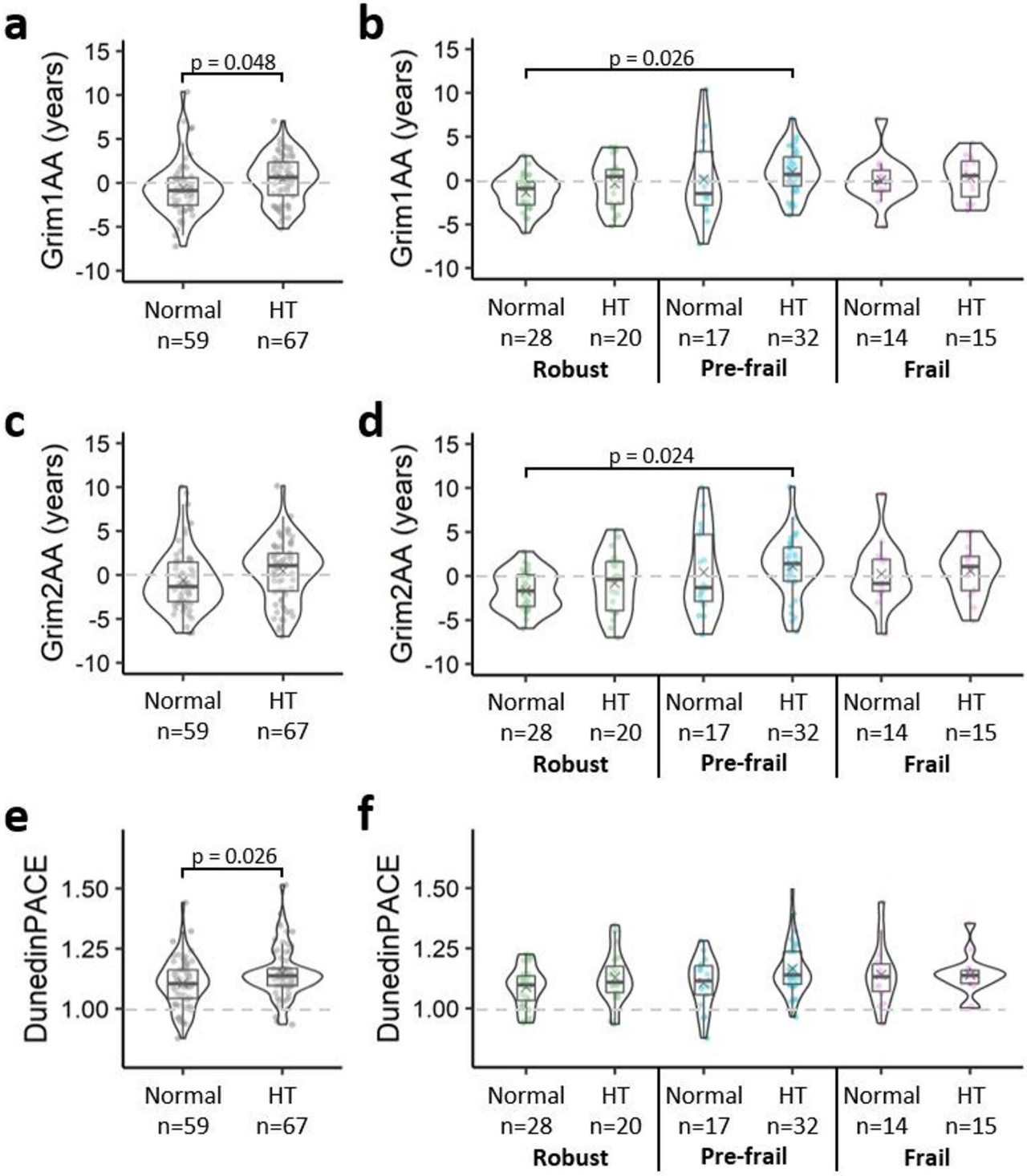

Fig. 3. Relationships between hypertension (HT) status and age accelerations (AA) (**a**, **c**, and **e**) and among robust, pre-frail, and frail individuals stratified by hypertension status (**b**, **d**, and **f**). **a – b** Grim1AA. **c – d** Grim2AA. **e – f** DunedinPACE.

which has been linked to blood pressure regulation. These findings suggest that antihypertensive treatment may reduce GrimAA differences, potentially mitigating the impact of hypertension on biological aging. Together, our finding suggests a potential association between hypertension, frailty, and accelerated biological aging, highlighting further studies to clarify these relationships and to explore the role of blood pressure management in frail populations. Although no statistically significant differences in Grim1AA and Grim2AA were observed between normotensive and hypertensive women within frailty groups, these results should be interpreted with

caution due to the limited sample size and potential lack of statistical power. Further research with larger, well-characterized cohorts is required to determine whether antihypertensive treatment influences biological aging.

Our study has taken a comprehensive approach to accurate assessment of frailty status, utilizing both Fried's phenotype and the Kihon Checklist. By combining Fried's phenotype with the Kihon Checklist, this approach allowed us to assess both physical and functional dimensions of frailty. These are core aspects of how frailty presents in older adults and reflect its inherently multidimensional nature. However, we recognize that restricting the analysis to participants with concordant classifications may have limited the representation of individuals with intermediate or discordant frailty profiles, who may also exhibit meaningful variations in biological aging. In addition to standard frailty measures by trained medical personnel, the study included further physical performance tests such as sit-and-reach, functional reach, and balance assessments. For safety reasons, some participants were excluded from these additional tests. Therefore, the small sample size may have limited the statistical power to detect significant differences among groups, as potentially demonstrated by non-significant increases in AA with hypertension by frailty status. To better understand the strength of our findings despite this limitation, we calculated effect sizes for AA in each group based on available sample sizes. These estimates provide additional context for interpreting the biological relevance of our results, even in the absence of statistical significance. We acknowledge, however, that larger studies are needed to confirm these associations and improve the precision of effect estimates. Additionally, despite the use of eight DNAm age estimators, mortality risk scores, which might be associated with frailty[56] could not be investigated due to missing probes in the Infinium Methylation EPIC v2.0. Finally, our study was cross-sectional, which limits our ability to conclude causality and the direction of effects.

Overall, this study highlights the importance of considering both physical and biological markers in assessing frailty and the impact of conditions such as hypertension in elderly populations. The observed associations between epigenetic clocks, frailty, and hypertension in this study demonstrate the potential value of considering multiple biological and clinical factors when investigating aging in older populations. Moreover, the differences in age acceleration observed across frailty groups, particularly with Grim2AA, indicate that combining DNA methylation clocks such as Grim2Age with physical and frailty assessments could improve the accuracy of frailty diagnosis and interventions. Future research should continue to explore these interactions to inform more effective interventions aimed at improving the health and longevity of elderly individuals. By further investigating the complex relationships among frailty, hypertension, and biological aging, researchers can identify potential strategies to slow biological aging and prevent the progression of frailty. This could lead to targeted therapies or lifestyle modifications that not only delay age-related decline but also enhance the overall quality of life in older adults.

In summary, this study provides valuable insights into the relationships among frailty, DNAm age, and hypertension in Thai elderly women. Although the prevalence of hypertension did not differ significantly across frailty groups, it is important to recognise that comorbid conditions like hypertension may still influence biological aging. Our analysis showed that healthy older adults without hypertension had lower epigenetic AA compared to pre-frail individuals with hypertension. This suggests that even when prevalence differences are absent, hypertension might contribute to subtle yet meaningful variations in biological aging, especially when combined with early signs of functional decline. Moreover, future research should explore these interactions to develop effective interventions that enhance health and longevity while addressing biological aging and frailty progression.

Data availability

Data related to epigenetic clock calculations are available from the corresponding author upon reasonable request.

Received: 25 May 2025; Accepted: 22 July 2025
Published online: 01 August 2025

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Acknowledgements

The authors are indebted to the Siriraj Clinical Molecular Pathology Laboratory team. C.S. acknowledges support from the Postdoctoral Fellowship Program at Siriraj Hospital, Mahidol University, through the Academic Development Scholarship.

Author contributions

C.S. provided conceptualization, funding acquisition, and supervision. P.C., C.S., W.K., A.D., and S.K. conducted the experiments. P.C., C.S., B.P., A.D., T.B., and V.K. performed the data analysis, interpreted the results, recruited the samples, and contributed to the drafting of the manuscript. P.C. created the figures and tables. C.S. had primary responsibility for the final content. All the authors read and approved the final manuscript.

Funding

This work was funded by the National Research Council of Thailand [grant number N34A660389] and the Siriraj Research Development Fund (IO) [grant number R016633018], Faculty of Medicine Siriraj Hospital, Mahidol University, awarded to C.S. The National Research Council of Thailand and the Siriraj Research Development Fund had no role in the design, analysis or writing of this article.

Declarations

Competing interests

The authors declare no competing interests.

Ethical approval and consent to participate

This study (COA no. Si 213/2021) received ethical approval from the Siriraj Institutional Review Board at Siriraj Hospital, Mahidol University, Bangkok, Thailand, and was registered with the Thai Clinical Trial Registry under the identifier TCTR20240626002. Prior to enrollment, all participants provided written informed consent for the collection of blood samples, clinical data, and genetic analysis.

Consent for publication

Not applicable.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-13175-0.

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