

# Metabolic syndrome accelerates epigenetic ageing in older adults: Findings from The Irish Longitudinal Study on Ageing (TILDA)

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## ABSTRACT

Metabolic syndrome (MetS) is a risk factor for the development of diabetes, cardiovascular disease, and all-cause mortality. It has an estimated prevalence of 40 % among older adults. Epigenetic clocks, which measure biological age based on DNA methylation (DNAm) patterns, are a candidate biomarker for ageing. GrimAge is one such clock which is based on levels of DNAm at 100 Cytosine-phosphate-Guanine (CpG) sites. This study hypothesised that those with MetS have 'accelerated ageing' (biological age greater than their chronological age) as indexed by GrimAge. This study examined MetS's association with GrimAge age acceleration (AA) using data from a subsample of 469 participants of the Irish Longitudinal Study on Ageing (TILDA). MetS was defined by National Cholesterol Education Program Third Adult Treatment Panel (ATP III) and International Diabetes Foundation (IDF) criteria, operationalised using the conventional binary cut-off, and as a count variable ranging from 0 to 5, based on the presence of individual components. This study also explored inflammation (as measured by C reactive protein) and metabolic dysfunction (as measured by adiponectin) as possible mediating factors between MetS and GrimAge AA. We found that MetS as defined by IDF criteria was associated with GrimAge AA of 0.63 years. When MetS was treated as a count, each unit increase in MetS score was associated with over 0.3 years GrimAge AA for both ATP III and IDF criteria. Inflammation mediated approximately one third of the association between MetS and GrimAge AA, suggesting that chronic subclinical inflammation observed in MetS has a relationship with DNAm changes consistent with a faster pace of ageing. Metabolic dysfunction mediated the association between MetS and GrimAge AA to a lesser extent (16 %). These data suggest that chronic subclinical inflammation observed in MetS has a relationship with DNAm changes consistent with a greater pace of ageing.

## 1. Introduction

Metabolic syndrome (MetS) is characterised by a cluster of inter-related cardiovascular risk factors — insulin resistance, central obesity, hypertension, elevated triglycerides and reduced high-density lipoprotein (Alberti et al., 2009). MetS is a risk factor for the development of diabetes and cardiovascular disease, and it is also associated with increased risk of numerous cancers and all-cause mortality (Reilly and Rader, 2003; Cornier et al., 2008; Mottillo et al., 2010). MetS has an estimated prevalence of 40 % among older adults and is associated with a higher likelihood of incident frailty (McCarthy et al., 2022a; McCarthy et al., 2022b).

The passage of time does not account for the ageing process on its

own. People of the same chronological age can have markedly different biological age with some individuals ageing at a much faster rate than others. Many biomarkers have been proposed to quantify the differences in actual calendar age and apparent biological age. Epigenetic clocks are one of the most promising candidate biomarkers of ageing. These clocks have been developed from observations that patterns of DNA methylation (DNAm) change with age (Horvath, 2013; Fitzgerald et al., 2021), with marked divergence in the degree of change among many individuals of the same chronological age. Higher levels of DNAm at gene promoter sites are associated with gene silencing (Razin, 1998), and these differences in gene regulation and expression may explain some of this divergence.

Patterns of DNAm at a small number of Cytosine-phosphate-Guanine

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(CpG) sites across the human methylome provide accurate measures of chronological age (Horvath, 2013; Hannum et al., 2013; Horvath and Raj, 2018). The residuals of the regression of DNAm age on calendar age has been hypothesised to represent the rate of biological ageing. Horvath's 'GrimAge' clock (Lu et al., 2019), a second-generation epigenetic clock based on levels of DNAm at 1030 CpG sites, is one such clock.

Given that MetS is associated with increased likelihood of morbidity and mortality, the consequences of increased biological ageing, we hypothesise it would associate with measurements of biological ageing such as DNAm. Measuring epigenetic age among those with MetS may identify and allow risk-stratification of those with the highest pace of ageing. Understanding why a relationship exists between MetS and epigenetic ageing may offer insight into possible causal mechanisms and therapies and address if inflammation and metabolic dysregulation are two possible avenues worth exploring (Tzika et al., 2018; Zhu et al., 2021; Raghuraman et al., 2016).

To address the role of inflammation and metabolic dysregulation in the causal pathway between MetS and epigenetic ageing we used C reactive protein (CRP) and Adiponectin as markers of inflammation and metabolic function, respectively. CRP is a pro-inflammatory plasma protein, largely produced and stored in the liver, whose concentration in blood rapidly increases as part of the 'acute-phase response', a coordinated response to certain pathogenic stimuli, before falling to near-undetectable levels once the immune response is no longer required (Du Clos and Mold, 2004). Elevated baseline CRP levels are associated with insulin resistance (de Rekeneire et al., 2006), incidence of diabetes (Dehghan et al., 2007; Thorand et al., 2003), and cardiovascular events, suggestive of a correlation between low level chronic inflammation and incident disease (Ridker et al., 1997). Adiponectin is a fat-derived hormone, produced and secreted by adipose tissue, with beneficial effects on numerous targets including the heart, liver, pancreatic  $\beta$ -cells, brain, kidney, and immune cells (Zhao et al., 2021). Circulating levels are inversely proportional to total adipose mass and also relate to the size and location of adipose cells rather than quantity of adipose tissue overall. Adiponectin enhances whole-body insulin sensitivity, suppresses hepatic gluconeogenesis, and improves nutrient utilization in skeletal muscle (Wang and Scherer, 2016). It plays an important role in metabolic regulation, and has been postulated as a biomarker for measurement of metabolic dysregulation (Mather and Goldberg, 2014).

This study has 2 major aims: (i) to examine whether MetS is associated with epigenetic ageing as indexed using the GrimAge clock among a sample of community dwelling older people participating in the Irish Longitudinal Study on Ageing (TILDA), and (ii) to explore potential inflammatory (i.e. CRP) and metabolic (i.e. adiponectin) pathways underlying any potential link between MetS and GrimAge AA.

2. Methods

2.1. Sample

This study uses data from the first wave of TILDA, a nationally representative prospective cohort study of 8170 community-dwelling adults living in the Republic of Ireland, aged  $\geq 50$  years old at baseline, collected between October 2009 and February 2011 (Whelan and Savva, 2013; Kearney et al., 2011). As part of this observational study, participants completed a Computer Assisted Personal Interview, gathering information relating to demographics, physical and mental health, physical activity, and medication use. Participants also completed a comprehensive health assessment which included anthropometrics and blood draws (Whelan and Savva, 2013).

This analysis utilises data for the epigenetic subsample of the baseline TILDA cohort ( $n = 469$ ), who were selected on the basis of their life-course social-class trajectory into four groups (stable high, upwardly mobile, stable low, and downwardly mobile) (McCrory et al., 2019a; McCrory et al., 2019b) and for whom measures of DNAm, CRP and adiponectin were available.

2.2. Primary predictor variable — MetS

MetS status was determined using TILDA-modified versions (McCarthy et al., 2022a) of the National Cholesterol Education Program Adult Treatment Panel III (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2001) (ATP III) and International Diabetes Federation (Alberti et al., 2005) (IDF) criteria (Table 1). Data included self-reported doctor diagnoses and regular prescribed medications and objective measures, including blood pressure, waist circumference and blood metabolic biomarker measurements as described in detail previously (McCarthy et al., 2022a). MetS was operationalised in two ways; firstly, using the conventional binary cut-off to define MetS (i.e.  $\geq 3$  components as per ATP III and IDF criteria, with the main difference between these two criteria being ATP III requiring any 3 components or more, whereas IDF requires central obesity and  $\geq 2$  other components, with lower waist circumference cut-offs for obesity) and secondly, as a count variable ranging from 0 to 5 to indicate the number of individual components present.

2.3. Outcome variable — GrimAge age acceleration

Epigenetic age acceleration was indexed using the second generation 'GrimAge' clock, based on levels of DNAm at 1030 CpG sites, designed using a two-stage process (Lu et al., 2019). Firstly, the levels of 88 plasma proteins and smoking pack-years were regressed on chronological age, sex and CpG levels. This identified CpG sites whose combination best predicted the corresponding plasma protein levels in the training dataset. 12 DNAm based surrogate biomarkers were identified to correlate  $\geq 0.35$  with their target biomarker in the training and test datasets. In the second stage, time to death (all-cause mortality) was regressed on age, sex, the 12 DNAm surrogate biomarkers of plasma protein levels and DNAm smoking pack-years, before elastic net Cox regression model selected age, sex, DNAm smoking pack-years and 7 of the DNAm based surrogate markers of plasma proteins: adrenomedullin, beta-2-microglobulin, cystatin C, growth differentiation factor 15, leptin, plasminogen activation inhibitor 1, and tissue inhibitor metalloproteinases 1.

Measurement of epigenetic age including laboratory techniques and use of algorithms for computing GrimAge were completed as described in detail previously (McCrory et al., 2019b; McCrory et al., 2020).

The residuals of the regression of GrimAge on calendar age defined 'GrimAge age acceleration' (GrimAge AA) with a positive residual representing a biological age greater than chronological age and a negative residual reflective of a biological age less than chronological. This clock

Table 1  
TILDA-modified ATP III and IDF criteria for diagnosis of metabolic syndrome.

|                                 | ATP III                                                                           | IDF                                                                               |
|---------------------------------|-----------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|
| Central obesity                 | WC $> 102$ cm (male) or $>88$ cm (female)                                         | BMI $> 30$ kg/m <sup>2</sup> or WC $\geq 94$ cm (male) or $\geq 80$ cm (female)   |
| Insulin resistance <sup>1</sup> | Diagnosis of diabetes, treatment for diabetes or HbA1c $\geq 39$ mmol/mol (5.7 %) | Diagnosis of diabetes, treatment for diabetes or HbA1c $\geq 39$ mmol/mol (5.7 %) |
| Blood pressure                  | SBP $\geq 130$ mmHg or DBP $\geq 85$ mmHg, or treatment                           | SBP $\geq 130$ mmHg or DBP $\geq 85$ mmHg, or treatment                           |
| Triglycerides                   | $\geq 1.7$ mmol/L                                                                 | $\geq 1.7$ mmol/L or treatment <sup>2</sup>                                       |
| High density lipoprotein        | $\leq 1.03$ mmol/L (male) or $\leq 1.29$ mmol/L (female)                          | $\leq 1.03$ mmol/L (male) or $\leq 1.29$ mmol/L (female)                          |

Note: TILDA = The Irish Longitudinal Study on Ageing; ATP III = National Cholesterol Education Program Third Adult Treatment Panel; IDF = International Diabetes Foundation; WC = waist circumference; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure. ATP III criteria is met if  $\geq 3$  of 5 components present; IDF criteria is met with central obesity plus  $\geq 2$  of remaining 4 components.

<sup>1</sup> Used as surrogate for raised fasting glucose ( $\geq 5.6$  mmol/L).

<sup>2</sup> Fibrates or nicotinic acid.

has been shown to predict age-related clinical phenotypes (e.g. walking speed, disability, polypharmacy) and mortality better than comparator clocks (McCorry et al., 2020).

## 2.4. Covariates

Age (years), sex (male, female), life-course social-class trajectory, smoking history, and physical activity were considered as covariates, due to possible confounding with regards to any association epigenetic clocks may have with MetS. Life-course social-class trajectory was categorised using the cross classification of father's social-class and the respondent's own contemporaneous social-class into four groups: stable high, upwardly mobile, stable low, and downwardly mobile (McCorry et al., 2019a). Smoking history was categorised into current smoker, heavy ex-smoker, light ex-smoker, and non-smoker, based on the number of cigarettes smoked per day, number of years smoking, and age at which they stopped smoking. Physical activity was categorised into low, moderate, and high levels, determined using the International Physical Activity Questionnaire short form, a validated tool to quantify physical activity (Craig et al., 2003).

## 2.5. Potential mediators

Measures of CRP and adiponectin were collected at baseline. We included them as markers of inflammation and metabolic function, respectively, and hypothesise that they mediate the relationship between GrimAge AA and MetS.

CRP concentrations were determined from plasma, using an immunoturbidimetric assay (Roche Cobas c701 analyser, Roche Diagnostics Ireland). The measurement range for the analyser was 0.3–350 mg/L. As this assay was not equivalent to the high sensitivity CRP assay, measures below 1.0 mg/L were assigned a value of 1.0 mg/L.

Adiponectin concentrations were determined from plasma, by electrochemiluminescence immunoassay using a custom R-Plex Enzyme-Linked Immunosorbent Assay (ELISA) kit (R-PLEX Human Biomarker Kit, Meso Scale Diagnostics, MSD, MD, USA). Plates were read using a MESO QuickPlex SQ 120MM instrument. Protein concentrations (pg/mL) were calculated using Meso Scale Diagnostics Discovery Workbench Software (v4.0). For participants with undetectable levels of analyte in plasma, these were replaced by the lower limit of detection for subsequent analysis.

Log-transformations were applied to both CRP and adiponectin due to right-skewed distributions.

## 2.6. Statistical analysis

Descriptive statistics were performed for baseline characteristics of TILDA participants including means and 95 % confidence intervals (CI) for continuous variables and percent for categorical variables. Participants were categorised by MetS status for both ATP III and IDF criteria. ANOVA and chi-square tests were used to test for differences between groups for continuous and categorical variables, respectively.

The GrimAge AA residual was regressed separately on each of the MetS measures in ordinary least squares regression while holding age and sex constant in minimally adjusted models (model 1), and then adjusting for the wider pool of covariates in the full multivariable adjusted models (model 2).

Finally, as our hypothesised causal model postulates that CRP and adiponectin serve as biological intermediaries between MetS and epigenetic AA, we tested this formally using the Karlson, Holm and Breen (KHB) Method (Kohler et al., 2011). It allows for decomposition of associations and a statistical test for determining whether the addition of a mediator leads to a statistically significant reduction in the relationship between an exposure (X) and an outcome (Y). With this framework, the direct effect represents the independent effect of X on Y with the mediator (Z) included as an explanatory variable. The indirect

effect describes the proportion of the effect that is relayed through the mediator. The total effect is the sum of the direct and indirect effects. The decomposition analysis shows the proportion of the total effect relayed by each of the mediators.

Stata/MP 14.1 was used for all statistical analysis (StataCorp, College station, TX).

## 2.7. Ethics

Ethical approval was obtained from the Faculty of Health Science Research Ethics Committee at Trinity College Dublin. Written informed consent was obtained from all participants.

## 3. Results

In the TILDA epigenetic cohort of 469 individuals, 142 (30.3 %) and 176 (37.5 %) had MetS, according to the ATP III and IDF criteria, respectively. When expressed as a count, only a small proportion of the sample – 11.7 % and 7.0 % for ATP III and IDF, respectively – had a score of zero, with the large majority having between 1 and 3 components of MetS (Table 2). Proportions of each component among participants are described in Table 3. Obesity, as defined using IDF cut-off points, was the most common component (78 %), closely followed by hypertension (75 %).

Baseline characteristics for the 469 participants, both overall and stratified by MetS status, are described in Table 4. Those with MetS were older, while those with MetS according to IDF were more predominately male and had heavier smoking histories. MetS was more common among those who were characterised as socio-economically disadvantaged across the life-course (i.e. stable low). Those meeting the ATP III criteria had a mean of 3.4 components, compared to a mean of 1.3 for those who did not meet these criteria. Similarly, those meeting the IDF criteria had a mean of 3.4 components compared to a mean of 1.5 for those who did not. GrimAge AA ranged from –9.9 years to 11.7 years with a mean of 0.04 years. Those with MetS had significantly higher mean GrimAge AA for both ATP III ( $p = 0.024$ ) and IDF ( $p = 0.007$ ) criteria. They also had higher levels of CRP and lower levels of adiponectin compared to those without MetS.

Fig. 1 depicts the GrimAge AA residual associated with MetS as defined using the ATP III and IDF binary cut-off as well as a count of deficits in minimally and full multi-variable adjusted models.

The GrimAge AA residual associated with MetS as defined using the ATP III and IDF binary cut-off, as well as a count of deficits in minimally and fully adjusted models (Fig. 1). When treated as a binary variable, MetS was associated with 1.00 years of GrimAge AA (95 % CI: 0.14, 1.86;  $p = 0.023$ ) when defined by ATP III criteria, and 1.16 years of GrimAge AA (95 % CI: 0.35, 1.98;  $p = 0.005$ ) when defined by IDF. When MetS was treated as a count variable, a one unit increase in ATP III score was associated with 0.55 years of GrimAge AA (95 % CI: 0.22, 0.88;  $p = 0.001$ ), while a one unit increase in IDF count was associated with 0.63 years of GrimAge AA (95 % CI: 0.28, 0.98;  $p < 0.001$ ). These

**Table 2**

Proportion of participants stratified by number of individual components of metabolic syndrome.

| No. of individual components | ATP III      | IDF          |
|------------------------------|--------------|--------------|
| 0                            | 55 (11.7 %)  | 33 (7.0 %)   |
| 1                            | 116 (24.7 %) | 84 (17.9 %)  |
| 2                            | 156 (33.3 %) | 172 (36.7 %) |
| 3                            | 94 (20.1 %)  | 125 (26.6 %) |
| 4                            | 38 (8.1 %)   | 43 (9.2 %)   |
| 5                            | 10 (2.1 %)   | 12 (2.6 %)   |

Note: Data presented as n (%); ATP III = National Cholesterol Education Program Third Adult Treatment Panel; IDF = International Diabetes Foundation; components include central obesity, insulin resistance, raised systolic or diastolic blood pressure, raised triglycerides, and reduced high density lipoprotein.

**Table 3**

Proportion of participants with each individual component of metabolic syndrome.

| Metabolic syndrome component | n   | Proportion (95 % confidence interval) |
|------------------------------|-----|---------------------------------------|
| Obesity (IDF)                | 366 | 78.0 % (74.0, 81.6)                   |
| Obesity (ATP III)            | 243 | 51.8 % (47.3, 56.3)                   |
| Insulin resistance           | 63  | 13.4 % (10.6, 16.8)                   |
| Hypertension                 | 353 | 75.3 % (71.1, 79.0)                   |
| Raised triglycerides         | 182 | 38.8 % (34.5, 43.3)                   |
| Reduced HDL                  | 71  | 15.1 % (12.2, 18.7)                   |

Note: Data presented as proportions with percentages with 95 % confidence intervals in brackets. ATP III = National Cholesterol Education Program Third Adult Treatment Panel; IDF = International Diabetes Foundation.

associations were attenuated appreciably when adjusted for the additional socio-demographic and lifestyle-related factors in the fuller models (model 2). Nevertheless, with the exception of the binary ATP III classification, MetS continued to be associated with GrimAge AA after adjustment for all of these potential confounders (Fig. 1, Supplementary Table S1).

To further explore underlying mechanisms, we proceeded to examine the extent to which CRP and adiponectin mediated the relationship between MetS and GrimAge AA in separate models using KHB mediation. The full multivariable adjusted models (model 2) served as the base for these analyses. Table 5 presents the results of the analysis and reveals that CRP had a statistically significant indirect effect, reducing the association of MetS with GrimAge by 47.3 % and 31.0 % for the binary ATP III and IDF classifications respectively; and by 36.7 % and 31.2 % when expressed as a count. There was no statistically significant direct path from MetS to GrimAge AA when CRP was added as a mediator. By contrast, the indirect path via adiponectin was not statistically significant when MetS was treated as a binary variable, but it was when treated as a count. Nevertheless, the proportion of the total effect mediated by adiponectin was much smaller than for CRP, reducing the association of the binary ATP III and IDF classifications with GrimAge by 17.0 % and 16.5 % respectively; and by 16.4 % and 15.6 % when expressed as a count. ATP III and IDF counts remained significantly associated with GrimAge AA (direct effect) when adjusted for levels of

adiponectin.

As a sensitivity check, we re-ran the analyses using the PhenoAge epigenetic clock (Levine et al., 2018), another second-generation clock, as the dependent variable (Supplementary Table S1). The overall pattern of results was ostensibly the same, although the proportion of the association mediated by both CRP and adiponectin was smaller on this occasion (Supplementary Table S2) and both the ATP III and IDF counts remained significantly associated with PhenoAge AA.

#### 4. Discussion

MetS is associated with earlier all-cause mortality (Mottillo et al., 2010). The GrimAge clock serves as an intermediate endpoint to show that MetS is associated with biological age acceleration. We have shown that meeting the IDF criteria for MetS was associated with GrimAge AA of 0.63 years. When MetS was treated as a count, each unit increase in MetS score was associated with over 0.3 years GrimAge AA for both ATP III and IDF criteria. Therefore, those with MetS appear to be ageing faster than would otherwise be expected, implying that it is a risk factor for earlier disease presentation, morbidity and mortality. Someone with all 5 components of MetS could be nearly 2 years older as measured by GrimAge than someone with none of the components of MetS which would seem important at both an individual and population level. Epigenetic alterations are considered a hallmark and potential underlying cause of disease, so public health strategies designed to act on the upstream causes of biological age acceleration, including MetS, may serve to ameliorate the overall burden of disease, maximise lifespan, and compress morbidity.

Our analysis revealed that inflammation, as measured by CRP, mediated nearly a third of the association between MetS and GrimAge AA, suggesting that chronic subclinical inflammation observed in MetS has a relationship with DNAM changes consistent with a greater pace of ageing. Adding CRP to the adjusted models reduced the GrimAge AA effect size by 31 % for IDF (for both binary and count variables) and by 37 % and 47 % for ATP III for count and binary variables respectively.

Metabolic dysfunction, as measured by adiponectin, mediated the association between MetS and GrimAge AA to a lesser extent than CRP, with the effect size reduced by 17 % when adiponectin was added to the

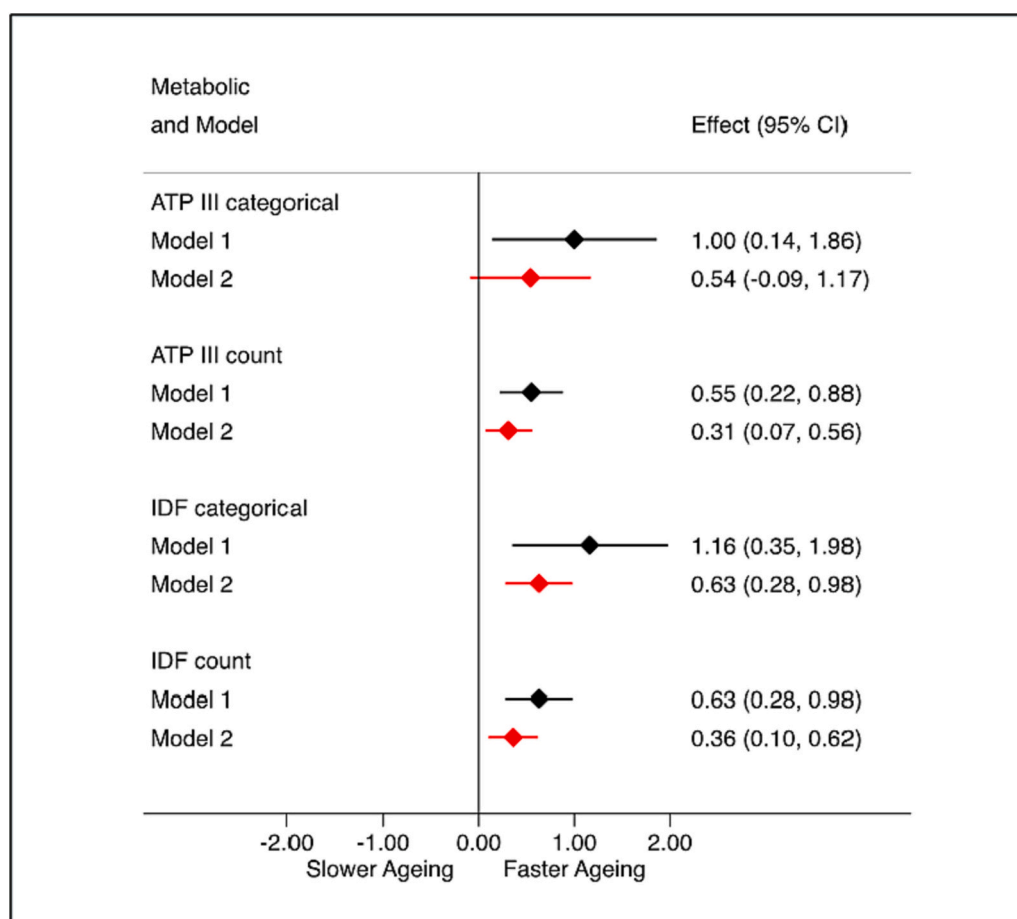
**Table 4**

Characteristics of the participants included in analyses, overall and stratified by metabolic syndrome status for both ATP III and IDF criteria.

| Characteristics       | Overall           | MetS Status       |                      |         | MetS Status       |                   |         |
|-----------------------|-------------------|-------------------|----------------------|---------|-------------------|-------------------|---------|
|                       | (n = 469)         | ATP III (n = 142) | No ATP III (n = 327) | p value | IDF (n = 176)     | No IDF (n = 293)  | p value |
| Age (years)           | 62.0 (61.2, 62.7) | 63.7 (62.3, 65.1) | 61.2 (60.4, 62.1)    | 0.003   | 63.0 (61.8, 64.3) | 61.4 (60.4, 62.3) | 0.030   |
| Sex (male, %)         | 49.9 (45.4, 54.4) | 52.1 (43.9, 60.2) | 48.9 (43.5, 54.4)    | 0.526   | 56.8 (49.4, 64.0) | 45.7 (40.1, 51.5) | 0.020   |
| Trajectory (%)        |                   |                   |                      |         |                   |                   |         |
| Downward              | 24.9 (21.2, 29.1) | 26.1 (19.5, 33.9) | 24.5 (20.1, 29.4)    | 0.013   | 26.1 (20.1, 33.2) | 24.2 (19.6, 29.5) | 0.005   |
| Stable high           | 25.3 (21.4, 29.3) | 19.0 (13.3, 26.4) | 27.8 (23.2, 33.0)    |         | 22.2 (16.6, 28.9) | 27.0 (22.2, 32.4) |         |
| Stable low            | 24.9 (21.2, 29.1) | 33.8 (26.5, 42.0) | 21.1 (17.0, 25.9)    |         | 32.9 (26.4, 40.3) | 20.1 (15.9, 25.1) |         |
| Upward                | 24.9 (21.2, 29.1) | 21.1 (15.2, 28.7) | 26.6 (22.1, 31.7)    |         | 18.8 (13.6, 25.2) | 28.7 (23.8, 34.1) |         |
| Smoking history (%)   |                   |                   |                      |         |                   |                   |         |
| Non-smoker            | 39.4 (35.1, 44.0) | 35.2 (27.8, 43.5) | 41.3 (36.0, 46.7)    | 0.056   | 35.8 (29.0, 43.2) | 41.6 (36.1, 47.4) | 0.013   |
| Light ex-smoker       | 17.3 (14.1, 21.0) | 14.1 (9.2, 20.9)  | 18.7 (14.8, 23.3)    |         | 13.1 (8.8, 18.9)  | 19.8 (15.6, 24.8) |         |
| Heavy ex-smoker       | 25.6 (21.8, 29.7) | 33.8 (26.5, 42.0) | 22.0 (17.8, 26.9)    |         | 33.5 (26.9, 40.9) | 20.8 (16.5, 25.9) |         |
| Current smoker        | 17.7 (14.5, 21.4) | 16.9 (11.6, 24.0) | 18.0 (14.2, 22.6)    |         | 17.6 (12.6, 24.0) | 17.8 (13.8, 22.6) |         |
| Physical activity (%) |                   |                   |                      |         |                   |                   |         |
| Low                   | 29.2 (25.3, 33.5) | 34.5 (27.1, 42.7) | 26.9 (22.4, 32.0)    |         | 33.0 (26.3, 40.3) | 27.0 (22.2, 32.4) |         |
| Moderate              | 35.8 (31.6, 40.3) | 34.5 (27.1, 42.7) | 36.4 (31.3, 41.8)    | 0.226   | 35.2 (28.5, 42.6) | 36.2 (30.9, 41.9) | 0.336   |
| High                  | 35.0 (30.8, 39.4) | 31.0 (23.9, 39.1) | 36.7 (31.6, 42.1)    |         | 31.8 (25.3, 39.1) | 36.8 (31.5, 42.6) |         |
| GrimAge AA (years)    | 0.0 (−0.4, 0.4)   | 0.7 (0.1, 1.4)    | −0.3 (−0.7, 0.2)     | 0.024   | 0.7 (0.1, 1.4)    | −0.4 (−0.9, 0.1)  | 0.007   |
| Log CRP               | 0.7 (0.7, 0.8)    | 1.0 (0.9, 1.1)    | 0.6 (0.5, 0.7)       | <0.001  | 0.9 (0.8, 1.1)    | 0.6 (0.5, 0.7)    | <0.001  |
| Log Adiponectin       | 17.1 (17.0, 17.2) | 17.0 (16.8, 17.1) | 17.2 (17.1, 17.2)    | 0.015   | 16.9 (16.8, 17.1) | 17.2 (17.1, 17.3) | <0.001  |
| Count ATP III         | 1.9 (1.8, 2.1)    | 3.4 (3.3, 3.5)    | 1.3 (1.2, 1.4)       | <0.001  | 3.1 (3.0, 3.2)    | 1.2 (1.2, 1.3)    | <0.001  |
| Count IDF             | 2.2 (2.1, 2.3)    | 3.5 (3.4, 3.6)    | 1.7 (1.6, 1.7)       | <0.001  | 3.4 (3.3, 3.5)    | 1.5 (1.4, 1.6)    | <0.001  |

Note: Data presented as means or proportions with percentages with 95 % confidence intervals in brackets. Metabolic syndrome (MetS) as per International Diabetes Foundation (IDF) and National Cholesterol Education Program Third Adult Treatment Panel (ATPIII) criteria. Between group differences were analysed using ANOVA with adjusted Wald test given weighted data, and Chi-Square tests as appropriate. AA = age acceleration (as measured by GrimAge epigenetic clock); CRP = C reactive protein.





**Fig. 1.** Association of metabolic syndrome (binary and count) with GrimAge age acceleration in minimally and full multivariable adjusted models. Note: Model 1 adjusted for age and sex; Model 2 additionally adjusted for life-course social-class trajectory, smoking history, and physical activity.

models. This would suggest that metabolic dysregulation also has a relationship with DNAm changes observed with age acceleration.

Notably, GrimAge version 2 has recently been developed, with the addition of two new DNAm based estimators of plasma proteins — high-sensitivity CRP and HbA1c, both log-transformed (Lu et al., 2022). GrimAge 2 outperforms GrimAge in terms of predicting mortality as well as heart disease and fatty liver. This is somewhat unsurprising given the importance of insulin resistance and inflammation in numerous disease processes.

Inflammation explains a significant amount of the association between GrimAge AA and MetS, which highlights the potential for using life-style modifications for prevention of AA, for example through physical activity and dietary modifications, both of which are associated with altered epigenetics (Kresovich et al., 2021; Ramos-Lopez et al., 2021). It also raises the possibility of treatments with anti-inflammatories. Studies have shown that cardiovascular risk reduction directly relates to CRP levels; those with highest levels of inflammation benefiting the most from aspirin (Ridker et al., 1997). Similarly, Rosuvastatin, a medication used to reduce low-density lipoprotein levels, reduced CRP levels by 37 % and the incidence of major cardiovascular events by 44 % compared to placebo (Ridker et al., 2008), with some of the beneficial effects of statins attributed to inhibition of the harmful effects of inflammation (Yeh, 2004).

While metabolic dysregulation, as measured by adiponectin, mediated the association between MetS and GrimAge AA, it did so to a lesser extent than inflammation (as measured by CRP). This could be explained by adiponectin affecting inflammation. Ceramides are a class of lipids involved in inflammation, insulin resistance, apoptosis, and atherosclerosis but which can be converted to beneficial lipids by ceramidase

(Chaurasia and Summers, 2015). Adiponectin receptors produce ceramidase activity, and adiponectin reduces ceramide levels (Wang and Scherer, 2016).

A strength of this study is that the sample used was a subsample of a robust dataset from a nationally representative population cohort, with comprehensive subjective and objective measurements, including socio-demographic, lifestyle-related and health. This allowed MetS to be operationalised using two separate criteria and modelled with two separate second generation epigenetic clocks. The availability of CRP and adiponectin allowed examination of putative biological intermediaries between MetS and epigenetic age acceleration. GrimAge is an applicable epigenetic clock to use given that it was developed from cohorts with a similar age profile to TILDA. Furthermore, the analysis using the PhenoAge epigenetic clock allowed validation of results on accelerated ageing and MetS observed with GrimAge.

The main limitation of this study is that the analyses are cross-sectional, and therefore causation cannot be inferred as the exposure, mediators and outcome were measured at the same time. A further limitation is that epigenetic data was available for a small subsample of the TILDA cohort. Future studies could aim to examine MetS and epigenetic age acceleration longitudinally to explore temporality, ideally using a larger sample size. The epigenetic subsample was designed to examine the impact of life course socioeconomic trajectories on pace of epigenetic ageing, while the ATP III and IDF criteria were not rigidly adhered to, with insulin resistance measured using diagnosis or treatment for diabetes and measured HbA1c, rather than fasting glucose, which could also be considered limitations.

**Table 5**

Association of metabolic syndrome with GrimAge epigenetic age acceleration and proportion of the total effect mediated by C-Reactive Protein and Adiponectin in separate models.

| Metabolic syndrome  | + CRP              | p value | + Adiponectin      | p value |
|---------------------|--------------------|---------|--------------------|---------|
| ATP III categorical |                    |         |                    |         |
| Total effect        | 0.54 (−0.08, 1.15) | 0.089   | 0.54 (−0.09, 1.16) | 0.093   |
| Direct effect       | 0.28 (−0.35, 0.91) | 0.380   | 0.44 (−0.18, 1.07) | 0.165   |
| Indirect effect     | 0.25 (0.08, 0.42)  | 0.003   | 0.09 (−0.01, 0.19) | 0.079   |
| % Mediated          | 47.3 %             |         | 17.0 %             |         |
| ATP III count       |                    |         |                    |         |
| Total effect        | 0.31 (0.07, 0.56)  | 0.012   | 0.31 (0.07, 0.56)  | 0.012   |
| Direct effect       | 0.20 (−0.05, 0.45) | 0.122   | 0.26 (0.02, 0.51)  | 0.037   |
| Indirect effect     | 0.12 (0.04, 0.19)  | 0.002   | 0.05 (0.00, 0.09)  | 0.050   |
| % Mediated          | 36.9 %             |         | 15.5 %             |         |
| IDF categorical     |                    |         |                    |         |
| Total effect        | 0.63 (0.04, 1.22)  | 0.035   | 0.63 (0.04, 1.23)  | 0.037   |
| Direct effect       | 0.44 (−0.16, 1.04) | 0.152   | 0.53 (−0.07, 1.13) | 0.085   |
| Indirect effect     | 0.20 (0.05, 0.34)  | 0.008   | 0.10 (−0.01, 0.21) | 0.056   |
| % Mediated          | 31.0 %             |         | 16.4 %             |         |
| IDF count           |                    |         |                    |         |
| Total effect        | 0.36 (0.10, 0.61)  | 0.006   | 0.36 (0.10, 0.62)  | 0.007   |
| Direct effect       | 0.25 (−0.02, 0.51) | 0.068   | 0.30 (0.03, 0.56)  | 0.027   |
| Indirect effect     | 0.11 (0.03, 0.18)  | 0.003   | 0.06 (0.00, 0.12)  | 0.047   |
| % Mediated          | 31.2 %             |         | 15.6 %             |         |

Note: Total effect model adjusted for: age, sex, life-course social-trajectory, smoking history, and physical activity levels; ATP III = National Cholesterol Education Program Third Adult Treatment Panel; IDF = International Diabetes Foundation.

## 5. Conclusion

This study provides new evidence that MetS, a highly prevalent condition, that increases with age, is associated with accelerated biological ageing as measured by GrimAge. AA as indexed by GrimAge was observed among participants with fewer components present than is needed to meet the criteria for MetS as defined by both ATP III and IDF criteria. A considerable amount of the reported AA was shown to be mediated by inflammation, as measured by CRP, and to a lesser extent by metabolic dysregulation as measured by adiponectin.

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## CRedit authorship contribution statement

KMC, CMC and RAK conceived the study and were responsible for methodology, validation, and data curation. KMC and CMC were responsible for statistical analysis. KMC and CMC had full access to the data and can verify the data presented. All authors had full access to the data, critically revised the manuscript content and gave final approval for the version to be published. All authors accept responsibility to submit for publication.

## Data sharing

TILDA data can be accessed via the Irish Social Science Data Archive ([www.ucd.ie/issda](http://www.ucd.ie/issda)). The publicly accessible dataset files are hosted by the Irish Social Science Data Archive based in University College Dublin, and the Interuniversity Consortium for Political and Social Research (ICPSR) based in the University of Michigan. Researchers wishing to access the data must complete a request form, available on either the ISSDA or ICPSR website.

## Declaration of competing interest

The authors have no conflicts of interest.

## Data availability

The data that has been used is confidential.

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