



Review article

Breaking new ground on human health and well-being with epigenetic clocks: A systematic review and meta-analysis of epigenetic age acceleration associations



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ABSTRACT

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Epigenetic clocks provide an accurate molecular readout of epigenetic age and epigenetic age acceleration (EAA) derived from DNA methylation data have shown promise as biomarkers of ageing. This systematic review synthesised research on associations between EAA measures and various physiological, cognitive, social, and environmental factors. A comprehensive search strategy identified 299 publications reporting 1050 unique EAA-factor associations based on 53 methylation clocks. Random-effects meta-analyses pooled results across studies for selected EAA-factor pairs. Significant pooled associations emerged, providing insights into relationships between specific factors and accelerated epigenetic ageing. We developed a novel four-level classification system to categorise this diverse range of factors and enable a structured synthesis. To aid further research planning in this rapidly evolving field, TEAPEE (Tracker of EAA Associations with Phenotype & Environmental Exposure) - an interactive, searchable web table detailing all EAA-factor associations - was developed, cataloguing the epigenetic clocks, associated factors, classification categories, and direct links to the original studies. This resource will empower future investigations into the multifaceted determinants of epigenetic ageing, contributing to a deeper understanding of the epigenome's sensitivity to various life experiences and exposures.

1. Introduction

Epigenetic changes, which involve modifications in gene expression without altering the DNA sequence, are recognised as key hallmarks of ageing (López-Otín et al., 2013). This area of research has gained prominence for its role in deciphering the molecular underpinnings of ageing and related diseases. Central to this research are epigenetic clocks, innovative tools that estimate an individual's biological age by analysing patterns of DNA methylation, a type of epigenetic regulation of gene expression. These clocks have attracted attention for their potential to predict life expectancy and the risk of developing age-associated diseases, often outperforming other biomarkers (Jylhävää et al., 2017).

Epigenetic Age Acceleration (EAA) refers to the discrepancy between an individual's biological age, as estimated by epigenetic clocks, and their chronological age. A biological age greater than the chronological age indicates faster ageing faster at a cellular level, potentially influenced by both intrinsic and extrinsic factors. Conversely, a younger biological age suggests slower ageing processes. Studies have linked slower biological ageing with longevity and reduced risk of age-related conditions (Elliott et al., 2021). EAA is a valuable biomarker for assessing an individual's health status beyond their chronological age, offering insights into their ageing process and susceptibility to diseases (Jain et al., 2022; Bell et al., 2019). Epigenetic clocks differ from each other in the algorithm which they use to estimate a person's biological age. Interestingly, certain epigenetic clocks have increased sensitivity for various age-related diseases, which has the potential to provide exciting insights

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| Abbreviations | |
|---------------|--|
| ACE | Adverse Childhood Experience |
| ADNI | Alzheimer's Disease Neuroimaging Initiative |
| BASE-II | Berlin Ageing Study II |
| AIC | Akaike Information Criterion |
| AMDTSS | Australian Mammographic Density Twins and Sisters Study |
| BIC | Bayesian Information Criterion |
| BLUP | Best Linear Unbiased Predictor |
| BMI | Body Mass Index |
| CARDIA | Coronary Artery Risk Development in Young Adults |
| CHICOS | Children of Immigrants Collaborating to Overcome Stress |
| COPD | Chronic Obstructive Pulmonary Disease |
| CpG | Cytosin-phosphate-Guanine |
| CSS | Cascading Style Sheets |
| DOAJ | Directory of Open Access Journals |
| DNA | Deoxyribonucleic Acid |
| DNAm | DNA methylation |
| EA | Epigenetic Age/Ageing |
| EAA | Epigenetic Age Acceleration |
| EN | Elastic Net |
| EPIGEN | also known as EPIGEN-Brasil, it is the Initiative in population genomics and Genetic Epidemiology |
| ESTHER | Epidemiologische Studie zu Chancen der Verhütung, Früherkennung und optimierten Therapie chronischer Erkrankungen in der älteren Bevölkerung |
| FACS | Family and Community Health Study |
| FHS | Framingham Heart Study |
| FTC | Finnish Twin Cohort |
| GENOA | Genetic Epidemiology Network of Arteriopathy |
| GRS | Generation R Study |
| HIV | Human Immunodeficiency Virus |
| HR | Hazard Ratio |
| HRS | Health and Retirement Study |
| HTML | HyperText Markup Language |
| JHS | Jackson Heart Study |
| KORA | Cooperative Health Research in the Region of Augsburg |
| LBC | Lothian Birth Cohort |
| MCCS | Melbourne Collaborative Cohort Study |
| NAS | Normative Ageing Study |
| MH | Mental Health |
| NCBI GEO | National Center for Biotechnology Information Gene Expression Omnibus |
| OR | Odds Ratio |
| PMC | PubMed Central |
| PRISMA | Preferred Reporting Items for Systematic Reviews and Meta-Analyses |
| PROSPERO | International prospective register of systematic reviews |
| RR | Risk Ratio |
| SES | Socioeconomic Status |
| SS | The Sister Study |
| TEAPEE | Tracker of EAA Associations with Phenotype & Environmental Exposure |
| TTP | Texas Twin Project |
| VET | Vietnam Era Twin |
| WHI | Women's Health Initiative |
| WHR | Waist-Hip Ratio |
| WoS | Web of Science |

into the molecular mechanisms of ageing.

Systematic reviews of epigenetic age acceleration have provided valuable insights into the associations between EAA and various biological, social, and environmental characteristics, which we refer to as *factors*. A review by (Oblak et al., 2021) found significant associations between EAA and factors such as sex, mortality, cardiovascular disease, cancer, and diabetes. Other studies have found that increased EAA, as measured by four different epigenetic clocks, was associated with lower odds of survival to the age of 90 among older US women (Zhang et al., 2016; Levine et al., 2022). Furthermore, associations between EAA scores and cardiometabolic phenotypes have been evaluated in population cohorts (McGuinness and Higgins, 2021). A comprehensive review of 156 studies (Simons et al., 2018) found significant associations of EAA with mortality and other social and environmental factors. Other reviews have highlighted the association between EAA and heavy drinking (Rosen et al., 2019) as well as cognitive performance (Levine et al., 2021). Transdiagnostic evaluations have also been conducted, revealing associations between EAA and the burden of psychiatric disorders (Stringhini et al., 2017). In addition to age-related pathology, associations have also been identified between EAA and environmental exposures (Bozack et al., 2022) as well as socioeconomic status (SES) (Schmitz et al., 2022; Fiorito et al., 2019). These associations are particularly intriguing, as they may provide insights into an individual's susceptibility to ageing and age-related phenotypes.

Despite the growing body of literature, the relationship between EAA and various factors continues to be an area for exploration. This systematic review aims to synthesise current knowledge on the association of EAA with different phenotypes, expanding upon the groundwork laid by previous reviews, in particular, the one conducted by (Oblak et al., 2021).

Our first objective is to present a comprehensive summary of studies that have reported associations between EAA and various factors since

the publication of Oblak's review in 2021. We have made a concerted effort to expand on previous work by including non-blood tissues, post-mortem samples, and the heritability of factor exposures. By categorising and classifying the existing research, we aim to provide a valuable resource for researchers.

Our second objective is to critically analyse the associations between EAA and various health-related factors. We seek to discern which aspects of human health are consistently associated with EAA across different studies and which associations are specific to certain epigenetic clocks. This includes evaluating the performance of clocks that were initially introduced in Oblak's review and have since been applied to a diverse range of factors.

To enhance the utility of our review, we have categorised the factors associated with EAA into broader categories such as cardiovascular health, metabolic conditions, and lifestyle factors. Furthermore, we have developed an interactive, searchable table that includes the epigenetic clock used, its associated factor, the category it falls under, and links to the corresponding publications. This tool is designed to aid researchers in planning or conducting research on EAA associations to various phenotypes or exposures.

Additionally, we have conducted a meta-analysis of the associations between EAA and these factors. This meta-analysis provides a quantitative synthesis of the evidence, offering a more robust understanding of the relationships at play.

By elucidating the complex interplay between EAA and a various diverse phenotypes, we aim to shed light on the potential use of epigenetic clocks as predictive tools for health outcomes, as well as to guide future research directions in this rapidly evolving field.

We followed the PRISMA 2020 statement (Page et al., 2021), an updated guideline for reporting systematic reviews, to ensure comprehensive, transparent, and standardised reporting in our systematic review. The study is registered in the International Prospective Register of

Systematic Reviews (PROSPERO), project ID CRD42023375986.

2. Methods

2.1. Publication search and preliminary screening

A literature search was performed on January 5th, 2023 using 8 databases: BioRxiv, Directory of Open Access Journals (DOAJ), Nature, PubMed, PMC, Scilit, Scopus, and Web of Science (WoS). The search terms used for our literature search were partially adapted from (Oblak et al., 2021), with changes to account for spelling variation. Searches were limited to studies published or pre-printed in the English language between 1 February 2021 and 1 January 2023, as studies published/pre-printed before February 2021 were reviewed in (Oblak et al., 2021). Following the search, the publication lists retrieved from the eight databases were combined and de-duplicated. In cases where both a pre-print and final publication were available, only the final publication was kept for further review.

In our systematic review, we focused on identifying studies that explored how various factors influence epigenetic ageing. For clarity, we define ‘factors’ as any characteristic (physiological, environmental, social, or pathological) that has been studied for its potential impact on epigenetic ageing. We describe epigenetic ageing as the process of comparing an individual’s chronological age to their epigenetic age. The latter is determined using epigenetic clocks, which are computational tools developed to analyse DNA methylation (DNAm) patterns at specific genomic sites. These tools estimate an individual’s biological age and assess the rate of ageing in relation to their chronological age, phenotypic age, or mortality risk.

The preliminary screening was performed by seven reviewers, based on the following **eligibility criteria**:

- An included paper must be a primary research study published in the English language;
- Based on human tissue samples from at least three different people;
- The results must contain quantitative measures of the relationship between epigenetic age acceleration and factors;
- The publication must either examine a pre-existing epigenetic clock OR clearly define a new one.

No restrictions were placed on characteristics such as sex, age, race, or ethnicity.

Studies were excluded if they:

- Were conducted exclusively on *in vitro* cell cultures;
- Had a sample size of < 3;
- Reported epigenetic age which was not adjusted to chronological age;
- Lacked key information such as sample size or sample type;
- Failed to examine a pre-existing epigenetic clock OR clearly define a new one.

Data was excluded if it was derived from non-human samples or if it pertained to measures derived from DNAm (e.g. DNAm-inferred cell fractions). We have provided specific rationale for each study excluded from the reviewing process.

2.2. Data retrieval

A framework for the collection of data from publications was collectively designed and agreed upon, and then shared as a table (Google Docs spreadsheet). This table was adapted from (Oblak et al., 2021) with several additional columns. Reviewers worked independently to manually retrieve data from publications and input all required information into the shared table. Once data collection was finished, all manual inputs were checked by other reviewers and standardised to

ensure consistency in reporting.

The information required from each publication included: clock, factor, tissue type, and sample size. Furthermore, we collected and recorded characteristics of the study cohort, such as the chronological age, sex, race/ethnicity, number of samples in the cohort, and whether the study was looking specifically at twins. Tissue type and tissue treatment were also recorded, as differences in clock performance have previously been linked to tissue type (Zhang et al., 2019).

Given the variety of statistical methods employed to investigate associations between factors and epigenetic age acceleration, studies reported a range of effect size measures and corresponding statistics. To accommodate this diversity, we meticulously extracted all available effect size measures and their statistical values, including the level of significance, from each study. The array of effect measures we recorded encompasses hazard ratios (HRs), odds ratios (ORs), risk ratios (RRs), regression coefficients (β), and scores from statistical hypothesis tests (e.g. t , z).

In instances where a publication reported multiple effect measures for the same EAA-factor association, we included each measure in our final data compilation, allocating them to separate entries to ensure comprehensive data accessibility and to mitigate reporting bias. For publications missing certain data, we left the corresponding entries in the table blank, refraining from making assumptions about the missing information.

The systematic review included two types of studies: those that used existing epigenetic clocks to study EAA-factor associations, and those that introduced novel epigenetic clocks. For the latter, we additionally reviewed the publications to extract clock development and performance-related metrics, which were recorded in a separate table (see Table A.2). While the EAA-factor association studies were subject to exclusion criteria, all publications introducing novel DNAm-based clocks were reviewed, regardless of their characteristics or performance.

2.3. Data categorisation and analyses

Upon completing the reviewing procedures, we thoroughly checked the resulting data table to ensure consistency and integrity. We then classified the data by grouping related factors (“Factor” column) into broader categories. Specifically, we organised factors into factor groups (“Factor Group” column) based on common characteristics. We further grouped these factor groups into factor categories (“Factor Category” column) that shared high-level similarities. Finally, we assigned each factor category to one of five major topics (“Topic” column), thus creating a four-level hierarchy for the data:

1. **Factors** - The individual data elements
2. **Factor groups** - Groups of related factors
3. **Factor categories** - Broader categories encompassing multiple factor groups
4. **Topics** - High-level categories comprising multiple factor categories

This hierarchical classification system enabled structured analysis and interpretation of the data aligned with key areas of interest.

The performed exploratory data analyses included calculating descriptive statistics (mean, spread, frequencies, etc.), and data visualisation. All the statistical data analyses were performed using R software version 4.3.1, the figures were prepared using R libraries ggplot2 (Wickham, 2016), maps(code by Richard A. Becker et al., 2023), and ggnanogram(Maag, 2018).

2.4. Meta-analysis

While conducting meta-analysis we mainly followed the procedures described in (Harrer et al., 2021). The dataset for meta-analyses was created by concatenation of our data with the table produced in the Oblak et al. systematic review (Oblak et al., 2021). The meta-analysis of

the EAA-factor associations was based on the “Factor Group” data. For the data added from the systematic review (Oblak et al., 2021), the factors were grouped in line with our data classification. The combined dataset was split into two subsets for the available effect size measures: hazard ratio data and regression coefficients data. Both parts of the data were further filtered to satisfy the following criteria:

1. presence of a 95 % confidence interval (95 % CI) or/and standard error (SE);
2. this combination of tissue-clock-factor was present in at least three papers.

Hazard ratios were log-transformed with the corresponding 95 % confidence intervals, and standard error was calculated for both subsets.

The `rma.mv()` function in the `metaforR` library (Viechtbauer, 2010) was used to fit a meta-regression linear mixed-effects model based

on residual maximum likelihood (REML) method (Patterson and Thompson, 1971). This function is a method for conducting multilevel meta-analyses, it was designed to deal with complex data structures which might involve multiple effect sizes per study, which is the case with our data. In particular, we used the parameter `random` to account for multiple effect sizes nested within the same study. The outcomes of the meta-analysis models were visualised using enhanced forest plots (rainforest plots) and enhanced funnel plots (sunset plots) implemented in the `metavizR` library (Dewey, 2019).

All data manipulations, statistical analyses, and visualisations were performed using R software version 4.3.1. Significance threshold for p -value is $\alpha = 0.05$.

2.5. Lookup table

The lookup table web application was developed to provide an

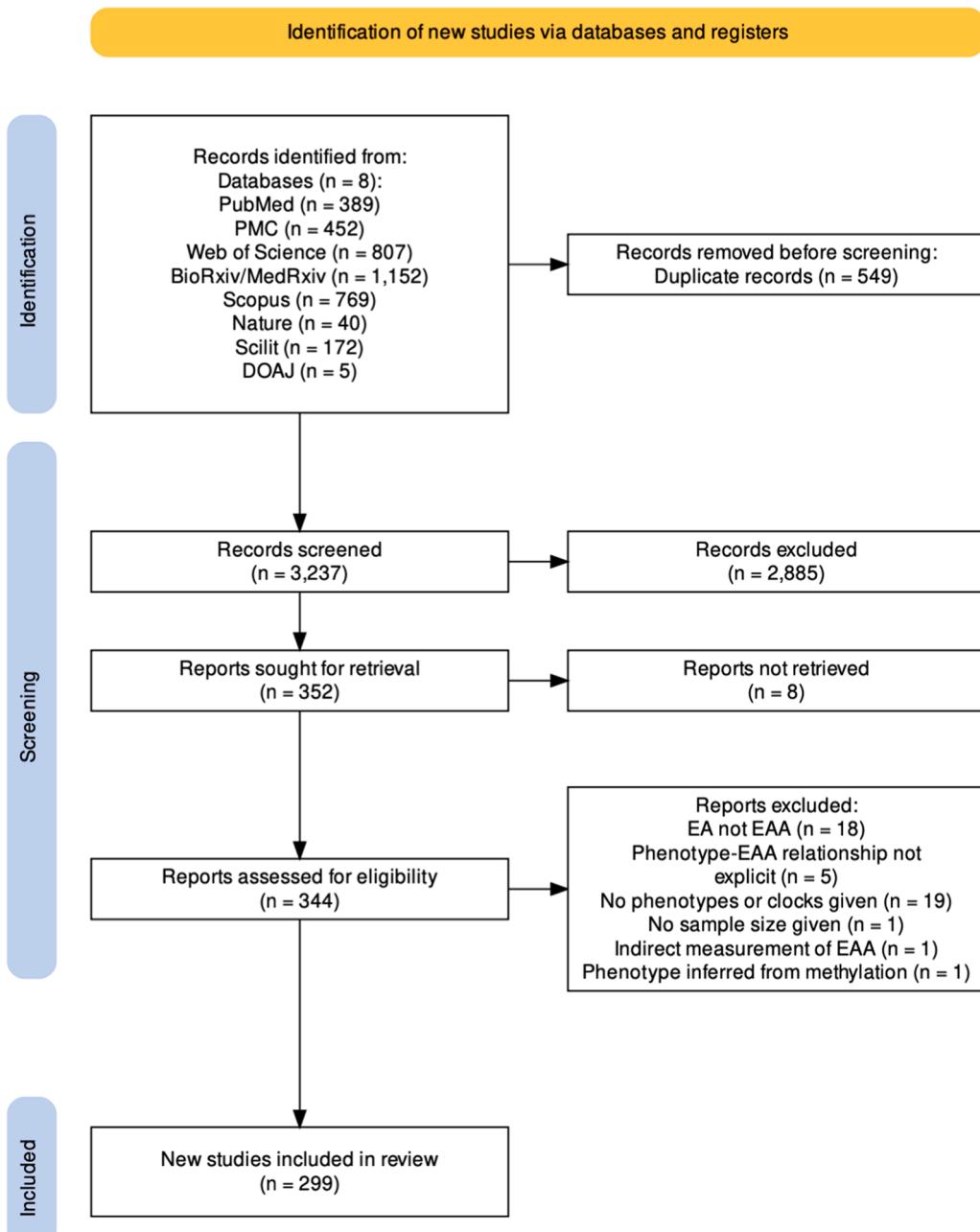


Fig. 1. PRISMA diagram of the publication search and selection.

interactive way of exploring the resulting review table, it works exclusively as a client-side-only application without the need to have any back-end server, making it completely host-independent.

The application utilises a combination of HTML(WWHATWG, 2024), CSS(W3C, 2024), and JavaScript(ECMA International, 2024) to render the review table. The DataTables(Ltd., 2024) library, a jQuery plugin, is integrated to enhance the table with features such as sorting, searching, and pagination. This library simplifies the process of handling large datasets by providing built-in functions for data manipulation. Some user interface features like row highlighting were implemented using pure jQuery(jQuery Foundation, 2024).

The application also uses the Bootstrap 5 framework (Bootstrap Team, 2024) and Bootstrap Toggle library (Bootstrap Toggle Contributors, 2024) for responsive design, ensuring that the application is accessible across various devices and screen sizes. To populate the table with data, the application employs the PapaParse(Holt, 2024) library to parse csv data and insert it into the DataTable. This approach allows for efficient handling of the dataset and dynamic updating of the table content as users interact with the application.

All code and csv table together with instructions to run it locally is available at <https://github.com/ucl-medical-genomics/TEAPEE>.

3. Results

3.1. Study selection and data extraction

The initial literature search returned 3786 publications, with 3237 unique values after the removal of duplicates. After the first screening, 2893 publications were removed due to not satisfying the eligibility criteria, and 344 papers were brought forward for review, see Fig. 1 for the summary of the selection process. The full table of collected variables is given in Supplementary Information file 1.

3.2. Studies overview/summary

The resulting review table includes 299 publications and consists of 11,028 rows of the reported EAA-factor associations' effect sizes (median (range) = 16 (1–836) rows per publication). All missing values in key columns (such as sample size, tissue, sex, ethnicity, and cohort details) were subject to an additional search. The resulting review table and the table with the references for the reviewed studies are provided in the Supplementary Materials files 1 and 4 respectively. A total of 1050 unique items are present in the "Factor" column and were assigned to

310 factor groups ("Factor group" column) as well as classified into 40 broader categories, which are contained in the column "Factor category". Note that some factors were classified as belonging to more than one category. In this case, the corresponding value in "Factor category" is "Multiple_groups", and the relevant categories are given in columns "Multiple_cat1", "Multiple_cat2", etc. For example, the factor "Cancer (colorectal)" was classified into the "Multiple_groups" category, with "Multiple_cat1" = "Cancer" and "Multiple_cat2" = "Gastrointestinal". The factor categories were then further classified into five wider topics named "Physical health", "Mental health", "Lifestyle", "Environment", and "Other", which form the top level of our factor classification. The entries from "Multiple_groups" were assigned to one of the topics based on the consensus of the multiple categories. The schematic overview of the top two levels of the classification ("Topic" and "Factor category") is presented in Fig. 2.

The published results were obtained using 53 different epigenetic clocks, with 6 clocks spanning more than 86 % of all EAA-factor associations covered in this review (Horvath (25.8 %), GrimAge (20.1 %), Hannum (15.5 %), PhenoAge (14.3 %), DunedinPoAm (6 %), SkinBlood (4.5 %)). A distribution of the EAA-factor association counts is presented as a 2D-histogram plot in Fig. 3A. Socioeconomic (11.2 %), Cognitive (8.6 %), Physical abilities/activity (7.2 %), Environment (6.6 %), Mental Health (MH, 5.6 %), Body Composition (5 %), Psychosocial (4.2 %), Cardiovascular (4.2 %), Smoking (3.3 %) and Healthspan/Lifespan/Mortality (3.2 %) are the top studied factor categories (not taking into account the Multiple_groups category, which was assigned to 17.3 % of associations). More than two-thirds (69.4 %) of the retrieved associations were discovered utilising datasets consisting of both males and females, whilst 23.7 % and 6.4 % of the associations were based on female only and male only data, respectively.

The DNAm profiles used in publications reviewed here were obtained from 13 different tissue types, nearly 93 % of the produced results were based on "liquid biopsies" which encompass blood (86.4 %) and saliva (6.4 %). The distribution of tissues featured in our systematic review is visualised using barplot and anatograms for both male and female human bodies, as shown in Fig. 3B. The data which was used in the publications covered by this review mostly originated from the established cohorts ($n = 148$ studies), active recruitment of study participants ($n = 76$ studies), clinical trials ($n = 6$ studies) as well as from the NCBI Gene Expression Omnibus (GEO, $n = 12$ studies) and the Cancer Genome Atlas (TCGA, $n = 1$ study) repositories. These data originated from 51 different countries/regions. The most utilised established cohorts were: Health and Retirement Study (HRS, $n = 10$

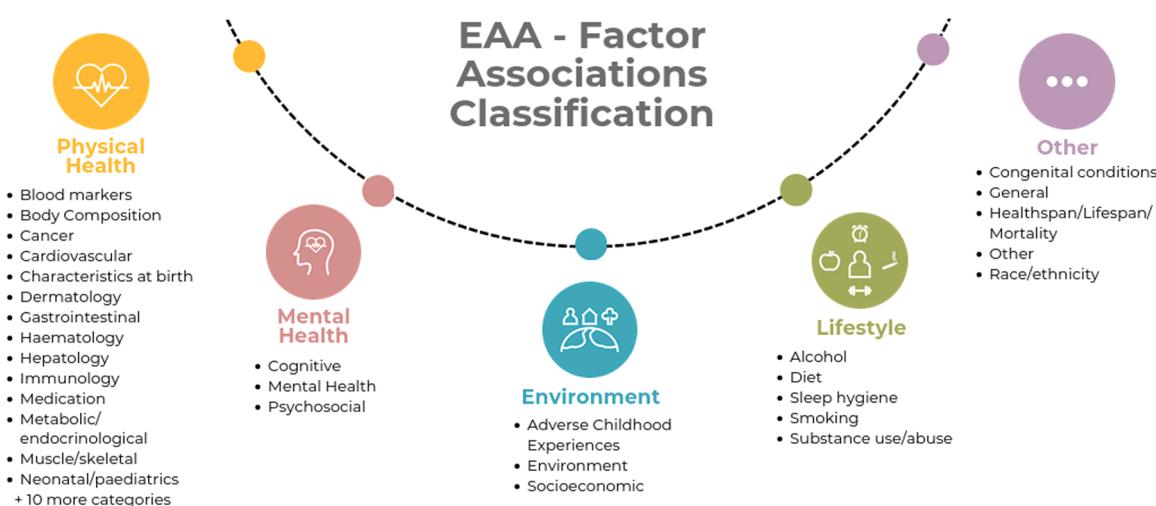


Fig. 2. Overview of the classification. The schematic shows the top two levels of the classification - Topics and Factor categories. The hidden "+10 categories" in the Physical Health topic are: Neurology, Obstetrics/gynaecology, Pain, Physical abilities/activity, Renal, Respiratory, Rheumatological, Viral, Vision, and Vitamin supplements.

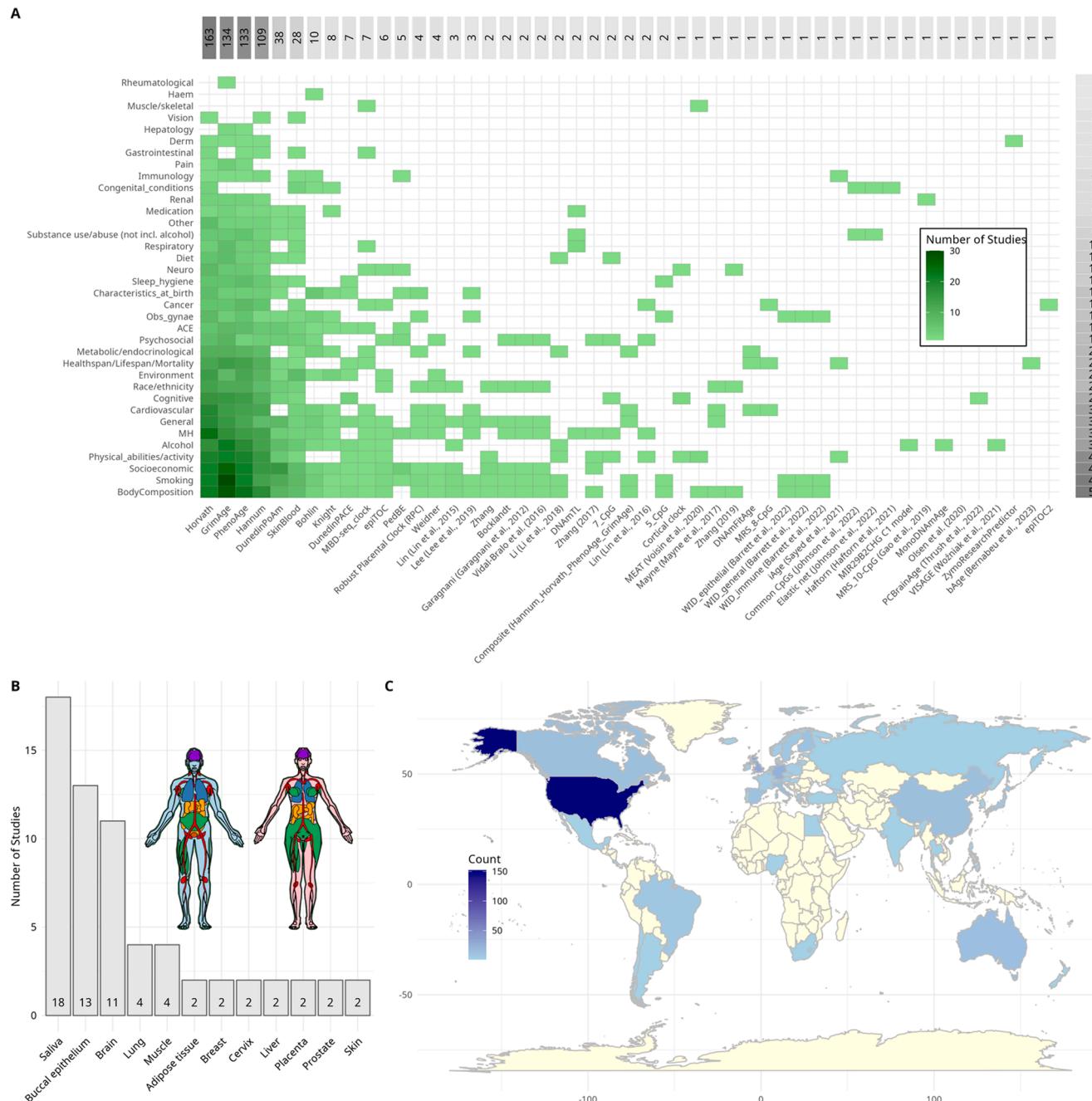


Fig. 3. Summary of the reviewed publications. (A) Distribution of the EAA-factor categories associations among the studies. The main heatmap illustrates the frequency of unique studies for each EAA-factor category pairing, with darker shades indicating a higher number of studies. The top panel displays the total number of unique studies associated with each clock, ordered by frequency. The right panel shows the total number of unique studies associated with each factor category, also ordered by frequency. This combined visualisation highlights the prevalence of study focus within the clock-category matrix and identifies the most studied clock and category elements. “Multiple groups” category is not included in this plot. (B) Bar plot of the tissue distribution across the publications and anatogram displaying the tissues used in the studies of EAA-factor associations. Blood data were featured in $n = 243$ (81.27 %) studies (not present on the bar plot). The height of the bar and the number inside it reflect the count of the unique related publications. (C) World map plot, displaying data sources distribution by country of origin with darker shades of blue colour indicating a higher number of data sources. Data from countries filled with light-yellow colour are not featured in any of the reviewed publications. This map plot is based on the data from Table A.3.

studies), Coronary Artery Risk Development in Young Adults (CARDIA, $n = 8$ studies), and Finnish Twin Cohort (FTC, $n = 7$ studies). The geographical distribution of the data sources by country is summarised in Fig. 3C and Table A.3.

Another table summarising newly developed clocks is given in Appendix A, see Table A.2. The data was retrieved from 44 papers and included 101 clocks, with more than half of those trained on blood and saliva DNA profiles. The majority of novel clocks were developed

based on human (84 %) or mouse (8 %) tissue data, and used chronological age (88 %) or all-cause mortality (3 %) as the training phenotype.

3.3. Meta-analysis

EAA-phenotype association meta-analyses were performed separately for studies that reported hazard ratios (HRs) (39 studies, of which

16 were added from the Oblak *et al.* systematic review [Oblak et al., 2021](#), and regression coefficients (168 studies, of them 52 were added from [Oblak et al., 2021](#)) as the effect measures. Meta-analyses were performed using multilevel mixed-effect models for each combination of tissue-clock-factor groups. In total, 13 (for HR subset) and 101 (for the regression coefficient subset) unique tissue-clock-factor group combinations were included in meta-analyses, and all of them except for one were based on blood DNAm data. Of those, three factor groups - Cancer, Cardiovascular disease and Mortality - were featured in the HR part for four clocks (Horvath, Hannum, PhenoAge and GrimAge) along with

GrimAge-Dementia, and 30 unique factor group-tissue combinations (29 of them are based on data from blood DNAm and one (SES) on saliva) involving seven clocks (Horvath, Hannum, PhenoAge, GrimAge, DunedinPoAm, Bohlin, SkinBlood) were present in the regression coefficients part. The presence of the EAA-factor association in meta-analysis is summarised in the heatmap on [Fig. 4A](#). The most frequent factor groups featured in meta-analysis in combination with at least 5 epigenetic clocks are “Smoking”, “BMI”, “SES”, “Alcohol consumption”, “Childhood adversity”, “Education”, and “Sex”.

The overview of the meta-analyses outcomes is visualised using the

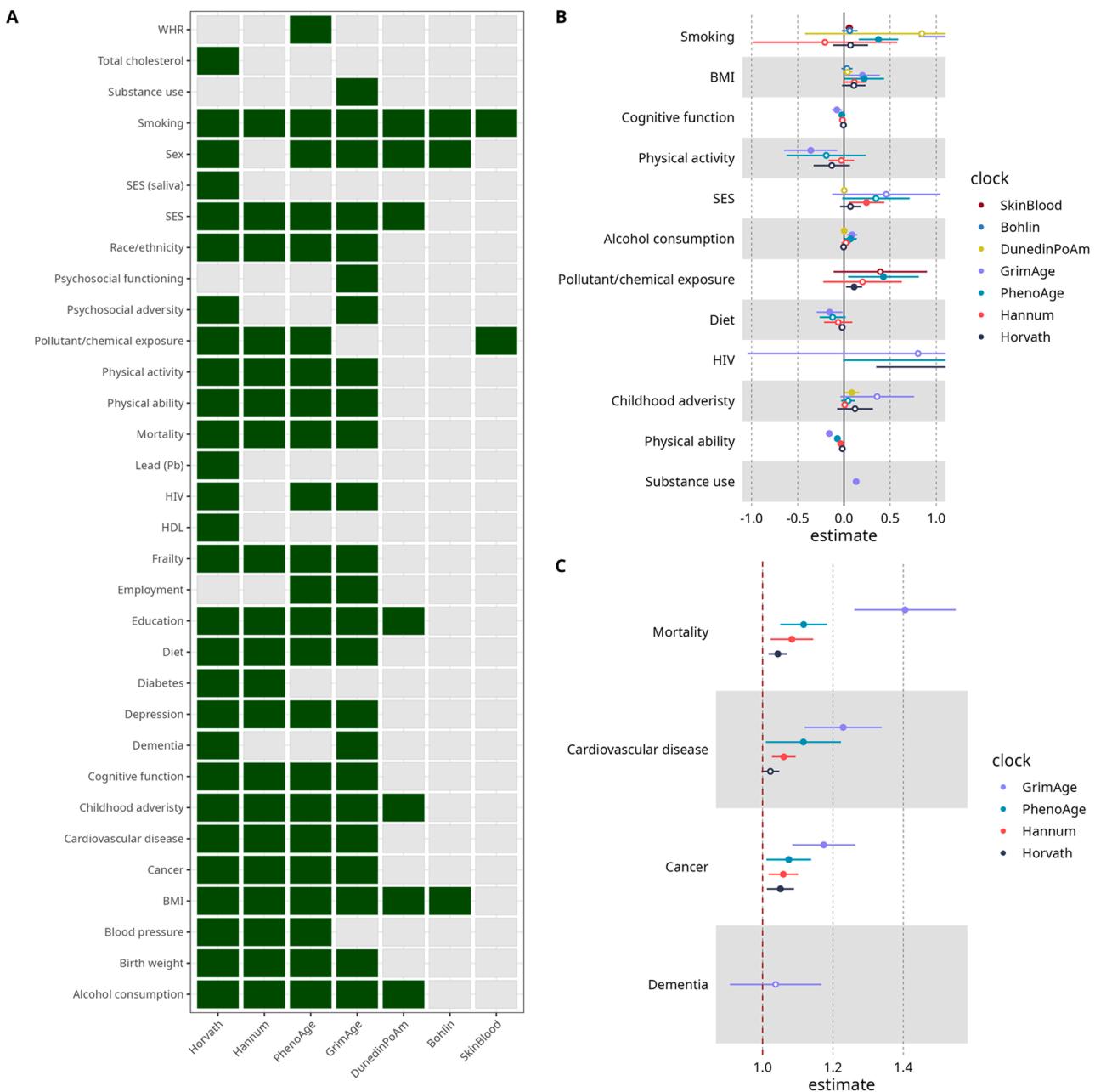


Fig. 4. Overview of the multilevel meta-analysis model outcomes. (A) Heatmap of Factor-Clock Pair Presence. Each cell represents the presence (green) or absence (white) of a specific factor-clock pair within the dataset. The x-axis categorises the unique ‘clock’ variables, while the y-axis lists the distinct ‘factor’ variables. (B) Forest plots of the EAA-factor associations effect sizes based on the regression coefficients data, which visually represents the magnitude, direction, and precision of the regression coefficients. Only the factors with significant results for at least one clock are included. Note that bars for five factor-clock associations go beyond the displayed area: GrimAge smoking ($\beta = 2.122, p < 0.01$) and HIV ($\beta = 0.803, p = 0.39$), DunedinPoAm smoking ($\beta = 0.844, p = 0.19$), PhenoAge HIV ($\beta = 3.633, p = 0.05$), and Horvath HIV ($\beta = 1.701, p = 0.01$). Based on the data from [Table A.5](#). (C) Forest plots of the EAA-factor associations effect sizes based on the hazard ratio data, which quantify how much the risk of outcomes like mortality or cancer incidence changes per unit increase in EAA. A vertical dashed line marks the line of no effect $x = 1$. Based on the data from [Table A.4](#).

forest plots (see Fig. 4C for HRs and Fig. 4B for the regression coefficients outcomes). In the latter, we only included factor groups that demonstrated statistically significant ($p < 0.05$) relationships with at least one type of epigenetic clock, aiming to make the visualisation more comprehensible.

The model coefficients for all the EAA-factor group associations included in the meta-analysis are presented in Tables A.4 and A.5 for HRs and regression coefficients subsets respectively. These tables also contain Cochran's Q test outcomes to assess heterogeneity among the studies, along with the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC) which could be used to compare the goodness of fit. Meta-analysis returned significant estimates for 11 factor-clock associations in the HR subset, and 21 in the regression coefficient subset. To summarise the outcomes of individual studies and assess the publication bias we generated enhanced forest and funnel (tipi-shaped) plots for each tissue-clock-factor group combination. The plots for meta-analysis outcomes for all EAA-factor group combinations are provided in the Supplementary Materials as two separate pdf files for HR and regression coefficients subsets (see Supplementary Information files 3). As an example, Fig. 5 provides funnel and forest plots (panels A and B respectively) for the meta-analysis outcomes of the associations of

cardiovascular disease (CVD) risk with GrimAge EAA calculated based on blood DNAm profiles. In particular, in this figure, one can observe a positive association of the CVD HR with the increased EAA.

3.4. Tracker of EAA Associations with Phenotype & Environmental Exposure (TEAPEE) web table

Tracker of EAA Associations with Phenotype & Environmental Exposure (TEAPEE) is an interactive web platform for users to explore the associations between epigenetic clocks and various factors as presented in the main review table (see Supplementary Information file 1). The application's interface is centered around a dynamic table that allows users to sort, search, and filter the data by columns such as "Factor Category", "Factor Group", "Clock", and others. In addition, text search is provided over the entire table.

Users can interact with TEAPEE through checkbox lists and input fields, enabling them to refine their search and focus on specific subsets of the data. For instance, the application allows filtering by categories, searching for specific terms, and setting minimum and maximum values for numerical data such as sample size “N total”.

The application also provides visual cues, such as row highlighting

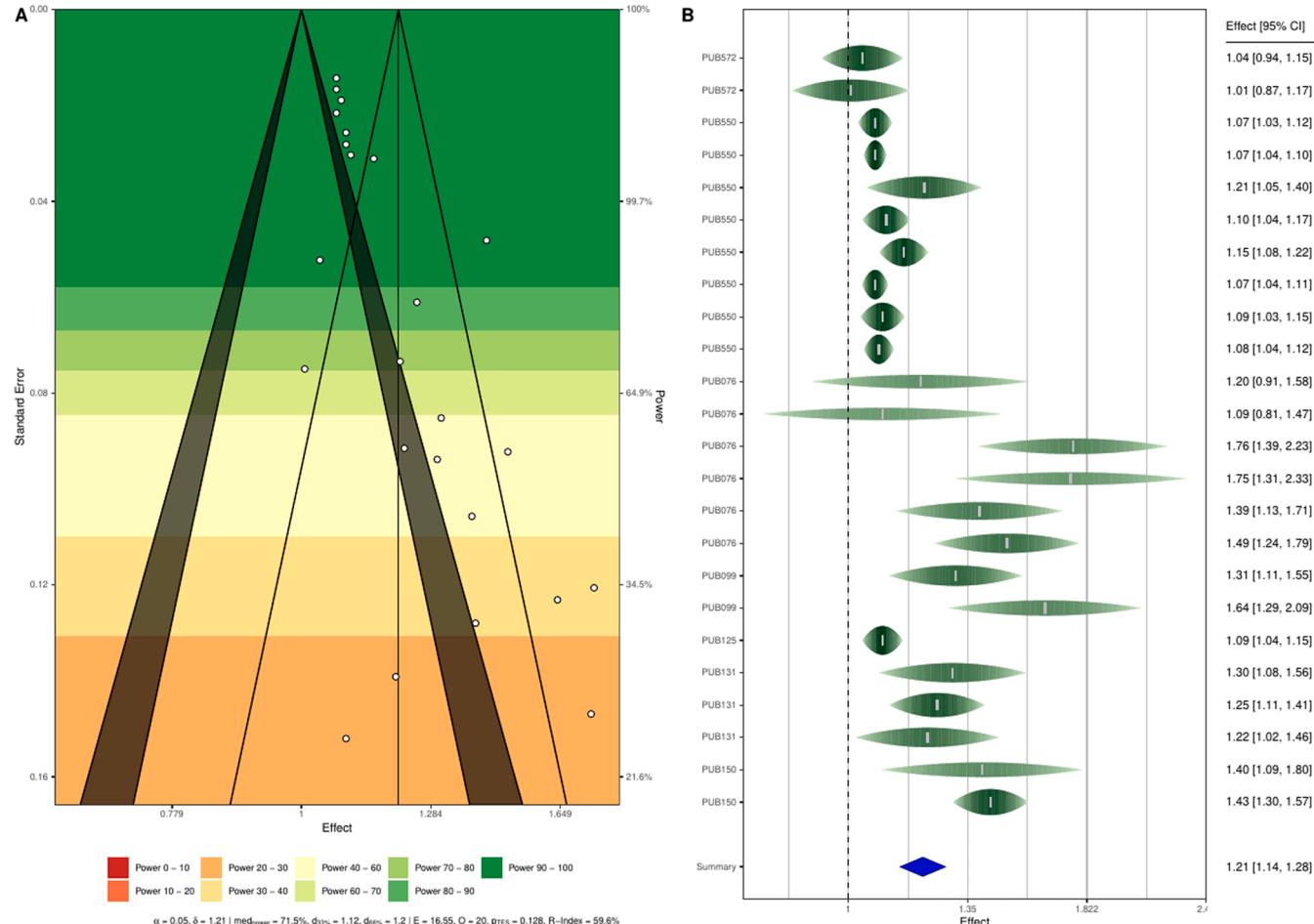


Fig. 5. Meta-analysis of the associations of cardiovascular disease (CVD) risk with GrimAge EAA calculated based on the blood DNAm profiles. (A) Power-enhanced funnel plot (sunset plot). For the given effect size and standard error the power is calculated from the two-sided Wald test and the meta-analytic summary effect as the assumed true effect, and the coloured regions shown in the funnel plot indicate different levels of power. The power-related statistics provided at the bottom of the funnel plot are the median power of all studies, the true effect size necessary such that the median power of the studies would have been 33 % or 66 %, results of a test of excess significance proposed in (Ioannidis and Trikalinos, 2007), the *R*-index for expected replicability as described in (Anusic and Schimmacik, 2016). (B) Rainforest plot. The y-axis represents the publication ID, which is a unique identifier assigned to each study included in the meta-analysis. The width of each raindrop is identical to the width of the confidence interval and the height of each raindrop is scaled concerning its relative meta-analytic weight considering all studies. Duplicate publication IDs appear when a study provided more than one effect estimate for the same EAA-factor association (e.g. when several data subsets were considered separately or when various adjustments were applied).

on hover, to assist users in navigating the data. Additionally, links to the original papers are embedded within TEAPEE, offering direct access to the source material for further reading.

The TEAPEE table application can be accessed at <https://ucl-medical-genomics.github.io/TEAPEE/>.

4. Discussion

Our systematic review of 299 publications on EAA-factor associations based on DNAm from human tissue samples has provided a comprehensive picture of the current state of research in this field. The review was inspired by the work of (Oblak et al., 2021), and aimed to collate and analyse the vast and sometimes contradictory findings reported in the literature. The interactive web table TEAPEE was developed to assist researchers in planning and conducting research on EAA associations with various phenotypes or exposures.

4.1. Clocks

The review underscored the prominence of six epigenetic clocks, namely SkinBlood, DunedinPoAm, PhenoAge, Hannum, GrimAge, and Horvath, in 88 % of the EAA-factor associations, highlighting their value as biomarkers across a diverse array of factors. However, the predominance of these six clocks might also be attributed to their longevity in the field. Older clocks, such as Horvath and Hannum, have been extensively utilised since their inception, contributing to their notable representation in the literature. Conversely, newer clocks like Zhang's EN and BLUP algorithms (Zhang et al., 2019), despite their promising outcomes, may not have achieved comparable exposure or usage yet (Milicic et al., 2022).

Moreover, it is crucial to acknowledge that many epigenetic clocks, including some of the six highlighted, were initially developed based on blood DNAm data, which may limit their direct applicability to other tissues. This specificity raises questions about their generalisability and effectiveness in non-blood tissues. On the other hand, the existence of multi-tissue clocks, such as the Horvath clock (Horvath, 2013) or AltunAge (de de Lima Camillo et al., 2022), trained across various tissues and cell types, demonstrates an effort to create more universally applicable ageing biomarkers. They were designed to predict chronological age across the lifespan, signify a step towards overcoming the limitations posed by tissue-specific clocks, and enhancing our understanding of biological ageing processes across various tissues.

Therefore, while the current dominance of the six aforementioned clocks in EAA-factor associations may reflect their historical usage and the datasets they were trained on, the development and increasing recognition of multi-tissue clocks suggest a potential shift in the landscape. As these more versatile clocks gain traction and are applied across a broader range of tissues, we may witness a change in the epigenetic clocks most frequently utilised in ageing research.

4.2. Sex

The data used in the publications showed that a significant proportion of the associations (69 %) were identified using datasets that included both males and females. We would like to point out that studies involving both genders are suitable for investigating the potential influence of gender on these associations. Indeed, research has shown (Kankaanpää et al., 2022) that there are sex differences in biological age measured by novel epigenetic clocks, with women generally living longer than men. However, the presentation of many diseases is directly influenced by biological factors as well as by gender through multiple routes, which are likely to contribute to the observed sex gaps in mortality and life expectancy (Kankaanpää et al., 2022).

More than 1.5 % of all associations were observed based on DNA methylation (DNAm) profiles of female-specific tissues, whilst < 0.1 % associations were based on the male reproductive system tissue DNAm.

This could be because women's fertility and sexual development traits may have implications for DNA methylation patterns (Zhang et al., 2023). However, it is important to note that gender or sex were not reported for all participants in some studies, making it challenging for researchers to judge whether the clock is likely to accurately estimate epigenetic age in populations with different sociodemographic characteristics (Watkins et al., 2023).

4.3. Tissue

The DNAm profiles used in the publications were obtained from 13 different tissue types, with nearly 93 % of the results based on "liquid biopsies" - blood (86.4 %) and saliva (6.3 %). This highlights the importance of these easily accessible tissue types in epigenetic research. However, it is important to keep in mind that the performance of epigenetic clocks can vary between tissues (Grodstein et al., 2021; Sillanpää et al., 2021). For example, a study on blood and skeletal muscle ageing (Sillanpää et al., 2021) determined by epigenetic clocks found that while these clocks perform well in estimating chronological age in muscle tissue, there are tissue-specific differences in ageing rates and associations with physical activity and functioning.

Research indicates that the pace of epigenetic ageing varies across different racial and ethnic groups as well as between genders (Horvath et al., 2016). This variability underscores the importance of selecting the most appropriate epigenetic clock based on the specific tissue type and demographic group being studied (Fang et al., 2023). Consequently, it is essential to account for the unique dynamics of tissue-specific ageing when utilising epigenetic clocks in research.

4.4. Factor classification

In the course of this systematic review, we developed a four-level factor classification system that we believe will be a valuable tool for future research in this field. This classification system was designed to categorise the various factors that have been studied in relation to epigenetic clocks, providing a structured and systematic approach to understanding the diverse range of variables that can influence epigenetic ageing. The most studied factor categories in our review were Socioeconomic, Cognitive, Physical Abilities/Activity, Environment, Mental Health (MH), Body Composition, Psychosocial, Cardiovascular, Smoking, and Healthspan/Lifespan/Mortality. These categories represent a broad range of social, environmental, physiological, and cognitive factors, reflecting the multifaceted nature of epigenetic ageing.

The use of this classification system has several potential benefits for future research. Firstly, it provides a clear and organised framework for understanding the diverse range of factors that have been studied in relation to epigenetic clocks. This can help researchers to identify gaps in the literature and to formulate research questions for future studies. Secondly, the classification system can facilitate comparisons between studies, as it provides a standardised way of categorising the factors that have been investigated. This can enhance the synthesis of findings across studies and support the development of a more comprehensive understanding of the relationships between these factors and epigenetic ageing.

4.5. TEAPEE web table

The classification system is presented in the TEAPEE (Tracker of EAA Associations with Phenotype & Environmental Exposure) - lookup table that allows users to search for relevant publications based on various parameters, including epigenetic clock, sample size, tissue, and cohort. This interactive tool provides a user-friendly way to navigate the extensive body of literature on this topic, making it easier for researchers to find studies that are relevant to their specific interests or research questions.

While our work was not implemented as a formal living systematic

review, we fully recognise the rapidly evolving nature of epigenetic clock research and the potential benefits of continuous updates. The development of our TEAPEE table tool was partly motivated by the community's need for an updateable resource in this field. TEAPEE's flexible structure allows the addition of new data in a relatively easy way. Its code is publicly available on GitHub, which enables community contributions. Although resource constraints currently prevent us from the regular formal updates commitments, we believe that TEAPEE provides a strong foundation for future expansions, for example, through periodic reviews or community-driven efforts. We encourage researchers to utilise and contribute to TEAPEE, which would aid in keeping this valuable resource current and relevant in this fast-paced field.

4.6. Meta-analysis

In our systematic review, we performed a meta-analysis on the combined dataset, which included our data and the data from (Oblak et al., 2021). This meta-analysis was conducted for each EAA-factor association that was featured in three or more publications, using the `rma` package in the `metafor` library to include random effects. The results of this meta-analysis revealed significant associations for some EAA-factor pairs, providing valuable insights into the relationships between these factors and epigenetic age acceleration.

As expected, most of the performed meta-analysis outcomes are in line with those reported in (Oblak et al., 2021). At the same time, several EAA-factor associations reported in (Oblak et al., 2021) were not included in our meta-analyses due to the inclusion criteria reported in section 2.4, which differ from those used in (Oblak et al., 2021). For example, COPD and lung function results were not meta-analysed in this study as all of them came from less than three different publication.

In addition, the use of random effects in our meta-analysis acknowledges the likelihood of heterogeneity among the included studies. This heterogeneity could be due to differences in study design, sample characteristics, or measurement methods among the studies. While the random effects model can account for some of this heterogeneity, it is important to interpret the results of the meta-analysis in light of these potential sources of variation.

Our systematic review and meta-analysis revealed several aspects of human health that showed consistent associations with EAA across multiple studies and epigenetic clocks. Cardiovascular health and mortality risk emerged as factors with robust positive associations with EAA, suggesting that accelerated epigenetic ageing might be a valuable predictor of cardiovascular disease and overall longevity. Several lifestyle factors (e.g. smoking and alcohol consumption) were also consistently positively associated with EAA. This could be viewed as a confirmation of the impact of these behaviours on the biological ageing processes. At the same time, less consistency was observed in associations between EAA and factors such as diet, education, and cognitive function. The differences in study design, population characteristics, or the specific clocks used could explain this. Further research is needed to clarify the complex relationships between EAA and various health-related factors and explore the potential for using EAA as a predictive or classifying tool in clinical settings.

4.7. Designing EAA association studies and interpreting the results

When designing EAA association studies, researchers should consider the following points, which are based on the strengths and drawbacks identified in the process of this systematic review. In particular, the heterogeneity in clocks' training datasets, methodologies, and tissue specificity could be the major sources of variability.

- Epigenetic clock selection should be based on the focus of the study such as specific tissue type, age range, and health outcomes.

- The methods of sample collection and processing should be compatible with the requirements of the chosen clock(s) (including factors such as DNA extraction methods and methylation profiling techniques).
- Interpretation of the results should take into account the limitations of the specific clock used, such as its training data and potential biases. For instance, clocks trained primarily on data from Caucasian populations may have limited generalisability to other ethnicities. Where possible, consideration should also be given to cell-type heterogeneity, feature selection, reverse causation and integrative analysis with other genetic and epigenetic data (reviewed in Teschendorff and Relton, 2018).
- Comparison of epigenetic age estimates across studies should be done with caution, as these estimates may not be directly comparable, particularly if studies are based on different clocks or tissue types. Differences in clock algorithms and tissue-specific ageing rates could affect the interpretation of the results.
- Reporting the outcomes requires detailed information on the clock used, sample characteristics, and any adjustments made for potential confounders. This contributes better comparison and replication of findings across the studies.
- EAA should be interpreted as a biomarker of biological ageing rather than a definitive measure of an individual's health status or future disease risk. EAA should be considered together with other relevant factors and biomarkers.

By following these guidelines, the design of future studies would be more robust and the reported results clearer and more informative.,

4.8. Limitations

This systematic review, while comprehensive, is subject to several limitations that should be taken into consideration. Firstly, despite our best efforts to ensure accuracy, errors in the reviewed papers and during the data extraction process may have occurred. Each paper was assessed independently by at least two reviewers, and papers that presented ambiguities or complexities were discussed in a group setting to reach a consensus. However, the potential for human error in interpreting and recording details from the papers cannot be entirely eliminated. This could lead to inaccuracies in the information presented in our review, particularly in the web table that catalogues paper details and results.

Moreover, our meta-analysis was constrained to EAA-factor associations featured in three or more publications. This criterion was established to ensure that the meta-analysis was based on a robust dataset. Nonetheless, it also means that associations reported in fewer publications were excluded. This approach may overlook emerging or less-studied associations that could be of significance, potentially biasing our understanding of EAA-factor relationships towards those more frequently studied.

Publication bias represents another significant limitation. The funnel plots generated in our meta-analysis suggested that larger effect sizes were predominantly reported by underpowered studies, hinting at the possibility of publication bias. This bias arises when studies with significant, positive results are more likely to be published than those with non-significant or negative results, leading to an overrepresentation of positive findings in the literature. Such bias can skew the meta-analysis results, overestimating the true effect sizes of EAA-factor associations. We did not perform a targeted search for unpublished studies, such as conference abstracts, due to their limited data availability. While we included preprints from bioRxiv, the exclusion of other unpublished studies may have introduced some publication bias into our results. However, given the comprehensive nature of our search strategy and the strict data requirements of our analyses, we believe that the impact of this potential bias is likely to be minimal. Future studies could explore ways to incorporate data from a wider range of unpublished sources, while ensuring the robustness and reliability of the analyses.

Additionally, other forms of bias, such as selection bias, measurement bias, and reporting bias, may also affect the outcomes of this systematic review and meta-analysis despite our best efforts to avoid them. Selection bias could occur if the studies included are not representative of all relevant research, potentially due to exclusion criteria or limited availability of publications. Measurement bias may arise from inconsistencies in how variables are defined and measured across the different studies. Reporting bias could result from the selective disclosure of results within individual studies, where studies with positive or statistically significant findings are more likely to be published compared to those with negative or non-significant results.

In conclusion, while this systematic review provides valuable insights into the associations between various factors and epigenetic age acceleration, it is important to interpret the findings in light of these limitations. Future research should aim to address these issues by including a broader range of studies, employing rigorous methods to minimise errors and biases, and exploring innovative approaches to mitigate the impact of publication bias. The new web tool TEAPEE will enable all researchers and the wider community to easily find, filter and assess ageing-related epigenetic associations relevant to human health and well-being.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of the manuscript, the authors used

Perplexity AI to enhance the readability and clarity of the text, as well as to reduce grammatical errors. This tool assisted in ensuring that the manuscript's statements were presented clearly and understandably, facilitating better communication of the research findings. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of Competing Interest

None.

Data Availability

The links to data and code are available in the manuscript.

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Appendix A. Additional information

Table A.1
Search terms.

| Database name | Search terms used | Number of hits |
|---|--|----------------|
| PubMed | (age[Title/Abstract]) OR aging[Title/Abstract] OR biological age[Title/Abstract] OR ageing[Title/Abstract] AND (methylation [Title/Abstract] OR methylated[Title/Abstract] OR methylome[Title/Abstract] OR DNAm[Title/Abstract]) AND (epigenetic age OR age estimator OR epigenetic clock OR biological clock OR methylation clock OR age predictor OR age estimation OR age acceleration OR "predict age" OR accelerated age OR "estimate age" OR "Dnam Age" OR "methylation age" OR methylation profile OR model of aging OR aging rate OR ageing rate OR ageing) AND (epigenetic) AND (human OR man OR men OR women OR woman OR male OR female OR males OR females) AND (CpG OR CpGs) | 389 |
| PMC | (age[Title/Abstract] OR aging[Title/Abstract] OR biological age[Title/Abstract] OR ageing[Title/Abstract]) AND ("methylation" OR "methylated" OR "DNAm") AND (human OR humans) AND ("epigenetic age" OR "age estimator" OR "epigenetic clock" OR "biological clock" OR "methylation clock" OR "age predictor" OR "age estimation" OR "age acceleration" OR "predict age" OR "accelerated age" OR "estimate age" OR "Dnam Age" OR "methylation age" OR "methylation profile" OR "model of aging" OR "aging rate" OR "ageing rate" OR "model of ageing") AND "CpG" AND "epigenetic" | 452 |
| Web of Science | ((epigenetic) AND (methylation OR methylated) AND (age estimator OR epigenetic clock OR methylation clock OR age predictor OR age estimation OR age estimation OR age acceleration OR DNAm age OR predict age OR DNAm OR CpG OR CpGs) AND human) | 807 |
| BioRxiv + MedRxiv | for abstract or title "epigenetic, age, cpg, methylation, human" (match any words) and full text or abstract or title "epigenetic, age, cpg, methylation, human, DNA" (match whole all) | 1152 |
| Scopus | ((epigenetic) AND (methylation OR methylated) AND (age estimator OR epigenetic clock OR methylation clock OR age predictor OR age estimation OR age estimation OR age acceleration OR DNAm age OR predict age OR DNAm OR CpG OR CpGs) AND human) | 769 |
| Nature | ((epigenetic) AND (methylation OR methylated) AND (age estimator OR epigenetic clock OR methylation clock OR age predictor OR age estimation OR age estimation OR age acceleration OR DNAm age OR predict age OR DNAm OR accelerated age OR methylation age OR ageing rate OR aging rate OR CpG OR CpGs) AND (human OR humans OR men OR women OR male OR female)) | 40 |
| Scilit Directory of Open Access Journals | For abstract or title "epigenetic, age, cpg, methylation, human" ((epigenetic) AND (methylation OR methylated) AND (age estimator OR epigenetic clock OR methylation clock OR age predictor OR age estimation OR age acceleration) AND (human OR humans OR men OR women OR male OR female)) | 172 5 |

The table contains full lists of search terms used to identify relevant publications in the eight databases, and the number of papers returned by the search.

Table A.2
Summary of the novel epigenetic clocks.

| First Author, Year | Clock name | Species | DNAm profiling technology | n of predictors | Training Phenotype | Tissue(s) | Sample size | R ² | r |
|---------------------|---------------------|--------------|--------------------------------|------------------|-----------------------------------|---------------------------|-------------|----------------|--------|
| Correira Dias, 2021 | BBT-APM | Homo sapiens | Sanger Sequencing | 7 | Chronological age | Blood, bone, tooth | 185 | 0.878 | 0.94 |
| Correira Dias, 2021 | BBT-APM | Homo sapiens | SNaPshot | 3 | Chronological age | Blood, bone, tooth | 168 | 0.847 | 0.922 |
| Sukawutthiya, 2021 | | Homo sapiens | Pyrosequencing | 2 | Chronological age | Blood | 100 | 0.733 | 0.865 |
| Fokias, 2023 | | Homo sapiens | Pyrosequencing | 5 | Chronological age | left hand finger nail | 99 | | |
| Fokias, 2023 | | Homo sapiens | Pyrosequencing | 5 | Chronological age | right hand finger nail | 100 | | |
| Fokias, 2023 | | Homo sapiens | Pyrosequencing | 7 | Chronological age | left foot toe nail | 99 | | |
| Fokias, 2023 | | Homo sapiens | Pyrosequencing | 5 | Chronological age | right foot toe nail | 98 | | |
| Keerthana, 2021 | | Homo sapiens | Illumina450k and SNaPshot | 6 | Chronological age | Saliva | 45 | | |
| Funayama, 2021 | | Homo sapiens | MiSeq | 4 | Chronological age | Blood | 60 | 0.939 | 0.969 |
| Funayama, 2021 | | Homo sapiens | MiSeq | 4 | Chronological age | Blood | 60 | 0.9395 | 0.9693 |
| Thrush, 2022 | PCBrainAge | Homo sapiens | Illumina450k and EPIC, Elisyum | 15PCs | Chronological age | Post mortem brain tissues | 399 | | 0.62 |
| Thrush, 2022 | DNAmClock Cortical | Homo sapiens | Illumina450k and EPIC, Elisyum | | Chronological age | Post mortem brain tissues | 1047 | | 0.79 |
| Mayer, 2022 | 11CpGs | Homo sapiens | Pyrosequencing | 11 | Chronological age | buccal mucosa | 199 | | |
| Mayer, 2022 | 5CpGs | Homo sapiens | Pyrosequencing | 5 | Chronological age | buccal mucosa | 199 | | |
| Huan, 2022 | | Homo sapiens | Illumina450k | 177 | All-cause mortality | Blood | 10715 | | |
| Huan, 2022 | | Homo sapiens | Illumina450k | 71 | All-cause mortality and CVD Death | Blood | 10715 | | |
| Huan, 2022 | | Homo sapiens | Illumina450k | 54 | Cancer death | Blood | 10715 | | |
| Haoliang, 2022 | | Homo sapiens | Pyrosequencing | 25 | Chronological age | Blood | 240 | 0.99 | |
| Haoliang, 2022 | | Homo sapiens | Pyrosequencing | 25 | Chronological age | Blood | 240 | 0.95 | |
| Haoliang, 2022 | | Homo sapiens | Pyrosequencing | 25 | Chronological age | Blood | 240 | 0.97 | |
| Haoliang, 2022 | | Homo sapiens | Pyrosequencing | 25 | Chronological age | Blood | 240 | 0.97 | |
| Miyano, 2021 | ELF5 Clock | Homo sapiens | Illumina450k | 5 | Chronological age | Breast tissue | 229 | 0.97 | 0.99 |
| Correira Dias, 2020 | | Homo sapiens | Sanger sequencing | 6 | Chronological age | Bone | 29 | 0.925 | 0.957 |
| Correira Dias, 2020 | | Homo sapiens | SNaPshot assay | 2 | Chronological age | Bone | 31 | 0.576 | 0.777 |
| Correira Dias, 2020 | | Homo sapiens | SNaPshot assay | 2 | Chronological age | Teeth | 24 | 0.764 | 0.886 |
| Anaya, 2021 | | Homo sapiens | Pyrosequencing | 5 | Chronological age | Blood | 160 | 0.759 | 0.873 |
| Schmidt, 2021 | | Homo sapiens | Illumina EPIC | 7 | Chronological age | 9 tissues from 13 studies | 301 | 0.43 | |
| Belsky, 2022 | DunedinPACE | Homo sapiens | Illumina EPIC x 450k | 173 | Pace of Aging | Blood | 1037 | | |
| Copeland, 2022 | | Homo sapiens | MBD-seq | 1000–75,000 loci | Chronological age | Blood | 381 | | 0.9 |
| Liu, 2022 | eClock balanced | Homo sapiens | Illumina27k and 450k | 46 | Chronological age | Placenta | 194 | 0.812 | |
| Liu, 2022 | eClock normal | Homo sapiens | Illumina27k and 450k | 39 | Chronological age | Placenta | 194 | 0.823 | |
| Liu, 2022 | eClock bootstrapped | Homo sapiens | Illumina27k and 450k | 48 | Chronological age | Placenta | 194 | 0.807 | |
| Liu, 2022 | eClock balanced | Homo sapiens | Illumina27k and 450k | 66 genes | Chronological age | Blood | 539 | 0.881 | |

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Table A.2 (continued)

| First Author, Year | Clock name | Species | DNAm profiling technology | n of predictors | Training Phenotype | Tissue(s) | Sample size | R ² | r |
|--------------------|--------------------------------------|--|----------------------------------|-----------------|-------------------------------|---------------------------------------|-------------|----------------|--------|
| Liu, 2022 | eClock normal | Homo sapiens | Illumina27k and 450k | 167 genes | Chronological age | Blood | 539 | 0.89 | |
| Liu, 2022 | eClock bootstrapped | Homo sapiens | Illumina27k and 450k | 70 genes | Chronological age | Blood | 539 | 0.867 | |
| Liu, 2022 | eClock balanced | Homo sapiens | Illumina450k | 60 DMRs | Chronological age | Brain | 145 | 0.853 | |
| Liu, 2022 | eClock normal | Homo sapiens | Illumina450k | 61 DMRs | Chronological age | Brain | 145 | 0.942 | |
| Liu, 2022 | eClock bootstrapped | Homo sapiens | Illumina450k | 50 DMRs | Chronological age | Brain | 145 | 0.928 | |
| Pisarek, 2021 | | Homo sapiens | Illumina EPIC | 6 | Chronological age | Semen | 288 | | |
| Horvath, 2022 | pan-tissue opossum clock | opossums | HorvathMammal MethylChip40 | 28 | Chronological age | Ear, Liver, Tail | 100 | 0.85 | |
| Horvath, 2022 | | Red kangaroo, Eastern grey kangaroo, Western grey kangaroo, Red-necked wallaby | HorvathMammal MethylChip40 | 42 | Chronological age | Blood | 59 | 0.91 | |
| Horvath, 2022 | | Red kangaroo | HorvathMammal MethylChip40 | 32 | Chronological age | Blood | 37 | 0.91 | |
| Horvath, 2022 | | Tasmanian devil | HorvathMammal MethylChip40 | 28 | Chronological age | Ear | 41 | 0.83 | |
| Horvath, 2022 | human-opossum clock for CA | Homo sapiens and opossums | HorvathMammalMethylChip40 | 506 | Chronological age | Ear, Liver, Tail | 1466 | 0.98 | |
| Horvath, 2022 | human-opossum clock for relative age | Homo sapiens and opossums | HorvathMammal MethylChip40 | 498 | Chronological age | Ear, Liver, Tail | 1466 | 0.96 | |
| Xiao, 2021 | male clock | Homo sapiens | Pyrosequencing and Illumina EPIC | 25 | Chronological age | Blood | 163 | 0.9522 | 0.976 |
| Xiao, 2021 | female clock | Homo sapiens | Pyrosequencing and Illumina EPIC | 23 | Chronological age | Blood | 141 | 0.949 | 0.9736 |
| Xiao, 2021 | combined clock | Homo sapiens | Pyrosequencing and Illumina EPIC | 45 | Chronological age | Blood | 304 | 0.9289 | 0.9615 |
| Vidaki, 2020 | | Homo sapiens | Illumina450k | 75 | Chronological age | Blood | 1057 | 0.653 | 0.81 |
| Vidaki, 2020 | | Homo sapiens | Illumina450k | 19 | Chronological age | Blood | 1057 | 0.53 | 0.73 |
| Voisin, 2021 | MEAT2 | Homo sapiens | Illumina27k, 450k and EPIC | 156 | Chronological age | Muscle | 1053 | | 0.69 |
| Steg, 2021 | Fetal Brain Clock (FBC) | Homo sapiens | Illumina450k and EPIC, Elisum | 107 | Chronological age (pre-natal) | embryonic and fetal brain and iPSC | 258 | | 0.8 |
| Li, 2022 | | Homo sapiens | Illumina450k | 35 | Chronological age | Blood | 656 | 0.931 | |
| Li, 2022 | | Homo sapiens | Illumina450k | 5 | Chronological age | Blood | 656 | 0.964 | |
| Koop, 2021 | | Homo sapiens | Pyrosequencing | 1 | Chronological age | Buccal cells | 215 | 0.85–0.90 | |
| Trapp, 2021 | scAge Liver model | mouse | RRBS and WGBS | | Chronological age | Liver | 196 | 0.952 | |
| Trapp, 2021 | scAge multi-tissue model | mouse | RRBS and WGBS | | Chronological age | Blood, liver, kidney, muscle and lung | 196 | 0.862 | |
| Trapp, 2021 | scAge blood model | mouse | RRBS and WGBS | | Chronological age | Blood | 196 | 0.363 | |
| Wang, Preprint2022 | CerebellumClock specific | Homo sapiens | Illumina450k and EPIC | 613 | Chronological age | Cerebellum | 752 | | 0.941 |
| Wang, Preprint2022 | CerebellumClock common | Homo sapiens | Illumina450k and EPIC | 201 | Chronological age | Cerebellum | 752 | | 0.868 |
| Wang, Preprint2022 | CortexClock common | Homo sapiens | Illumina450k and EPIC | 201 | Chronological age | Brain tissues | 752 | | 0.865 |
| Pilsner, 2022 | SEA clock CpG | Homo sapiens | Illumina EPIC | 120 | Chronological age | Semen | 379 | | 0.83 |
| Pilsner, 2022 | SEA clock DMR | Homo sapiens | Illumina EPIC | 318 | Chronological age | Semen | 379 | | 0.79 |
| Barrett, 2022 | WID general | Homo sapiens | Illumina EPIC | 759 | Chronological age | Cervical samples | 869 | | 0.95 |

(continued on next page)

Table A.2 (continued)

| First Author, Year | Clock name | Species | DNAm profiling technology | n of predictors | Training Phenotype | Tissue(s) | Sample size | R ² | r |
|--|---|-----------------------|-------------------------------|-----------------|--|--|-------------|----------------|--------|
| Barrett, 2022 | WID-epithelial | Homo sapiens | Illumina EPIC | 759 | Chronological age | Cervical samples | 869 | 0.67 | |
| Barrett, 2022 | WID-Immune | Homo sapiens | Illumina EPIC | 759 | Chronological age | Cervical samples | 869 | 0.58 | |
| Barrett, 2022 | WID-REA | Homo sapiens | Illumina EPIC | 759 | Cancer risk | Cervical samples | 869 | | |
| Aliferi, 2022 | | Homo sapiens | Illumina27k and 450k | 11 | Chronological age | Blood | 200 | | |
| Mammalian Methylation Consortium, Preprint2021 | universal naïve clock (Clock1) | 185 mammalian species | Illumina450k, Mammalian array | | Chronological age | 59 tissue types | 11754 | | > 0.96 |
| Mammalian Methylation Consortium, Preprint2021 | universal relative age clock (Clock 2) | 185 mammalian species | Illumina450k, Mammalian array | | individual age relative to the maximum lifespan of its species | 59 tissue types | 11754 | | > 0.96 |
| Mammalian Methylation Consortium, Preprint2021 | universal log-linear transformed age clock (Clock3) | 185 mammalian species | Illumina450k, Mammalian array | | Chronological age | 59 tissue types | 11754 | | > 0.96 |
| Franzen, 2021 | BA-seq clock | Homo sapiens | BA-seq | 3 | Chronological age | Blood | 40 | 0.88 | |
| Graw, 2021 | PMA-450k | Homo sapiens | Illumina450k | 409 | Post-menstrual gestational age | Buccal mucosa | 542 | 0.93 | |
| Graw, 2021 | PMA-EPIC | Homo sapiens | Illumina EPIC | 522 | Post-menstrual gestational age | Buccal mucosa | 542 | 0.93 | |
| Graw, 2021 | PNA-450l | Homo sapiens | Illumina450k | 303 | Postnatal age | Buccal mucosa | 542 | 0.93 | |
| Graw, 2021 | PNA-EPIC | Homo sapiens | Illumina EPIC | 509 | Postnatal age | Buccal mucosa | 542 | 0.94 | |
| Galkin, 2021 | DeepMAge | Homo sapiens | Illumina27k and 450k | 1000 | Chronological age | Blood | 4930 | 0.96 | 0.98 |
| Freire-Aradas, 2022 | QRNN clock | Homo sapiens | EpiTYPER mass spectrometry | 7 | Chronological age | Blood | 1097 | 0.9669 | |
| Freire-Aradas, 2022 | QRSVM clock | Homo sapiens | EpiTYPER mass spectrometry | 7 | Chronological age | Blood | 1097 | 0.9679 | |
| Gensous, 2022 | Targeted Epigenetic Clock | Homo sapiens | EpiTYPER mass spectrometry | | Chronological age | Blood | 278 | 0.89 | |
| Ying, Preprint2022 | CausAge | Homo sapiens | Illumina450k | | Chronological age | Blood | 2664 | 0.93 | |
| Ying, Preprint2022 | AdaptAge | Homo sapiens | Illumina450k | | Chronological age | Blood | 2664 | 0.83 | |
| Ying, Preprint2022 | DamAge | Homo sapiens | Illumina450k | | Chronological age | Blood | 2664 | 0.84 | |
| Kuzub, 2022 | | Homo sapiens | Pyrosequencing | 4 | Chronological age | Blood | 153 | 0.92 | |
| Kuzub, 2022 | | Homo sapiens | Pyrosequencing | 2 | Chronological age | Blood | 153 | 0.85 | |
| Griffin, Preprint2021 | TIME-Seq rDNA Clock | Mouse | TIME-seq | 232 | Chronological age | Blood | 182 | 0.95 | |
| Griffin, Preprint2021 | TIME-Seq Mouse Multi-tissue clock, | Mouse | TIME-seq | 419 | Chronological age | blood, liver, skin, kidney, and white adipose tissue (WAT) | 860 | 0.89 | |
| Griffin, Preprint2021 | TIME-Seq Mouse Blood Clock | Mouse | TIME-seq | | Chronological age | Blood | 198 | 0.93 | |
| Griffin, Preprint2021 | TIME-Seq Mouse Skin Clock | Mouse | TIME-seq | | Chronological age | Skin | 250 | 0.95 | |
| Griffin, Preprint2021 | TIME-Seq Mouse Liver Clock | Mouse | TIME-seq | | Chronological age | Liver | 208 | 0.94 | |
| Griffin, Preprint2021 | TIME-Seq Human Blood Clock | Homo sapiens | TIME-seq | 405 | Chronological age | Blood | 1056 | 0.96 | |
| Liang, 2022 | MonoDNAMAge | Homo sapiens | Illumina450k and EPIC | 186 | Chronological age | Blood monocytes | 2242 | 0.86 | |
| Adiv, 2022 | Common CpGs clock | Homo sapiens | Illumina450k | 130 | Chronological age | Buccal cells | 431 | 0.95 | |
| Adiv, 2022 | Elastic Net CpGs clock | Homo sapiens | Illumina450k | 335 | Chronological age | Buccal cells | 431 | 0.94 | |
| Ambroa-Conde, 2022 | | Homo sapiens | Illumina450k | 7 | Chronological age | Saliva and buccal swabs | 368 | | |

(continued on next page)

Table A.2 (continued)

| First Author, Year | Clock name | Species | DNAm profiling technology | n of predictors | Training Phenotype | Tissue(s) | Sample size | R ² | r |
|--------------------|------------|--------------|---------------------------|--------------------------------|-------------------------------------|------------------|-------------|----------------|------|
| Bernabeu, 2022 | cAge | Homo sapiens | Illumina450k and EPIC | 2330, incl. squared components | Chronological age | Blood and Saliva | 24674 | | 0.96 |
| Bernabeu, 2022 | bAge | Homo sapiens | Illumina450k and EPIC | 36 | All-cause mortality (time to event) | Blood | 22499 | | |
| Lu, 2022 | GrimAge2 | Homo sapiens | Illumina450k and EPIC | | All-cause mortality | Blood | 13399 | | |
| Woźniak, 2021 | VISAGE | Homo sapiens | Sanger sequencing | 6 | Chronological age | Blood | 160 | | |
| Woźniak, 2021 | VISAGE | Homo sapiens | Sanger sequencing | 5 | Chronological age | Buccal cells | 160 | | |
| Woźniak, 2021 | VISAGE | Homo sapiens | Sanger sequencing | 6 | Chronological age | Bones | 161 | | |

Unless specified, the predictors are CpGs. R^2 and r denote the coefficient of determination and correlation coefficient respectively. Additional information and statistics are provided in the Supplementary Materials file 2.

Table A.3

Distribution of data sources by country.

| Country | Count | Country | Count | Country | Count |
|----------------|-------|-------------|-------|--------------|-------|
| Argentina | 1 | Germany | 24 | Poland | 4 |
| Australia | 13 | Greece | 5 | Portugal | 1 |
| Austria | 3 | Hungary | 1 | Puerto Rico | 1 |
| Belgium | 2 | Iceland | 1 | Qatar | 1 |
| Brazil | 7 | India | 1 | Russia | 3 |
| Canada | 14 | Ireland | 4 | Rwanda | 1 |
| Chile | 1 | Israel | 3 | Singapore | 3 |
| China | 14 | Italy | 15 | South Africa | 3 |
| Costa Rica | 1 | Japan | 8 | South Korea | 1 |
| Croatia | 1 | Lithuania | 3 | Spain | 12 |
| Czech Republic | 3 | Luxembourg | 1 | Sweden | 8 |
| Denmark | 5 | Mexico | 1 | Switzerland | 2 |
| Egypt | 1 | Netherlands | 18 | Taiwan | 4 |
| Estonia | 2 | New Zealand | 1 | Thailand | 1 |
| Finland | 12 | Nigeria | 2 | Turkey | 2 |
| France | 4 | Norway | 4 | UK | 29 |
| Georgia | 1 | Philippines | 2 | USA | 152 |

The table contains countries of data origin, and the count of the publications, that used data from these countries.

Table A.4

Multilevel meta-analysis model results for all EAA-factor associations with available hazard ratio coefficient data.

| Clock | Factor | N papers (rows) | Estimate (95 %CI) | z | p | Cochran Q | AIC | BIC |
|----------|------------------------|-----------------|----------------------|------|------|------------|---------|---------|
| GrimAge | Cancer | 8 (36) | 1.174 (1.074, 1.284) | 3.52 | 0.00 | 236.533*** | 87.53 | 92.20 |
| Hannum | Cancer | 9 (24) | 1.059 (1.015, 1.104) | 2.66 | 0.01 | 34.337 | -51.56 | -48.16 |
| Horvath | Cancer | 8 (23) | 1.051 (1.011, 1.092) | 2.52 | 0.01 | 22.577 | -52.46 | -49.19 |
| PhenoAge | Cancer | 7 (34) | 1.075 (1.008, 1.145) | 2.22 | 0.03 | 70.407*** | -35.84 | -31.36 |
| GrimAge | Cardiovascular disease | 7 (24) | 1.229 (1.102, 1.371) | 3.70 | 0.00 | 113.894*** | -38.91 | -35.50 |
| Hannum | Cardiovascular disease | 9 (25) | 1.060 (1.025, 1.097) | 3.39 | 0.00 | 39.223* | -66.84 | -63.30 |
| Horvath | Cardiovascular disease | 9 (25) | 1.022 (0.997, 1.048) | 1.70 | 0.09 | 36.054 | -67.44 | -63.90 |
| PhenoAge | Cardiovascular disease | 7 (24) | 1.116 (1.003, 1.242) | 2.02 | 0.04 | 110.480*** | -54.32 | -50.91 |
| GrimAge | Dementia | 4 (6) | 1.037 (0.910, 1.181) | 0.55 | 0.58 | 10.874 | 0.42 | -0.75 |
| GrimAge | Mortality | 12 (68) | 1.405 (1.216, 1.623) | 4.62 | 0.00 | 573.383*** | -63.04 | -56.43 |
| Hannum | Mortality | 11 (33) | 1.083 (1.019, 1.151) | 2.58 | 0.01 | 101.271*** | -76.03 | -71.64 |
| Horvath | Mortality | 13 (66) | 1.043 (1.016, 1.071) | 3.15 | 0.00 | 76.166 | -135.87 | -129.35 |
| PhenoAge | Mortality | 12 (45) | 1.117 (1.045, 1.193) | 3.25 | 0.00 | 238.471*** | -112.20 | -106.84 |

All associations were calculated from blood DNA methylation profiles. Notation:

* $p < 0.05$, ** $p < 0.01$,

*** $p < 0.001$.

Table A.5

Multi-level meta-analysis model results for all EAA-factor associations with available regression coefficient data.

| Clock | Factor | N papers (rows) | Estimate (95 %CI) | z | p | Cochran Q | AIC | BIC |
|-------------|---------------------|-----------------|------------------------|-------|------|------------|---------|---------|
| DunedinPoAm | Alcohol consumption | 5 (16) | 0.003 (0.000, 0.005) | 2.36 | 0.02 | 41.209*** | -45.482 | -43.358 |
| GrimAge | Alcohol consumption | 14 (31) | 0.089 (0.033, 0.145) | 3.12 | 0.00 | 123.475*** | 33.742 | 37.946 |
| Hannum | Alcohol consumption | 8 (23) | 0.023 (-0.026, 0.072) | 0.92 | 0.36 | 40.298* | 10.489 | 13.762 |
| Horvath | Alcohol consumption | 11 (38) | -0.004 (-0.033, 0.026) | -0.25 | 0.80 | 48.660 | 19.264 | 24.097 |

(continued on next page)

Table A.5 (continued)

| Clock | Factor | N papers (rows) | Estimate (95 %CI) | z | p | Cochran Q | AIC | BIC |
|-------------|-----------------------------|-----------------|-------------------------|--------|------|-------------|----------|----------|
| PhenoAge | Alcohol consumption | 11 (27) | 0.072 (0.006, 0.139) | 2.13 | 0.03 | 79.194*** | 45.095 | 48.870 |
| GrimAge | Birth weight | 4 (7) | -0.060 (-0.259, 0.140) | -0.59 | 0.56 | 15.249* | 4.733 | 4.108 |
| Hannum | Birth weight | 4 (7) | -0.072 (-0.184, 0.039) | -1.27 | 0.20 | 13.900* | 12.294 | 11.669 |
| Horvath | Birth weight | 3 (7) | 0.011 (-0.030, 0.051) | 0.51 | 0.61 | 1.136 | -3.996 | -4.620 |
| PhenoAge | Birth weight | 4 (7) | -0.046 (-0.093, 0.002) | -1.90 | 0.06 | 19.098** | 20.160 | 19.536 |
| Hannum | Blood pressure | 4 (10) | -0.050 (-0.261, 0.161) | -0.46 | 0.64 | 25.950*** | -10.244 | -9.652 |
| Horvath | Blood pressure | 5 (29) | 0.139 (-0.139, 0.416) | 0.98 | 0.33 | 180.165*** | 16.873 | 20.870 |
| PhenoAge | Blood pressure | 3 (9) | 0.042 (-0.027, 0.111) | 1.18 | 0.24 | 28.883*** | -11.096 | -10.857 |
| Bohlin | BMI | 4 (7) | 0.033 (-0.027, 0.094) | 1.08 | 0.28 | 4.885 | -1.376 | -2.000 |
| DunedinPoAm | BMI | 5 (15) | 0.039 (-0.013, 0.091) | 1.48 | 0.14 | 68.993*** | -46.830 | -44.913 |
| GrimAge | BMI | 12 (32) | 0.201 (0.014, 0.388) | 2.10 | 0.04 | 479.150*** | 146.919 | 151.221 |
| Hannum | BMI | 10 (30) | 0.113 (-0.013, 0.240) | 1.75 | 0.08 | 108.966*** | 35.644 | 39.746 |
| Horvath | BMI | 14 (55) | 0.107 (-0.020, 0.235) | 1.65 | 0.10 | 245.980*** | 89.957 | 95.924 |
| PhenoAge | BMI | 13 (39) | 0.220 (0.005, 0.435) | 2.01 | 0.04 | 406.565*** | 110.604 | 115.517 |
| Hannum | Cancer | 3 (39) | 0.623 (-0.175, 1.422) | 1.53 | 0.13 | 77.143*** | 123.009 | 127.922 |
| Horvath | Cancer | 3 (34) | 0.731 (-0.073, 1.535) | 1.78 | 0.07 | 58.624** | 85.029 | 89.518 |
| PhenoAge | Cancer | 4 (18) | 0.355 (-0.041, 0.750) | 1.76 | 0.08 | 116.296*** | 129.718 | 132.217 |
| GrimAge | Cardiovascular disease | 5 (25) | -0.175 (-0.586, 0.236) | -0.83 | 0.40 | 1457.317*** | 121.979 | 125.513 |
| Hannum | Cardiovascular disease | 7 (30) | 0.134 (-0.070, 0.337) | 1.29 | 0.20 | 97.725*** | 5.830 | 9.932 |
| Horvath | Cardiovascular disease | 9 (41) | -0.014 (-0.116, 0.088) | -0.27 | 0.78 | 126.749*** | 13.487 | 18.554 |
| PhenoAge | Cardiovascular disease | 5 (13) | -0.037 (-0.290, 0.217) | -0.28 | 0.78 | 121.516*** | -8.601 | -7.146 |
| DunedinPoAm | Childhood adversity | 5 (44) | 0.087 (0.006, 0.167) | 2.12 | 0.03 | 107.991*** | 11.416 | 16.700 |
| GrimAge | Childhood adversity | 7 (41) | 0.359 (-0.040, 0.759) | 1.77 | 0.08 | 1100.214*** | 426.429 | 431.496 |
| Hannum | Childhood adversity | 4 (10) | 0.009 (-0.024, 0.042) | 0.52 | 0.60 | 3.985 | -14.580 | -13.989 |
| Horvath | Childhood adversity | 4 (13) | 0.120 (-0.074, 0.314) | 1.22 | 0.22 | 19.586 | -0.875 | 0.579 |
| PhenoAge | Childhood adversity | 5 (12) | 0.045 (-0.030, 0.120) | 1.18 | 0.24 | 10.764 | -6.154 | -4.960 |
| GrimAge | Cognitive function | 10 (70) | -0.077 (-0.130, -0.023) | -2.81 | 0.00 | 681.182*** | -178.417 | -171.714 |
| Hannum | Cognitive function | 6 (64) | -0.016 (-0.041, 0.009) | -1.22 | 0.22 | 94.202** | -302.724 | -296.295 |
| Horvath | Cognitive function | 9 (80) | -0.005 (-0.023, 0.012) | -0.61 | 0.54 | 203.763*** | -218.721 | -211.613 |
| PhenoAge | Cognitive function | 9 (52) | -0.023 (-0.043, -0.003) | -2.24 | 0.02 | 116.449*** | -187.741 | -181.946 |
| Horvath | Dementia | 3 (4) | 0.025 (-0.022, 0.072) | 1.05 | 0.29 | 3.394 | 1.828 | -0.876 |
| GrimAge | Depression | 4 (10) | 0.148 (-0.174, 0.471) | 0.90 | 0.37 | 310.700*** | 100.763 | 101.355 |
| Hannum | Depression | 3 (8) | 0.088 (-0.111, 0.287) | 0.87 | 0.39 | 32.976*** | 17.503 | 17.341 |
| Horvath | Depression | 3 (8) | 0.195 (-0.697, 1.087) | 0.43 | 0.67 | 42.809*** | 25.360 | 25.198 |
| PhenoAge | Depression | 4 (10) | 0.204 (-0.281, 0.689) | 0.83 | 0.41 | 106.976*** | 7.150 | 7.742 |
| Hannum | Diabetes | 4 (19) | 0.021 (-0.061, 0.102) | 0.50 | 0.62 | 67.597*** | -30.445 | -27.774 |
| Horvath | Diabetes | 4 (21) | 0.008 (-0.001, 0.016) | 1.81 | 0.07 | 9.822 | -70.115 | -67.128 |
| GrimAge | Diet | 6 (13) | -0.155 (-0.295, -0.015) | -2.17 | 0.03 | 84.735*** | -1.825 | -0.370 |
| Hannum | Diet | 3 (8) | -0.062 (-0.215, 0.091) | -0.79 | 0.43 | 12.804 | 4.341 | 4.179 |
| Horvath | Diet | 5 (29) | -0.020 (-0.057, 0.017) | -1.04 | 0.30 | 20.296 | 41.126 | 45.123 |
| PhenoAge | Diet | 5 (10) | -0.122 (-0.263, 0.018) | -1.71 | 0.09 | 36.143*** | -1.344 | -0.753 |
| DunedinPoAm | Education | 4 (25) | 0.339 (-0.335, 1.013) | 0.99 | 0.32 | 83.221*** | -60.524 | -56.990 |
| GrimAge | Education | 10 (34) | 0.595 (-0.102, 1.293) | 1.67 | 0.09 | 644.377*** | 137.390 | 141.879 |
| Hannum | Education | 4 (49) | -0.010 (-0.038, 0.018) | -0.71 | 0.48 | 86.826** | 86.952 | 92.565 |
| Horvath | Education | 6 (12) | 0.002 (-0.051, 0.055) | 0.09 | 0.93 | 17.392 | 19.387 | 20.581 |
| PhenoAge | Education | 8 (19) | 0.399 (-0.049, 0.846) | 1.75 | 0.08 | 94.019*** | 29.510 | 32.181 |
| GrimAge | Employment | 5 (36) | 0.178 (-0.010, 0.367) | 1.85 | 0.06 | 243.357*** | 48.462 | 53.128 |
| PhenoAge | Employment | 4 (23) | 0.030 (-0.020, 0.081) | 1.18 | 0.24 | 13.358 | 11.509 | 14.782 |
| GrimAge | Frailty | 7 (35) | -0.150 (-0.363, 0.064) | -1.37 | 0.17 | 430.738*** | 190.182 | 194.761 |
| Hannum | Frailty | 7 (41) | -0.005 (-0.033, 0.022) | -0.39 | 0.69 | 91.355*** | -103.363 | -98.297 |
| Horvath | Frailty | 10 (49) | 0.025 (-0.084, 0.134) | 0.45 | 0.65 | 110.299*** | -78.714 | -73.101 |
| PhenoAge | Frailty | 7 (41) | -0.006 (-0.030, 0.018) | -0.48 | 0.63 | 141.912*** | -32.295 | -27.229 |
| Horvath | HDL | 3 (4) | 0.000 (-0.004, 0.003) | -0.21 | 0.84 | 8.110* | 2.568 | -0.136 |
| GrimAge | HIV | 3 (8) | 0.803 (-1.043, 2.649) | 0.85 | 0.39 | 99.613*** | 98.919 | 98.757 |
| Horvath | HIV | 5 (23) | 1.701 (0.350, 3.052) | 2.47 | 0.01 | 156.088*** | 148.905 | 152.178 |
| PhenoAge | HIV | 3 (9) | 3.633 (-0.011, 7.277) | 1.95 | 0.05 | 172.280*** | 73.877 | 74.116 |
| Horvath | Lead (Pb) | 4 (16) | 0.000 (-0.001, 0.001) | 0.53 | 0.60 | 4.917 | -102.224 | -100.100 |
| GrimAge | Physical ability | 5 (23) | -0.158 (-0.175, -0.142) | -19.23 | 0.00 | 66.743*** | -6.708 | -3.435 |
| Hannum | Physical ability | 4 (13) | -0.036 (-0.057, -0.015) | -3.36 | 0.00 | 18.879 | -8.042 | -6.587 |
| Horvath | Physical ability | 4 (13) | -0.017 (-0.046, 0.012) | -1.15 | 0.25 | 6.774 | -19.806 | -18.352 |
| PhenoAge | Physical ability | 4 (13) | -0.071 (-0.095, -0.046) | -5.71 | 0.00 | 14.153 | -9.410 | -7.955 |
| GrimAge | Physical activity | 7 (37) | -0.359 (-0.647, -0.071) | -2.44 | 0.01 | 123.237*** | 127.233 | 131.984 |
| Hannum | Physical activity | 6 (33) | -0.028 (-0.167, 0.111) | -0.40 | 0.69 | 17.012 | 54.393 | 58.790 |
| Horvath | Physical activity | 7 (74) | -0.131 (-0.328, 0.065) | -1.31 | 0.19 | 77.471 | 48.066 | 54.938 |
| PhenoAge | Physical activity | 7 (34) | -0.191 (-0.620, 0.237) | -0.87 | 0.38 | 594.511*** | 31.036 | 35.525 |
| Hannum | Pollutant/chemical exposure | 5 (15) | 0.202 (-0.224, 0.627) | 0.93 | 0.35 | 35.664** | 42.467 | 44.384 |
| Horvath | Pollutant/chemical exposure | 8 (50) | 0.110 (0.023, 0.197) | 2.49 | 0.01 | 135.875*** | 138.531 | 144.206 |
| PhenoAge | Pollutant/chemical exposure | 5 (20) | 0.428 (0.046, 0.811) | 2.19 | 0.03 | 44.878** | 26.916 | 29.749 |
| SkinBlood | Pollutant/chemical exposure | 4 (103) | 0.394 (-0.112, 0.900) | 1.52 | 0.13 | 98.728 | -341.653 | -333.778 |
| GrimAge | Psychosocial adversity | 3 (13) | 0.029 (-0.189, 0.247) | 0.26 | 0.79 | 43.703*** | 7.600 | 9.055 |
| Horvath | Psychosocial adversity | 3 (14) | -0.118 (-0.276, 0.040) | -1.47 | 0.14 | 29.876** | 16.821 | 18.516 |
| GrimAge | Psychosocial functioning | 5 (12) | -0.003 (-0.094, 0.089) | -0.06 | 0.95 | 56.749*** | 32.669 | 33.863 |
| GrimAge | Race/ethnicity | 5 (8) | -0.152 (-0.832, 0.527) | -0.44 | 0.66 | 24.682** | 21.297 | 21.135 |
| Hannum | Race/ethnicity | 3 (6) | -0.874 (-1.911, 0.164) | -1.65 | 0.10 | 37.135*** | 25.345 | 24.174 |
| Horvath | Race/ethnicity | 7 (14) | 0.293 (-0.510, 1.097) | 0.72 | 0.47 | 27.598* | 40.854 | 42.549 |
| PhenoAge | Race/ethnicity | 6 (10) | -0.556 (-2.016, 0.904) | -0.75 | 0.46 | 45.254*** | 29.588 | 30.179 |

(continued on next page)

Table A.5 (continued)

| Clock | Factor | N papers (rows) | Estimate (95 %CI) | z | p | Cochran Q | AIC | BIC |
|-------------|-------------------|-----------------|------------------------|-------|------|--------------|----------|----------|
| DunedinPoAm | SES | 5 (58) | 0.003 (-0.003, 0.010) | 1.05 | 0.30 | 348.891 *** | -216.278 | -210.149 |
| GrimAge | SES | 8 (93) | 0.458 (-0.127, 1.043) | 1.54 | 0.12 | 1534.188 *** | 555.761 | 563.326 |
| Hannum | SES | 10 (79) | 0.245 (0.052, 0.437) | 2.49 | 0.01 | 231.918 *** | 66.156 | 73.226 |
| Horvath | SES | 8 (52) | 0.071 (-0.042, 0.184) | 1.24 | 0.22 | 72.305 * | 68.396 | 74.192 |
| PhenoAge | SES | 8 (70) | 0.346 (-0.018, 0.711) | 1.86 | 0.06 | 318.898 *** | 148.546 | 155.249 |
| Horvath | SES (saliva) | 3 (10) | 0.021 (-0.029, 0.071) | 0.84 | 0.40 | 4.149 | 17.286 | 17.877 |
| Bohlin | Sex | 4 (8) | -0.044 (-0.116, 0.028) | -1.20 | 0.23 | 9.262 | 15.878 | 15.715 |
| DunedinPoAm | Sex | 3 (4) | -0.028 (-0.117, 0.062) | -0.60 | 0.55 | 7.982 * | -1.023 | -3.727 |
| GrimAge | Sex | 6 (6) | -0.505 (-1.240, 0.230) | -1.35 | 0.18 | 143.795 *** | 20.562 | 19.390 |
| Horvath | Sex | 10 (13) | -0.102 (-0.729, 0.526) | -0.32 | 0.75 | 76.684 *** | 42.245 | 43.700 |
| PhenoAge | Sex | 6 (6) | -0.028 (-0.154, 0.098) | -0.43 | 0.66 | 34.534 *** | 14.944 | 13.772 |
| Bohlin | Smoking | 4 (6) | 0.063 (-0.023, 0.149) | 1.43 | 0.15 | 2.750 | 7.671 | 6.499 |
| DunedinPoAm | Smoking | 5 (19) | 0.844 (-0.420, 2.109) | 1.31 | 0.19 | 1689.220 *** | 1110.429 | 1113.100 |
| GrimAge | Smoking | 14 (30) | 2.122 (0.809, 3.435) | 3.17 | 0.00 | 5078.044 *** | 1848.732 | 1852.833 |
| Hannum | Smoking | 8 (21) | -0.206 (-0.988, 0.577) | -0.52 | 0.61 | 69.124 *** | 50.952 | 53.939 |
| Horvath | Smoking | 13 (41) | 0.072 (-0.118, 0.262) | 0.74 | 0.46 | 65.819 ** | 78.212 | 83.279 |
| PhenoAge | Smoking | 12 (27) | 0.374 (0.161, 0.587) | 3.44 | 0.00 | 166.026 *** | 80.545 | 84.319 |
| SkinBlood | Smoking | 3 (17) | 0.058 (0.017, 0.099) | 2.79 | 0.01 | 21.129 | -5.196 | -2.879 |
| GrimAge | Substance use | 4 (15) | 0.132 (0.104, 0.160) | 9.34 | 0.00 | 60.788 *** | 50.168 | 52.085 |
| Horvath | Total cholesterol | 4 (7) | 0.026 (-0.362, 0.415) | 0.13 | 0.89 | 12.330 | 12.099 | 11.475 |
| PhenoAge | WHR | 3 (10) | 0.516 (-0.069, 1.101) | 1.73 | 0.08 | 94.451 *** | 10.056 | 10.648 |

Unless specified in **Factor** column, associations were calculated from blood DNA methylation profiles. Notation:

* $p < 0.05$,
** $p < 0.01$,
*** $p < 0.001$.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.arr.2024.102552.

References

- Anusic, I., Schimmack, U., 2016. Stability and change of personality traits, self-esteem, and well-being: Introducing the meta-analytic stability and change model of retest correlations. *J. Personal. Soc. Psychol.* 110, 766.
- Bell, C.G., Lowe, R., Adams, P.D., Baccarelli, A.A., Beck, S., Bell, J.T., Christensen, B.C., Gladyshev, V.N., Heijmans, B.T., Horvath, S., et al., 2019. DNA methylation aging clocks: challenges and recommendations. *Genome Biol.* 20, 1–24.
- Bootstrap Team, 2024.Bootstrap 5. (<https://getbootstrap.com/>). accessed: 2024-02-08.
- Bootstrap Toggle Contributors, 2024.Bootstrap Toggle. (<https://www.bootstraptoggle.com/>). accessed: 2024-02-08.
- Bozack, A.K., Boileau, P., Hubbard, A.E., Sillé, F.C., Ferreccio, C., Steinmaus, C.M., Smith, M.T., Cardenas, A., 2022. The impact of prenatal and early-life arsenic exposure on epigenetic age acceleration among adults in northern chile. *Environ. Epigenetics* 8 dvac014.
- code by Richard A. Becker, O.S., version by Ray Brownrigg. Enhancements by Thomas P Minka, A.R.W.R., by the CRAN team., A.D.F., 2023.maps: Draw Geographical Maps. (<https://CRAN.R-project.org/package=maps>).r package version 3.4.1.1.
- Dewey, M., 2019.metaviz: Forest Plots, Funnel Plots, and Visual Funnel Plot Inference for Meta-Analysis. (<https://CRAN.R-project.org/package=metaviz>).r package version 0.3.0.
- ECMA International, 2024. ECMAScript 2024 Language Specification. (<https://www.ecma-international.org/ecma-262/>). accessed: 2024-02-08.
- Elliott, M.L., Caspi, A., Houts, R.M., Ambler, A., Broadbent, J.M., Hancox, R.J., Harrington, H., Hogan, S., Keenan, R., Knodt, A., et al., 2021. Disparities in the pace of biological aging among midlife adults of the same chronological age have implications for future frailty risk and policy. *Nat. Aging* 1, 295–308.
- Fang, F., Zhou, L., Perng, W., Marsit, C.J., Knight, A.K., Cardenas, A., Aung, M.T., Hirvitt, M.F., Aris, I.M., Goodrich, J.M., et al., 2023. Evaluation of pediatric epigenetic clocks across multiple tissues. *Clin. Epigenetics* 15, 142.
- Fiorito, G., McCrory, C., Robinson, O., Carmeli, C., Rosales, C.O., Zhang, Y., Colicino, E., Dugué, P.A., Artaud, F., McKay, G.J., et al., 2019. Socioeconomic position, lifestyle habits and biomarkers of epigenetic aging: a multi-cohort analysis. *Aging (Albany NY)* 11, 2045.
- Grodstein, F., Lemos, B., Yu, L., Iatrou, A., De Jager, P.L., Bennett, D.A., 2021. Characteristics of epigenetic clocks across blood and brain tissue in older women and men. *Front. Neurosci.* 14, 555307.
- Harrer, M., Cuijpers, P., Furukawa, T., Ebert, D., 2021. Doing meta-analysis with R: A hands-on guide. Chapman and Hall/CRC.
- Holt, M., 2024.Papa Parse: Powerful, In-Browser CSV Parser for Big Boys and Girls. (<https://www.papaparse.com/>). accessed: 2024-02-08.
- Horvath, S., 2013. DNA methylation age of human tissues and cell types. *Genome Biol.* 14, 1–20.
- Horvath, S., Gurven, M., Levine, M.E., Trumble, B.C., Kaplan, H., Allayee, H., Ritz, B.R., Chen, B., Lu, A.T., Rickabaugh, T.M., et al., 2016. An epigenetic clock analysis of race/ethnicity, sex, and coronary heart disease. *Genome Biol.* 17, 1–23.
- Ioannidis, J.P., Trikalinos, T.A., 2007. An exploratory test for an excess of significant findings. *Clin. Trials* 4, 245–253.
- Jain, P., Binder, A.M., Chen, B., Parada, H., Gallo, L.C., Alcaraz, J., Horvath, S., Bhatti, P., Whitsel, E.A., Jordahl, K., et al., 2022. Analysis of epigenetic age acceleration and healthy longevity among older US women. *JAMA Netw. Open* 5, e2223285.
- jQuery Foundation, 2024.jQuery: The Write Less, Do More, JavaScript Library. (<https://jquery.com/>). accessed: 2024-02-08.
- Jylhävä, J., Pedersen, N.L., Hägg, S., 2017. Biological age predictors. *EBioMedicine* 21, 29–36.
- Kankaanpää, A., Tolvanen, A., Saikonen, P., Heikkilä, A., Laakkonen, E.K., Kaprio, J., Ollikainen, M., Sillampää, E., 2022. Do epigenetic clocks provide explanations for sex differences in life span? A cross-sectional twin study. *J. Gerontol.: Ser. A* 77, 1898–1906.
- Levine, M., Lu, A., Bennett, D., Horvath, S., 2021. Epigenetic Age Acceleration and Hearing. *NCBI*. <https://doi.org/10.1093/gerona/glab155>.
- Levine, M., Lu, A., Quach, A., Chen, B., Assimes, T., Bandinelli, S., Hou, L., Baccarelli, A., Stewart, J., Li, Y., Whitsel, E., Wilson, J., Reiner, A., Aviv, A., Lohman, K., Liu, Y., Ferrucci, L., Horvath, S., 2022. Analysis of Epigenetic Age Acceleration and Healthy Longevity Among Older US Women. *JAMA Netw. Open* 5, e2219755. <https://doi.org/10.1010/jamanetworkopen.2022.19755>.
- de Lima Camillo, L.P., Lapierre, L.R., Singh, R., 2022. A pan-tissue DNA-methylation epigenetic clock based on deep learning. *npj Aging* 8, 4.
- López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M., Kroemer, G., 2013. The hallmarks of aging. *Cell* 153, 1194–1217.
- Ltd, S., 2024.DataTables: Table plug-in for jQuery. (<https://datatables.net/>). accessed: 2024-02-08.
- Maag, J.L.V., 2018. ggenatogram: An R package for modular visualisation of anatograms and tissues based on ggplot2. *F1000Research* 7. (<https://f1000research.com/articles/7/1576>).
- McGuinness, L., Higgins, J., 2021. Association of cardiovascular health and epigenetic age acceleration. *Clin. Epigenetics* 13, 42. <https://doi.org/10.1186/s13148-021-01028-2>.
- Milicic, L., Vacher, M., Porter, T., Doré, V., Burnham, S.C., Bourgeat, P., Shishegar, R., Doecke, J., Armstrong, N.J., Tankard, R., et al., 2022. Comprehensive analysis of epigenetic clocks reveals associations between disproportionate biological ageing and hippocampal volume. *Geroscience* 44, 1807–1823.
- Oblak, L., van der Zaag, J., Higgins-Chen, A.T., Levine, M.E., Boks, M.P., 2021. A systematic review of biological, social and environmental factors associated with epigenetic clock acceleration. *Ageing Res. Rev.* 69, 101348. <https://doi.org/10.1016/j.arr.2021.101348>.
- Page, M.J., McKenzie, J.E., Bossuyt, P.M., Boutron, I., Hoffmann, T.C., Mulrow, C.D., Shamseer, L., Tetzlaff, J.M., Akl, E.A., Brennan, S.E., Chou, R., Glanville, J., Grimshaw, J.M., Hróbjartsson, A., Lalu, M.M., Li, T., Loder, E.W., Mayo-Wilson, E., McDonald, S., McGuinness, L.A., Stewart, L.A., Thomas, J., Tricco, A.C., Welch, V.A., Whiting, P., Moher, D., 2021. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *PLOS Med.* 18, e1003583. <https://doi.org/10.1371/journal.pmed.1003583>.

- Patterson, H.D., Thompson, R., 1971. Recovery of inter-block information when block sizes are unequal. *Biometrika* 58, 545–554.
- Rosen, A., Robertson, K., Hlady, R., Muench, C., Lee, J., Philibert, R., Horvath, S., Kaminsky, Z., 2019. Epigenetic aging is accelerated in alcohol use disorder and regulated by genetic variation in APOL2. *Neuropsychopharmacology* 44, 1593–1601. <https://doi.org/10.1038/s41386-019-0380-9>.
- Schmitz, L.L., Zhao, W., Ratliff, S.M., Goodwin, J., Miao, J., Lu, Q., Guo, X., Taylor, K.D., Ding, J., Liu, Y., et al., 2022. The socioeconomic gradient in epigenetic ageing clocks: evidence from the multi-ethnic study of atherosclerosis and the health and retirement study. *Epigenetics* 17, 589–611.
- Sillanpää, E., Heikkilä, A., Kankaanpää, A., Paavilainen, A., Kujala, U.M., Tammelin, T.H., Kovánen, V., Sipilä, S., Pietiläinen, K.H., Kaprio, J., et al., 2021. Blood and skeletal muscle ageing determined by epigenetic clocks and their associations with physical activity and functioning. *Clin. Epigenetics* 13, 110.
- Simons, R., Lei, M., Beach, S., Philibert, R., Cutrona, C., Gibbons, F., Barr, A., 2018. DNA methylation age - environmental influences, health impacts, and its role in environmental epidemiology. *Curr. Environ. Health Rep.* 5, 317–327. <https://doi.org/10.1007/s40572-018-0204-3>.
- Stringhini, S., Polidoro, S., Sacerdote, C., Kelly, R., van Veldhoven, K., Agnoli, C., Grioni, S., Tumino, R., Giurdanella, M., Panico, S., Mattiello, A., Palli, D., Masala, G., Gallo, V., Castagné, R., Paccaud, F., Campanella, G., Chadeau-Hyam, M., Vineis, P., 2017. Social adversity and epigenetic aging: a multi-cohort study on socioeconomic differences in peripheral blood DNA methylation. *Sci. Rep.* 7, 16266. <https://doi.org/10.1038/s41598-017-16391-5>.
- Teschendorff, A.E., Relton, C.L., 2018. Statistical and integrative system-level analysis of DNA methylation data. *Nat. Rev. Genet.* 19, 129–147.
- Viechtbauer, W., 2010.metafor: Meta-Analysis Package for R. (<https://CRAN.R-project.org/package=metafor>).r package version 1.9-9.
- W3C, 2024.Cascading Style Sheets Level 2 Revision 2 (CSS 2.2) Specification. (<https://www.w3.org/TR/CSS22/>).accessed: 2024-02-08.
- Watkins, S.H., Testa, C., Chen, J.T., De Vivo, I., Simpkin, A.J., Tilling, K., DiezRoux, A.V., DaveySmith, G., Waterman, P.D., Suderman, M., et al., 2023. Epigenetic clocks and research implications of the lack of data on whom they have been developed: a review of reported and missing sociodemographic characteristics. *Environ. Epigenetics* 9 dvad005.
- WHATWG, 2024.HTML Living Standard. (<https://html.spec.whatwg.org/>).accessed: 2024-02-08.
- Wickham, Hadley, 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag, New York. (<https://ggplot2.tidyverse.org>).
- Zhang, B., Yuan, Q., Luan, Y., Xia, J., 2023. Effect of women's fertility and sexual development on epigenetic clock: Mendelian randomization study. *Clin. Epigenetics* 15, 154.
- Zhang, Q., Vallerga, C.L., Walker, R.M., Lin, T., Henders, A.K., Montgomery, G.W., He, J., Fan, D., Fowdar, J., Kennedy, M., Pitcher, T., Pearson, J., Halliday, G., McRae, A.F., Visscher, P.M., Brown, M.A., Long, Q., Yang, J., Powell, J.E., Hewitt, A.W., Mackey, D.A., McIntosh, A.M., Whiteman, D.C., Zondervan, K.T., Martin, N.G., Ferreira, M.A., Vinkhuyzen, A.A.E., Nyholt, D.R., Medland, S.E., Madden, P.A., Heath, A.C., Montgomery, G.W., Martin, N.G., Wright, M.J., Bates, T.C., Golding, J., Lawlor, D.A., DaveySmith, G., Hagg, S., Mill, J., Gordon, S.D., Porteous, D.J., Hayward, C., Vitart, V., Hill, W.D., Goddard, M.E., Visscher, P.M., Yang, J., 2019. Improved precision of epigenetic clock estimates across tissues and its implication for biological ageing. *Genome Med.* 11, 54. <https://doi.org/10.1186/s13073-019-0667-1>.
- Zhang, Y., Wilson, R., Heiss, J., Breitling, L., Saum, K., Schöttker, B., Holleczek, B., Waldenberger, M., Peters, A., Brenner, H., 2016. Epigenetic age acceleration predicts cancer, cardiovascular, and all-cause mortality in a German case cohort. *Clin. Epigenetics* 8, 64. <https://doi.org/10.1186/s13148-016-0228-z>.