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Review

# A systematic review of biological, social and environmental factors associated with epigenetic clock acceleration

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ABSTRACT

Aging involves a diverse set of biological changes accumulating over time that leads to increased risk of morbidity and mortality. Epigenetic clocks are now widely used to quantify biological aging, in order to investigate determinants that modify the rate of aging and to predict age-related outcomes. Numerous biological, social and environmental factors have been investigated for their relationship to epigenetic clock acceleration and deceleration. The aim of this review was to synthesize general trends concerning the associations between human epigenetic clocks and these investigated factors. We conducted a systematic review of all available literature and included 156 publications across 4 resource databases. We compiled a list of all presently existing blood-based epigenetic clocks. Subsequently, we created an extensive dataset of over 1300 study findings in which epigenetic clocks were utilized in blood tissue of human subjects to assess the relationship between these clocks and numeral environmental exposures and human traits. Statistical analysis was possible on 57 such relationships, measured across 4 different epigenetic clocks (Hannum, Horvath, Levine and GrimAge). We found that the Horvath, Hannum, Levine and GrimAge epigenetic clocks tend to agree in direction of effects, but vary in size. Body mass index, HIV infection, and male sex were significantly associated with acceleration of one or more epigenetic clocks. Acceleration of epigenetic clocks was also significantly related to mortality, cardiovascular disease, cancer and diabetes. Our findings provide a graphical and numerical synopsis of the past decade of epigenetic age estimation research and indicate areas where further attention could be focused in the coming years.

## 1. Introduction

Aging is a relevant risk factor for a wide range of medical conditions (Jaul and Barron, 2017). Intervening in the aging process to reduce disease will require identifying targetable causal factors, as well as improving our understanding of the specific biological changes that lead to age-related pathology. However, doing so requires biomarkers that can quantify or characterize biological aging, a challenging task given the complexity and heterogeneity of human aging.

Changes in DNA methylation (DNAm) patterns with age have been widely used to quantify aging (Jones et al., 2015). Epigenetic alterations (including DNAm) are one of the nine hallmarks of aging—biological processes that change with age and can be targeted to influence health and lifespan (López-Otín et al., 2013). DNA methylation involves the addition of a methyl group to the 5′ position on cytosines in cytosine guanine dinucleotides, referred to as CpGs (Jung et al., 2017). Epigenetic clocks predict age or age-related phenotypes by combining the methylation values of tens to hundreds of CpGs selected using machine learning approaches. The first epigenetic clock for saliva samples, defined as a multivariate predictor of age, was published by a UCLA research team in 2011 (Bocklandt et al., 2011). In 2012, an Italian team published the first epigenetic clock for blood based on a single CpG (Garagnani et al., 2012). The first pan-tissue clock was published in 2013 by Steve Horvath. This clock showed impressive accuracy, predicting age with a correlation of 0.96 and a median error of 3.6 years across a wide array of diverse tissues and cells (Horvath, 2013). A number of other clocks have since been developed, many utilizing different CpG sites (Bell et al., 2019). Across these, there is a general

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trend suggesting that increased epigenetic age relative to chronological age (also known as age acceleration) is associated with a multitude of adverse health outcomes (Fransquet et al., 2019). Nevertheless, the various epigenetic clocks exhibit different degrees of associations with aging risk factors and outcomes (Horvath and Raj, 2018), suggesting they may also capture distinct aspects of aging.

This study aims to comprehensively summarize existing research findings on biological, social and environmental factors associated with aberrant epigenetic aging in blood. Such an approach extends previous meta-analyses such as those by Fransquet et al. (2019) by incorporating new epigenetic clocks and more factors in order to accommodate the rapidly growing body of research. To achieve this goal, an extensive systematic review on all present epigenetic age estimation literature is conducted, followed by a statistical analysis of the reported effects on epigenetic age. This provides a relevant snapshot of the current state of the field, sheds light on gaps and conflicting findings, and ultimately aids in directing future age estimation research.

## 2. Methods

This review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for protocol, search strategy, and risk of bias assessment (Moher et al., 2009).

### 2.1. Search strategy

A comprehensive literature search was performed on February 1st, 2021. The search was performed in the PubMed, PMC, Web of Science and BioRxiv online databases. The search string can be found in Supplementary File B. After the study search was complete, titles were screened according to the inclusion criteria, followed by abstract and title screening. A visual representation of the search strategy is shown in Fig. 1.

### 2.2. Inclusion criteria

We limited our search to primary studies available in the English language that concerned human subjects. There was no restriction on demographic characteristics, all ages, sexes and ethnicities were included. An epigenetic clock was defined as any tool utilizing CpG methylation patterns to assess the epigenetic age of human subjects. Only clocks trained to predict age from blood samples were included. Multi-tissue clocks were included provided that they were suited for prediction from blood samples. Clocks which predict either chronological age, mortality risk, phenotypic age (a metric first proposed by Levine et al. (2018), which utilizes chemistry biomarkers to differentiate mortality risk in persons of the same chronological age) or any feature which can be used as a measure of (ab)normal aging were accepted.

For the purposes of this review, every type of potential age-associated factor was included in the search, as long as there was a measured relationship between this factor and DNA methylation-based change in epigenetic age. A factor was defined as any biological, sociological, medical or psychological variable which may be associated with the rate of epigenetic aging.

### 2.3. Data extraction

Studies that fit the inclusion criteria were first classified as primary studies in which a new age estimation clock had been developed, or secondary studies in which existing clocks were utilized for further research. In both classes data was gathered on the general publication details of each study, number of subjects and subject demographics (age, sex, ethnicity).

In primary studies, we extracted the training phenotype of the clocks, number of CpGs in the clock, and the age prediction accuracy of the clock (Table 1, Supplementary Table A). In secondary studies, we additionally collected information on the specific epigenetic clock used, aging factors and the means of measuring them, and their corresponding statistics (Supplementary Table B). We extracted original measures of age acceleration (*AgeAccel*) wherever possible, as these were the most commonly reported. In cases where intrinsic and extrinsic age acceleration (*IEAA* and *EEAA*) were reported, we extracted only IEAA. All data was extracted and checked independently (LO and JvdZ).

### 2.4. Data synthesis and statistical analysis

Upon extraction, factors with sufficient observations were selected for further analysis. A factor was considered to have sufficient observations when it contained data points from at least 3 distinct samples measured by the same epigenetic clock. If a factor was measured in multiple ways (e.g., education score and years of education), only one measure was included per dataset to avoid biased representation. Efforts were made to avoid reporting on the same variable from one cohort multiple times, as many investigations gathered their samples from large

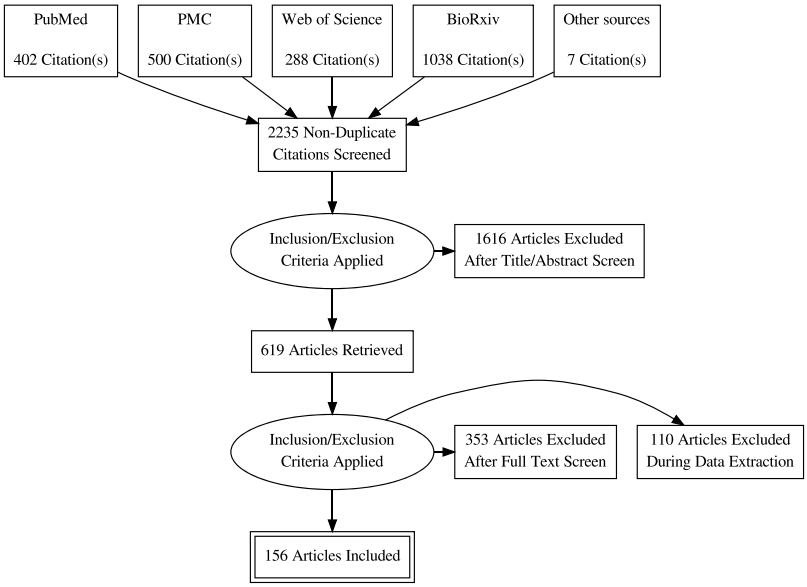


**Fig. 1.** PRISMA diagram of the search strategy and data extraction.

**Table 1**
Descriptive information of the primary epigenetic clock data extracted from the literature search. Accuracy of age prediction is reported based on validation data. Additional statistics are reported in Supplementary Table A.

| First Author | Publication Year | # of CpGs | Training Phenotype | Tissue | n of subjects | Age range | Accuracy of age prediction (r) |
|---|---|---|---|---|---|---|---|
| Correia | 2020 | 4 | Chronological Age | Blood | 53 | 1–93 | 0.977 |
| Han | 2020 | 9 | Chronological Age | Blood | 973 | 1–101 | 0.943 |
| Lee | 2020 | 1791 | Chronological Age | Blood | 2227 | 19–66 | 0.970 |
| Thong | 2021 | 3 | Chronological Age | Blood | 196 | 0–88 | 0.969 |
| Horvath | 2020 | 36,000 | Chronological Age | Multiple | 850 | 0–93 | 0.990 |
| Lu | 2021 | 54 | Chronological Age | Multiple | 7812 | 0–139 | 0.958 |
| Horvath | 2013 | 353 | Chronological Age | Multiple | 3931 | 0–100 | 0.960 |
| Horvath | 2018 | 391 | Chronological Age | Multiple | 278 | 19–82 | 0.980 |
| Koch | 2011 | 5 | Chronological Age | Multiple | 130 | 0–78 | 0.825 |
| Zhang | 2019 | 514 | Chronological Age | Multiple | 13,566 | 9–75 | 0.990 |
| Bekaert | 2015 | 4 | Chronological Age | Whole blood | 206 | 0–91 | 0.973 |
| Florath | 2014 | 17 | Chronological Age | Whole blood | 249 | 50–75 | 0.880 |
| Freire-Aradas | 2018 | 6 | Chronological Age | Whole blood | 180 | 2–18 | 0.893 |
| Garagnani | 2012 | 1 | Chronological Age | Whole blood | 64 | 9–99 | 0.920 |
| Han | 2020 | 66 | Chronological Age | Whole Blood | 973 | 1–101 | 0.906 |
| Hannum | 2013 | 71 | Chronological Age | Whole blood | 482 | 19–101 | 0.905 |
| Li | 2018 | 83 | Chronological Age | Whole blood | 90 | 6–17 | 0.930 |
| Li | 2018 | 6 | Chronological Age | Whole blood | 539 | 18–81 | 0.960 |
| Li | 2018 | 239 | Chronological Age | Whole blood | 1322 | 0–103 | – |
| Naue | 2017 | 13 | Chronological Age | Whole blood | 208 | 18–69 | – |
| Vidaki | 2017 | 16 | Chronological Age | Whole blood | 1156 | 2–90 | 0.980 |
| Vidal-Bralo | 2016 | 8 | Chronological Age | Whole blood | 390 | 20–70 | 0.775 |
| Weidner | 2014 | 3 | Chronological Age | Whole blood | 575 | 45–75 | – |
| Weidner | 2014 | 99 | Chronological Age | Whole blood | 656 | 19–101 | 0.933 |
| Wu | 2019 | 111 | Chronological Age | Whole Blood | 716 | 0–18 | 0.980 |
| Xu | 2015 | 6 | Chronological Age | Whole blood | 16 | 27–54 | – |
| Zbieć-Piekarska | 2015 | 1 | Chronological Age | Whole blood | 300 | 2–75 | – |
| Zubakov | 2016 | 8 | Chronological Age | Whole blood | 216 | 4–82 | 0.933 |
| Bohlin | 2016 | 132 | Gestational Age | Cord Blood | 685 | Neonates | 0.819 |
| Knight | 2016 | 148 | Gestational Age | Cord Blood | 207 | 24–44 weeks | 0.910 |
| Levine | 2018 | 513 | Lifespan (mortality risk score) | Multiple | 9926 | 0–100 | 0.710 |
| Lu | 2019 | 1030 | Lifespan (mortality risk score) | Whole blood | 6935 | 46–78 | – |
| Zhang | 2017 | 10 | Lifespan (mortality risk score) | Whole blood | 1000 | 32–81 | – |
| Belsky | 2020 | 46 | Pace of Aging | Whole Blood | 810 | 26–38 | 0.330 |

longitudinal cohorts (such as WHI, InCHIANTI, NAS; dbGaP study accession numbers phs001077.v1.p1, phs000215.v2.p1, phs000853.v1.p1, respectively).

Studies which did not examine a linear relationship were not taken into account. Values of p which were only reported as being smaller than a certain benchmark value (either $< 0.05$ or $< 0.001$) were rounded down to the nearest decimal place for the most conservative estimation (a value of $< 0.05$ would therefore become 0.049). In studies which presented results in terms of change-per-SD, we have re-derived the raw reported values in order to make them comparable with the rest of the datapoints, the majority of which were reported in terms of change-per-year. Data analysis was performed using JASP statistical software and plotted using Photoshop CC software.

#### 2.4.1. Weighted Z-scores

Inverse variance weighting was used to calculate a weighted effect size measure for each dataset. This weighted effect size was then converted into a z-score, as described in Lee et al. (2016). The z-value of +/- 1.96, which corresponds to a p-value of 0.05 assuming normal distribution, was determined as a benchmark for statistical significance.

#### 2.4.2. Meta-analysis

All meta-analyses were performed using JASP Statistical software (Love et al., 2019). JASP is an open-source program which offers standard analysis procedures in both frequentist and Bayesian form. A random effects analysis with a restricted maximum likelihood estimator (REML) was conducted to best account for the differences in study sizes, demographic characteristics and methodology. Forest plots were generated and heterogeneity estimates were calculated in the form of $\tau^2$, $I^2$, $H^2$ and Q. To assess publication bias, we generated funnel plots for each analysis and performed rank correlation tests for funnel plot asymmetry.

Due to constraints of simultaneously analysing multiple types of effect size measures, only the most frequently used Effect Size (ES) measure per individual dataset was selected for meta-analysis. Unstandardized regression coefficients (B), odds ratios (OR) and hazard ratios (HR) were used most frequently and were therefore the main effect sizes analysed in our dataset. Where standard errors were not reported, they were calculated from confidence intervals or estimated from their corresponding p-values (Higgins and Green, 2011).

## 3. Results

### 3.1. Study selection and data extraction

A total of 2235 studies were retrieved in the initial search. After the abstract and full-text screening, 266 studies were left which fit the inclusion criteria and from which data was extracted. 110 articles were removed during data extraction, yielding a final set of 156 articles that were included in further analyses (Fig. 1).

### 3.2. Descriptive statistics

Upon completing the data extraction, there was a final set of 34 publications in the primary clock dataset, and a set of 124 publications in the secondary literature dataset. We identified 34 publications in which epigenetic clocks were developed. Out of these, 28 are trained to estimate chronological age, 3 estimate mortality risk or time-to-death, 2 estimate gestational age, and 1 clock estimates the pace of aging. While the focus of this review is on secondary literature and factors interacting with epigenetic age rates, we present general descriptive information of the primary epigenetic clocks in Table 1 (for additional information see Supplementary Table A). Upon extraction, 1342 observations provided information on different aging factors. Similar measures were grouped,

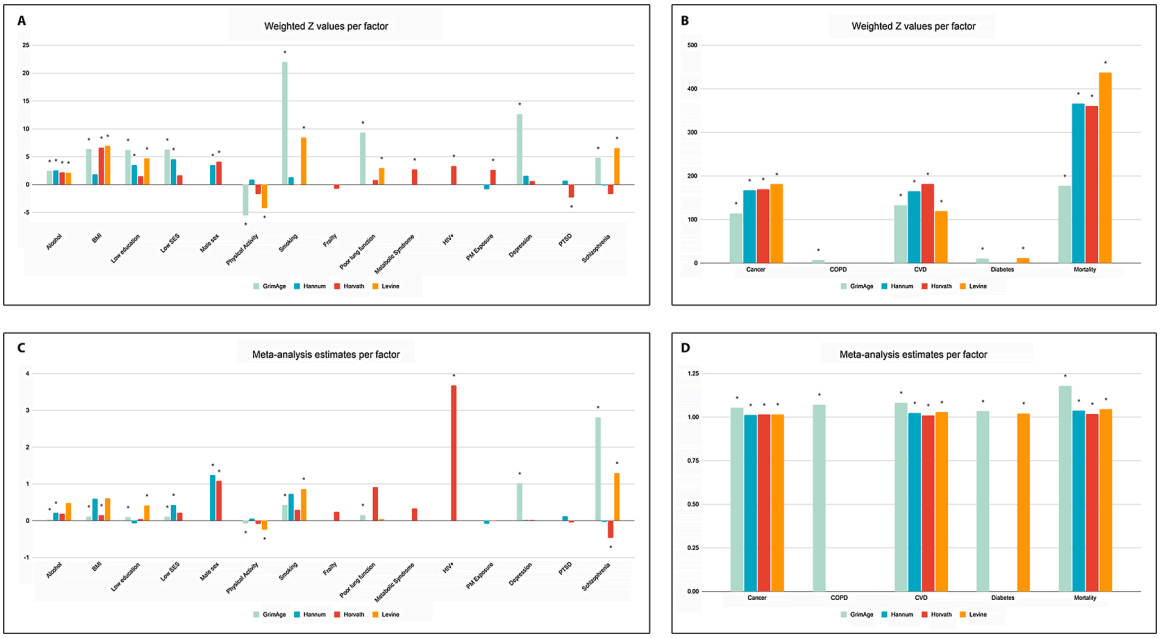


**Fig. 2.** Plots of calculated average effect sizes. Panels A and B show weighted-z values per factor, as assessed by the different epigenetic clocks. Values below -1.96 and above 1.96 are considered statistically significant. Plots C and D show meta-analysis effect size values per factor. Values marked with an asterisk correspond to a p-value $\leq 0.05$. Factors evaluated with regression models (panel A and C) are plotted separately from factors evaluated through hazard and odds ratios (panel B and D).

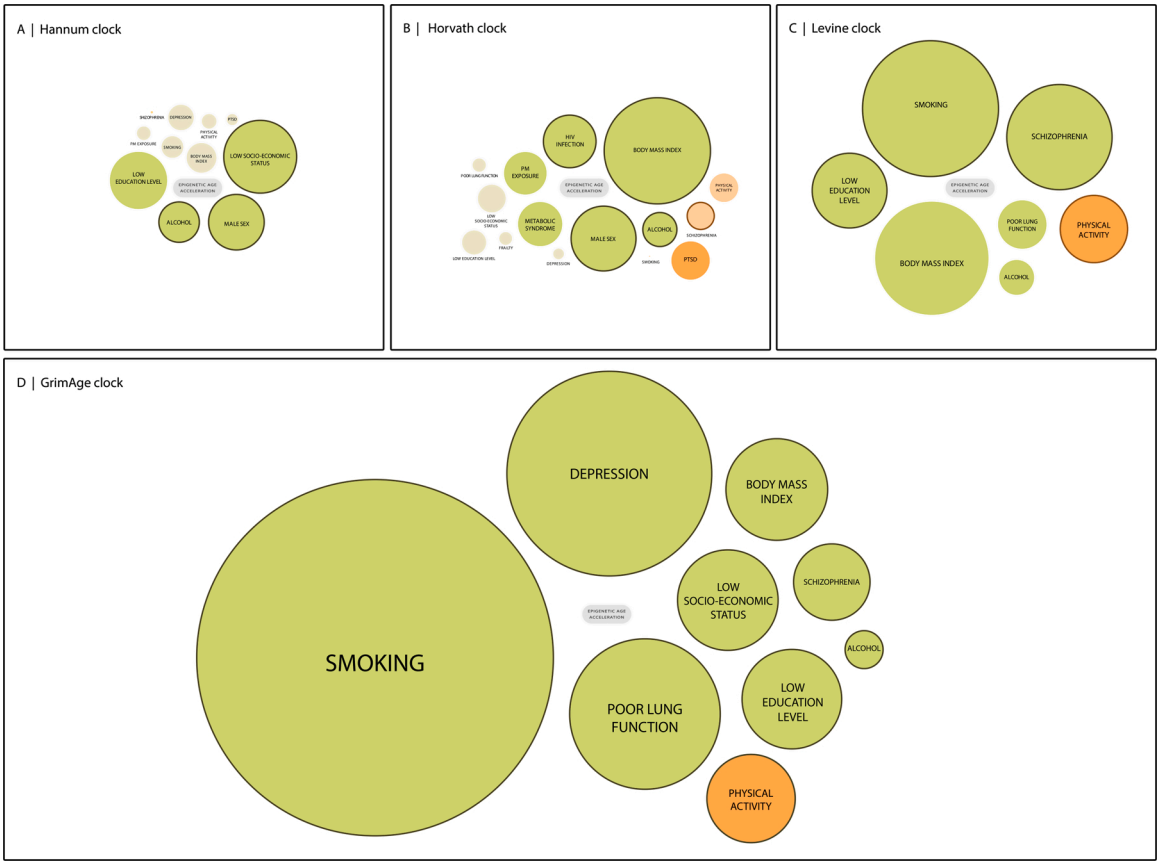


**Fig. 3.** Visual representation of the effect sizes found with the four epigenetic clocks. A: Hannum age acceleration. B: Horvath age acceleration. C: Levine age acceleration. D: GrimAge age acceleration. Circle diameter represents the calculated weighted z-value of the factor. The diameters are presented on an equal scale across all four panels to better visualize the effect sizes found across different clocks. Positive effect directions are represented in green colour; orange colour represents a negative effect direction. A dark outline indicates that a significant effect was also found in the meta-analysis. A visual representation of cancer, COPD, CVD, diabetes and mortality was constructed separately as these factors utilized a different category of effect size measures (see Fig. 4).

yielding a total set of 127 factors examined for their association with the rate of epigenetic aging. A total of 57 datasets of 20 distinct factors (relating to Horvath, Hannum, Levine and GrimAge clocks) contained sufficient observations for further analysis (Supplementary table C).

### 3.3. Weighted Z-scores

Among the 57 factors analysed, 42 factors passed the significance benchmark of +/− 1.96 in our weighted z-score dataset (Figs. 2 and 3, Supplementary Table D).

### 3.4. Meta-analysis

57 factors were meta-analysed. Among them, 36 showed statistically significant relationships with epigenetic age acceleration. These factors were $Alcohol_{GrimAge}$ (bicor = 0.037, p = 0.024), $Alcohol_{Hannum}$ (B = 0.217, p = 0.010), $Alcohol_{Horvath}$ (B = 0.193, p = 0.04), $BMI_{GrimAge}$ (bicor = 0.115, p < 0.001), $BMI_{Horvath}$ (B = 0.145, p = 0.001), $Cancer_{GrimAge}$ (HR = 1.054, p < 0.001), $Cancer_{Hannum}$ (HR = 1.012, p < 0.001), $Cancer_{Horvath}$ (HR = 1.014, p < 0.001), $Cancer_{Levine}$ (HR = 1.016, p < 0.001), $COPD_{GrimAge}$ (OR = 1.072, p < 0.008), $CVD_{GrimAge}$ (HR = 1.083, p < 0.001), $CVD_{Hannum}$ (HR = 1.024, p < 0.001), $CVD_{Horvath}$ (HR = 1.011, p < 0.001), $CVD_{Levine}$ (HR = 1,028, p < 0.001), $Depression_{GrimAge}$ (OR = 1.013, p < 0.001), $Diabetes_{GrimAge}$ (OR = 1.034, p < 0.001), $Diabetes_{Levine}$ (OR = 1.022, p < 0.001), $Education_{GrimAge}$ (bicor=-0.093, p < 0.001), $Education_{Levine}$ (B=-0.415, p = 0.038), $HIV_{Horvath}$ (B = 3.679, p = 0.014), $LungFunction_{GrimAge}$ (B = 0.154, p = 0.018), $MaleSex_{Hannum}$ (B = 1.246, p = 0.001), $MaleSex_{Horvath}$ (B = 1.079, p < 0.001), $Mortality_{GrimAge}$ (HR = 1.180, p < 0.001), $Mortality_{Hannum}$ (HR = 1.037, p < 0.001), $Mortality_{Horvath}$ (HR = 1.019, p < 0.001), $Mortality_{Levine}$ (HR = 1.046, p < 0.001), $Physical\ Activity_{GrimAge}$ (bicor=-0.073, p = 0.032), $Physical\ Activity_{Levine}$ (B=-0.245, p < 0.001), $Schizophrenia_{GrimAge}$ (B = 2.814, p = 0.011), $Schizophrenia_{Horvath}$ (B=-0.471, p = 0.02), $Schizophrenia_{Levine}$ (B = 1.299, p < 0.001), $SES_{GrimAge}$ (bicor = 0.105, p < 0.001), $SES_{Hannum}$ (B = 0.430, p < 0.001), $Smoking_{GrimAge}$ (bicor = 0.424, p < 0.001) and $Smoking_{Levine}$ (B = 0.866, p = 0.018) (Figs. 2 and 3, Supplementary Table D).

For binary variables such as sex, a B of 1.246 signifies that male sex is associated with a 1.246 increase in epigenetic age (as measured by the Hannum clock) compared to female sex. For continuous variables a one unit increase in the variable corresponds to a B-fold increase in epigenetic age.

## 4. Discussion

### 4.1. Summary of findings

This review systematically examined 156 studies and found strong associations of epigenetic clock acceleration with mortality and other social and clinical factors. On average, we observed a high coherence between the two methods of assessing effect sizes used in this review. There was an 86.0% agreement between the weighted z-score results and the meta-analytic results (Supplementary Table D). The level of agreement was calculated as the percentage of cases in which both methods have indicated the correlation as either significant or insignificant.

### 4.2. Associations with epigenetic clock acceleration

**Mortality** was the outcome most reliably correlated with accelerated epigenetic clocks. The correlation was tested in all four available clocks, and out of eight separate analyses, all showed a statistically significant effect. With mortality being the absolute outcome of the aging process, this result comes as no surprise and gives support to the underlying idea of epigenetic clocks reflecting the aging processes of an organism.

Several epigenetic clocks have been constructed to estimate mortality risk or time-to-death rather than biological age (Levine et al., 2018; Lu et al., 2019; Zhang et al., 2017), and we expect there will be many more studies published in the coming years confirming their strong associations with mortality. The mortality clocks may shed light on which age-related changes contribute to age-related morbidity and

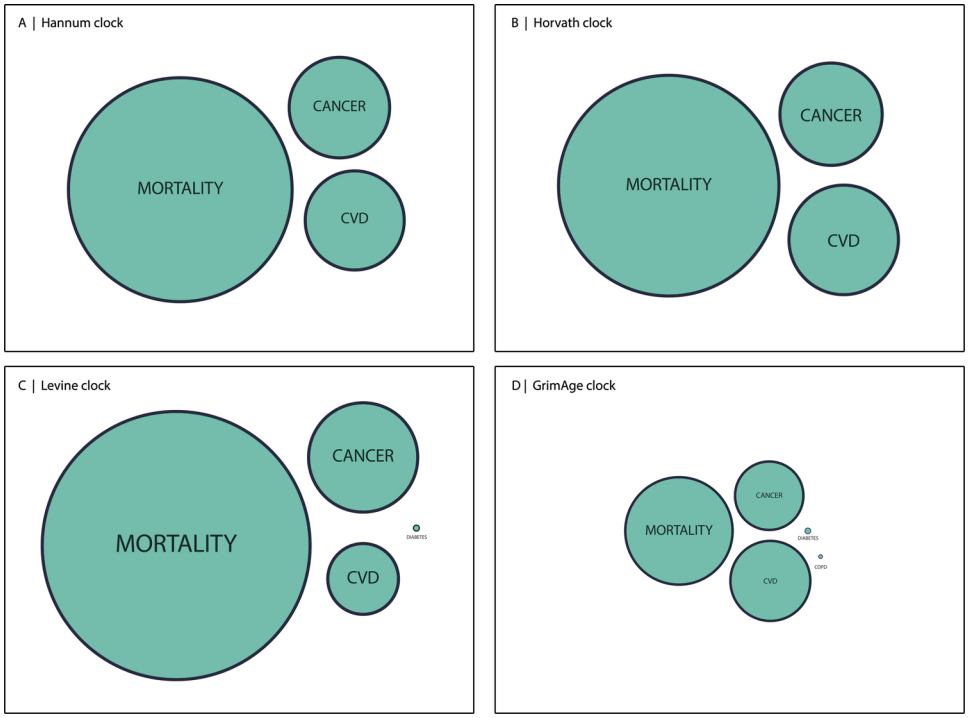


**Fig. 4.** Visual representation of the odds ratio and hazard ratio-based effect sizes found with the four epigenetic clocks. Circle diameter represents the calculated weighted z-value of the factor. The diameters are presented on an equal scale across all four panels to better visualize the effect sizes found across different clocks. A dark outline indicates that a significant effect was found in the meta-analysis. A: Hannum epigenetic clock, B: Horvath epigenetic clock, C: Levine epigenetic clock, D: GrimAge epigenetic clock.

mortality (as opposed to changes that are neutral or even beneficial), and may be particularly useful in guiding new treatments for age-related pathology.

**Male sex** was associated with increased epigenetic aging. This factor showed consistent effect sizes and statistical significance in all conducted analyses. This agrees with well-established observations that males have shorter lifespans, in spite of females experiencing higher rates of disability and poor health; a phenomenon known as the male-female health survival paradox (Alberts et al., 2014). Higher male epigenetic age could reflect greater rates of change, a higher baseline for epigenetic age, or some combination thereof depending on stage of life. A 20-year longitudinal study indicated higher male epigenetic age is already established by age 50, but average rates of epigenetic age change are not significantly different in males and females after the age 50 (Li et al., 2020). A possible explanation for this could be that males experience a faster pace of aging before age 50, whereas female aging increases in pace after menopause, and consequently minimizes this difference in epigenetic age between the sexes. Consistent with this theory, there is evidence that menopause increases epigenetic age (Levine et al., 2016). A faster male biological clock could be related to human males experiencing greater levels of age-associated genomic instability, including DNA methylation changes, though results are much more mixed in animal models (Fischer and Riddle, 2017). A more detailed comparative analysis of male and female epigenetic aging rates throughout the lifespan is warranted.

**Body Mass Index (BMI)** was correlated with increased epigenetic age according to the Horvath, Levine and GrimAge clocks, but not the Hannum clock. Indeed, it is possible to predict nearly 20 % of variance in BMI from DNA methylation (McCartney et al., 2018). The exact connection between BMI and methylation levels remains poorly understood, and it is difficult to determine a causal relationship. Furthermore, BMI itself is a result of many factors including adiposity, lean body mass, sex, nutrition, hormonal signalling, psychosocial factors, smoking and medications, and it is unclear which of these may mediate the connection between BMI and epigenetic age. However, data from a longitudinal study suggests that high BMI is a cause rather than a consequence of DNA methylation changes (Sun et al., 2019).

Closely related to BMI is **metabolic syndrome**, in which we observed a marginally significant relation with epigenetic age acceleration. In our weighted z-score analysis, we found a significant correlation between metabolic syndrome and epigenetic age acceleration, but this was not replicated in our meta-analytic approach. This was likely due to heterogeneity, as one study in young adults found a highly significant effect (Nannini et al., 2019), whereas the two other studies in older adults (de Toro-Martín et al., 2019; Gao et al., 2018) did not report a significant effect. Future studies should examine the relationship between epigenetic age and metabolic syndrome in different age groups, as well as adjust for covariates such as obesity and alcohol.

The relation between **physical activity** and epigenetic clocks was less uniform. The two mortality-trained clocks showed significant associations between physical activity and epigenetic clock deceleration in both the meta-analyses and the weighted z dataset. Horvath and Hannum clocks on the other hand did not show any significant associations; though the Horvath clock also showed a decelerating trend and was nearing the significance benchmark with its weighted z-score. Taken together, these results point toward epigenetic clock deceleration in relation to increased amounts of exercise. Further studies may confirm this trend, and may further support the benefits of physical activity for general health and many clinical conditions.

**Socio-economic status** impacts many aspects of physical and psychological health, and the correlation between low SES and shorter life expectancy has been observed repeatedly (Robertson et al., 2013). A logical expectation would therefore be an age acceleration in groups with lower SES. This was indeed the case in the Hannum and GrimAge clocks, while the effects of the Horvath clock did not reach statistical significance (albeit still showed a trend of age acceleration in lower SES). Analysis of the effects of **education level,** which typically correlates positively with SES, unveiled age acceleration in response to lower education in the Hannum, Levine and GrimAge clocks. Again, the Horvath estimator showed a concordant effect direction, but was not statistically significant. Given that the GrimAge and Levine clocks show more stable effects across our two analyses, they may be better suited for the appraisal of SES and education. Further studies are needed to corroborate this.

**Alcohol use** (including hazardous drinking and alcohol use disorder) is a major factor contributing to shorter life expectancy and increased risk of aging-related conditions, especially cardiovascular disease, cancer, and liver disease (Stevenson, 2005). However, alcohol's relationship to epigenetic clock acceleration is less clear and appears to be nonlinear (Beach et al., 2015), with age acceleration seen at low and high levels of alcohol intake, but no clear effects observed in moderate drinking. These nonlinear results may mirror the trends found in research connecting alcohol intake to general health outcomes (Griswold et al., 2018; Kunzmann et al., 2018). In our review, all four investigated clocks pointed to accelerated aging in alcohol users. Out of eight analyses conducted, seven were statistically significant, with the exception of the Levine meta-analysis. In further research, alcohol consumption should be carefully phenotyped and analysed for both linear and nonlinear effects on epigenetic age acceleration, ideally in a standardized way to further enhance comparison across studies.

**Smoking** is a leading risk factor for many major age-related diseases, especially cancer, cardiovascular disease, and chronic obstructive pulmonary disease (Lee and Pausova, 2013). It is well-established that smoking alters DNA methylation (Ambatipudi et al., 2016; Elliott et al., 2014; Wan et al., 2012), allowing for the construction of various robust methylation-based smoking scores (Elliott et al., 2014; Teschendorff et al., 2015; McCartney et al., 2018; Lu et al., 2019). In fact, these smoking scores can be better predictors of mortality than self-reported smoking (Lu et al., 2019), and are becoming standard smoking assessments to complement self-report in research studies. The effects of smoking found in our review were mixed. We once again observed that the two mortality clocks showed significant age acceleration in all analyses, while the chronological clocks did not reach significance in any of the analyses. This result is consistent with prior comparisons (Horvath and Raj, 2018). It is not surprising that Horvath and Hannum do not significantly reflect smoking, as they are meant to predict chronological age. In order to assign the same value to same-age subjects, Horvath and Hannum must largely exclude smoking-related methylation changes, as these would create variation among same-age subjects. In contrast, Levine was designed to predict mortality (through an intermediate measure termed phenotypic age), so it would be expected to be influenced by smoking. A smoking methylation proxy is a component of the GrimAge clock, which explains its strong correlations with smoking found in this review.

**HIV infection** causes signs of premature aging, especially early dementia. Infected patients are now achieving longer life expectancy due to antiretroviral therapy. However, in spite of the absence of AIDS related symptoms, these patients are experiencing an increased rate of diseases typically associated with aging. Only the Horvath clock had sufficient studies for our analysis, but we confirmed prior results that the Horvath clock is accelerated in HIV infection (Horvath and Levine, 2015). Accelerated epigenetic aging in HIV was further confirmed by several other research works which did not advance to the analysis phase of our data collection (Boulias et al., 2016; Gross et al., 2016; Horvath et al., 2018). Accelerated epigenetic aging in both blood and brain may be related to immune system changes as well as DNA double-strand breaks induced by the HIV virus, but further studies are needed (Horvath and Levine, 2015).

**Particulate matter (PM) exposure** from air pollution may affect aging, particularly extrinsic skin aging (Ding et al., 2017; Vierkötter et al., 2010; Park et al., 2018). PM exposure was previously linked to epigenetic aging (White et al., 2019; Wang et al., 2020). Our synthesis of

this correlation however only revealed a significant age acceleration effect in one of the four conducted analyses (the Horvath z-score). This could be due to heterogeneity in PM classification within different publications. The studies included in our analyses used three measures of PM exposure: PM2.5, PM10 and cadmium exposure. It should also be noted that research in the field of PM exposure is thus far much more abundant when investigating short term effects (Wyzga and Rohr, 2015), whereas a controlled longitudinal study design would be best fitted to assess the possible detrimental effects of particulate matter exposure on aging.

**Frailty** is a hallmark consequence of biological aging, and is widely used in assessment of age-related fitness. Our review only allowed us to conduct an analysis of frailty in Horvath clock literature, which somewhat surprisingly did not yield any significant effects. A possible explanation for this could be the variation in the frailty assessments of the studies included in the analysis: these included measures of hand grip strength as well as composite frailty index scores.

Aging is a strong risk factor for **cancer** (White et al., 2014; Centers for Disease Control and Prevention, 2021). Accordingly, our review identified a significant correlation between accelerated epigenetic age and future cancer incidence in all four clocks, with both z-scores and meta-analyses showing significant effects for every single age estimator. It is also notable that DNA methylation is profoundly altered in cancer cells (Fraga et al., 2007; Fraga and Esteller, 2007; Horvath, 2013). Others have constructed epigenetic clocks to predict "mitotic age" or the number of cell divisions in a tissue (Yang et al., 2016; Youn and Wang, 2018). Mitotic age is accelerated in tissues exposed to carcinogens, precancerous tissues and cancer. In the current study, the relationship between epigenetic age acceleration and incidence of any type of cancer was analysed based exclusively on blood tissue. However, it will be important to further characterize relationships with specific cancer types across different tissues, considering the highly heterogeneous epidemiology and molecular mechanisms of cancer. Tissue-specific age estimation could aid future efforts for early cancer screening, diagnosis and treatment.

Similar to the findings in cancer, **cardiovascular disease** was significantly related to all four epigenetic clocks using both of our datasets. Levine and GrimAge estimators, along with other mortality clocks, may be particularly suited to detecting cardiovascular disease and cancer, as these are the most common causes of death at older ages.

Altered DNA methylation patterns in **diabetes** patients compared to controls are well-established and are an active area of research (Ling and Rönn, 2019). Multiple studies previously found roles for epigenetic aging in diabetes pathogenesis, using tissues relevant to diabetes, such as adipose tissue, skeletal muscle, liver tissue and pancreatic islets (Bacos et al., 2016; Bysani et al., 2017; Ling et al., 2007; Ronn et al., 2015). In agreement, all analyses of the GrimAge and Levine clocks showed a significant correlation between the accelerated epigenetic age and diabetes in blood tissues.

In **lung function**, our results showed a consistent correlation of epigenetic clock acceleration with a decrease in lung function measured by forced expiratory volume (FEV). Our results have shown significant effects in both analyses of GrimAge data and in the z-score analysis of the Levine clock, but did not reach significance in studies using Horvath. These results are consistent with an age-related decrease in lung function, a well-documented consequence of aging (Lowery et al., 2013; Sharma and Goodwin, 2006; Thomas et al., 2019). Consistent with lung deterioration contributing to **chronic obstructive pulmonary disease (COPD)** (Kukrety et al., 2018), a positive correlation between COPD and accelerated epigenetic age was shown in our data.

This review has revealed somewhat unusual results concerning the epigenetic effects in relation to **PTSD** diagnosis. PTSD is positively correlated with acceleration of the Hannum clock, but deceleration in the Horvath clock, though only the Horvath z-score was statistically significant. The small overall effect sizes reported for PTSD compared to other health outcomes point to the conflicting trends found by primary research so far. Two studies included in our dataset both found the same pattern with regard to the correlation of trauma and PTSD symptoms to epigenetic clocks (Boks et al., 2015; Verhoeven et al., 2018): age acceleration was seen in traumatized participants without PTSD, whereas age deceleration was seen in those with a PTSD diagnosis. Another recent study by Yang et al. (2020) has performed multiple epigenetic clock assessments in a single cohort and has found a significant age acceleration effect in the GrimAge clock, but noted a negative correlation between PTSD and the Horvath, Levine and Hannum clocks. Previous research has shown that PTSD diagnosis is related to an increased all-cause mortality risk (Boscarino, 2006). However, it is worthy to note that a majority of the PTSD studies utilized healthy (that is, non-traumatized) control subjects, which may have tangled the role of PTSD in aging with that of traumatic stress. These results, together with our seemingly conflicting findings, lay an important foundation for further research into the exact nature of PTSD and its biological consequences on the aging organism.

Similarly, complex results are present for **schizophrenia**. A statistically significant positive correlation between advanced epigenetic age and schizophrenia in the Levine and GrimAge clocks, and a significant negative correlation was found in the Horvath clock. The Hannum clock also indicated a negative correlation, but did not reach statistical significance. A recent study from the authors of this review pointed out that several past studies using the Horvath clock found no evidence of age acceleration, in spite of various evidence involving other biomarkers and clinical outcomes that supported the age-acceleration hypothesis of schizophrenia (Higgins-Chen et al., 2020). These observations were addressed by simultaneously examining many different types of epigenetic clocks in three schizophrenia cohorts. The authors found consistent evidence of acceleration in mortality clocks including GrimAge and Levine related to smoking and proteins dysregulated in schizophrenia, but deceleration in mitotic clocks, indicating lower cancer risk related to anti-tumour immune cells. Chronological age clocks including Horvath and Hannum were specifically decelerated in schizophrenia patients receiving clozapine, which may explain the negative correlation we see in this review. Importantly, the various findings were consistent with the existing literature on schizophrenia, including higher all-cause mortality, lower cancer risk and unique clozapine effects, suggesting the epigenetic clocks can indeed be used to study aging in schizophrenia. However, the study by Higgins-Chen suggests different aspects of schizophrenia are linked to different types of epigenetic clocks, and future studies should be designed to account for this complexity.

In **depression**, the GrimAge clock showed a statistically significant positive correlation with increased epigenetic age, while no visible effect could be seen in the Horvath and Hannum clocks, showing a trend much like the one observed in schizophrenia. It would be valuable to implement a study design similar to the Higgins-Chen et al. (2020) schizophrenia study to assess similar trends in depression. One could then link specific factors associated with depression, such as symptomatology, substance use, medications, psychosocial factors or medical co-morbidities to different epigenetic clocks.

It is noteworthy that differences between chronological age-trained clocks and mortality-trained clocks are most prominent in psychiatric outcomes, while the clocks are relatively synchronized the effect directions of physical factors. This could partly reflect greater heterogeneity and complexity of mental health conditions. It would be valuable to focus future research efforts on the nature of this divergence, to uncover novel insights into highly complex psychiatric conditions. Conversely, comparative analyses of the clocks in psychiatric disorders could offer the chance to dissect the shared and distinct biological mechanisms underpinning different epigenetic clocks.

## 5. Limitations

The most prominent limitation of our review were the inconsistencies in effect size reporting and methodology. Our datasets

contained several different effect size measures, some of which could not be compared (e.g., we could not reliably compare a B coefficient with an odds ratio). We consequently decided to only analyse the most frequently occurring effect size measure within each dataset, which constricted the amount of data ultimately available for individual analyses. We have made an effort towards minimizing this issue in our analysis, but (as many others before us) urge towards a more systematic and complete approach to result reporting, as it is a small but immensely crucial step towards facilitating further meta-analytic research and data synthesis.

Due to the nature of the topic, the majority of the reviewed literature used regression models, and consequently reported regression coefficients as their measure of effect size. These statistics are influenced by the specific covariates used in their respective models, which differed among different studies, and are hence not entirely the same. This is a particular problem considering that many variables associated with the clocks are collinear, making it difficult to tease out their independent effects. Additionally, interactions among different factors are not well reported.

Our analysis criteria demanded observations in at least 3 cohorts per factor, which in some cases allowed large, multi-cohort publications to contribute a significant amount of data to our analyses. One such case was an extensive study conducted by Hillary et al. (2020). Its multi-cohort report resulted in this publication being the sole source of data for 4 of the 57 analysed factors, namely $COPD_{GrimAge}$, $Depression_{GrimAge}$, $Diabetes_{GrimAge}$, and $LungFunction_{GrimAge}$. This also occurred in the $PTSD_{Hannum}$, $SES_{GrimAge}$, $Schizophrenia_{GrimAge}$ and $Schizophrenia_{Hannum}$ datasets, where the sole contributing publications were Wolf et al. (2017); Lu et al. (2019); Higgins-Chen et al. (2020) and Ori et al. (2019), respectively. While we were able to synthesise multiple sources in the majority of our analyses, we point the reader towards the aforementioned publications for further information regarding the factors listed here.

It must also be noted that the threshold for analysis - a minimum of 3 separate cohorts using the same epigenetic clock - may have neglected otherwise relevant results. While the Horvath clock remains the most frequently used, other clocks have also shown promising results and should not be overlooked. An additional explanation for this imbalance is likely the amount of time that has passed since the creation of each clock, as newer clocks have had less time to accumulate literature.

A final limitation pertains to the fact that due to the number of factors and clocks that have been studied, type I error may be inflated, as no adjustment for multiple comparison has been implemented.

## 6. Conclusions

The associations of environmental and clinical factors with epigenetic clocks presented here are welcome results, as they point to the relevant biology that CpG-based age estimators are able to tap into, and represent a relevant target for the future of geriatric and developmental research.

The biological mechanisms underlying the epigenetic clocks are active areas of investigation, including why exposures might lead to epigenetic age acceleration, and why epigenetic aging can predict morbidity and mortality. Ultimately, a molecular and cellular understanding of the clocks (Raj and Horvath, 2020; Bell et al., 2019) will need to be linked to other biological hallmarks of aging, phenotypic aging, and functional aging (Ferrucci and Orini, 2018; Han et al., 2019; López-Otín et al., 2013). Further investigation must also be made into why various clocks differ in their relationships with the factors reported here.

By identifying relationships not only on the clock-level, but on the level of smaller subsets of CpG sites, and by gathering longitudinal experimental data, the epigenetic clocks may help us understand the factors that play a role in aging in even more detail. Epigenetic clocks are therefore a promising research tool for the coming years, and may bring us closer than ever to understanding the complexities of aging.

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## Declaration of Competing Interest

The authors report no declarations of interest.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.arr.2021.101348.

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