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Differential white blood cell count and epigenetic clocks: a bidirectional Mendelian randomization study

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Abstract

Background Human aging and white blood cell (WBC) count are complex traits influenced by multiple genetic factors. Predictors of chronological age have been developed using epigenetic clocks. However, the bidirectional causal effects between epigenetic clocks and WBC count have not been fully examined.

Methods This study employed Mendelian randomization (MR) to analyze summary statistics from four epigenetic clocks involving 34,710 participants, alongside data from the Blood Cell Consortium encompassing 563,946 individuals. We primarily explored bidirectional causal relationships using the random-effects inverse-variance weighted method, supplemented by additional MR methods for comprehensive analysis. Additionally, multivariate MR was applied to investigate independent effects of WBC count on epigenetic age acceleration.

Results In the two-sample univariate MR (UVMR) analysis, we observed that a decrease in lymphocyte count markedly accelerated aging according to the PhenoAge, GrimAge, and HannumAge metrics (all $P < 0.01$, $\beta < 0$), though it did not affect Intrinsic Epigenetic Age Acceleration (IEAA). Conversely, an increase in neutrophil count significantly elevated PhenoAge levels (β : 0.38; 95% CI 0.14, 0.61; $P = 1.65E-03 < 0.01$). Reverse MR revealed no significant causal impacts of epigenetic clocks on overall WBC counts. Furthermore, in multivariate MR, the impact of lymphocyte counts on epigenetic aging metrics remained statistically significant. We also identified a marked causal association between neutrophil counts and PhenoAge, GrimAge, and HannumAge, with respective results showing strong associations (PhenoAge β : 0.78; 95% CI 0.47, 1.09; $P = 8.26E-07$; GrimAge β : 0.55; 95% CI 0.31, 0.79; $P = 5.50E-06$; HannumAge β : 0.42; 95% CI 0.18, 0.67; $P = 6.30E-04$). Likewise, eosinophil cell count demonstrated significant association with HannumAge (β : 0.33; 95% CI 0.13, 0.53; $P = 1.43E-03 < 0.01$).

Conclusion These findings demonstrated that within WBCs, lymphocyte and neutrophil counts exert irreversible and independent causal effects on the acceleration of PhenoAge, GrimAge, and HannumAge. Our findings highlight the critical role of WBCs in influencing epigenetic clocks and underscore the importance of considering immune parameters when interpreting epigenetic age.

Keywords Mendelian randomization (MR), White blood cell, Lymphocyte cell, Neutrophil cell, Aging, Epigenetic age acceleration

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Introduction

Aging in humans is a multifaceted process involving the gradual loss of physiological integrity, diminished immune function, and increased susceptibility to mortality over time [1]. This immune deterioration with age leads to heightened vulnerability to infections, reduced vaccine efficacy, and a greater risk of conditions like osteoporosis, Alzheimer's disease, and cancer in elderly populations [2, 3].

Peripheral white blood cell (WBC) counts serve as essential indicators for evaluating inflammatory, immune, allergic, and hematological states [4]. The five primary types of WBCs—lymphocytes, basophils, eosinophils, monocytes, and neutrophils—are pivotal in both innate and adaptive immune responses. These cells engage in complex interactions, functioning as an integrated system within the immune framework to mount a comprehensive defense against pathogens. Specifically, lymphocytes, which encompass T cells, B cells, and NK cells, are central to the adaptive immune response [5]. Monocytes can differentiate into macrophages or dendritic cells; macrophages are phagocytic cells that engulf and digest pathogens, while dendritic cells help activate other immune cells [6]. Basophils, although present in the smallest quantities in the blood, are involved in the inflammatory responses [7]. Eosinophils are particularly important in fighting parasitic infections and are also involved in allergic reactions and asthma [8]. Neutrophils, the most abundant type of WBC, are essential to the body's innate immune system [9].

Empirical data corroborate the link between aging and variations in WBC count. Notably, research has shown that the absolute count of lymphocytes diminishes with age [10, 11]. Among lymphocyte subtypes, T cells and B cells exhibit a decline, while natural killer (NK) cells tend to increase with aging [12]. Neutrophil and monocyte counts also increase with age [13], while eosinophils and basophils remain relatively unchanged throughout life [12]. A multi-omic study suggested that immune cells are associated with epigenetic clocks through cell-type enrichment analysis [14]. In a cohort of 2996 women from Quebec aged from 55 to 101 years, it was demonstrated that aging is associated with elevated neutrophil and monocyte counts, while lymphocyte count decreases [13].

Epigenetic clocks have emerged as effective indicators of biological age, providing insights that may differ from chronological age. These clocks are based on DNA methylation patterns at specific CpG sites that correlate with aging [15]. The first-generation epigenetic clocks, such as HannumAge, trained on blood samples [16], and Intrinsic HorvathAge, a multi-tissue predictor [17], exhibit strong correlations with chronological age. The

second-generation clocks, including PhenoAge [18] and GrimAge [19], go beyond estimating chronological age by integrating data on disease and mortality risk factors, such as smoking habits, plasma protein levels, and WBC counts, to better predict health outcomes and longevity. Although epigenetic clocks were developed using bulk methylation data, which is inherently confounded by WBC counts, the causal relationship between epigenetic clocks and WBC counts remains relatively unexplored.

Mendelian randomization (MR) offers a method to establish causality between exposures and outcomes through genetic variants [20]. UVMR (two-sample univariable MR) uses two different samples to focus on one exposure variable, while multivariate MR (MVMR) concurrently investigates the causal effects of multiple exposures on an outcome. McCartney et al. utilized the two-sample MR approach to study the genetic predictors of lymphocyte count on epigenetic age acceleration [21]. Expanding on this analysis, we incorporate additional common WBC counts (neutrophil, monocyte, eosinophil, and basophil counts), leveraging the latest aggregated data, including from the Blood Cell Consortium, which integrate meta-analysis from the UK Biobank and other cohorts [22–24] (Additional file 3: Supplementary Table 1–2).

Method

Study design

To ensure unbiased estimates in MR, we adhered to three essential assumptions [25]. First, the genetic variants used as instruments must exhibit a strong correlation with the exposure. Second, these genetic instruments must be unaffected by any confounders that could affect the outcome. Third, the instruments should influence the outcome only through the exposure.

In our research, two-sample univariable MR was employed to investigate the bidirectional causal relationships between WBC counts and epigenetic age acceleration. Additionally, multivariate MR analyses were used to explore the independent effects of each WBC subtype on outcomes due to the interactions among the five different subtypes. Figure 1 showcases the design for the MR study.

Data sources

We sourced extensive genome-wide association study (GWAS) summary data on blood traits for a sizable cohort ($N=563,946$) from the Blood Cell Consortium, accessible via IEU Open GWAS Project. This consortium encompasses an additional 26 discovery cohorts beyond the UK Biobank [22–24]. Furthermore, GWAS summary statistics for four epigenetic age measures (GrimAge, PhenoAge, intrinsic epigenetic

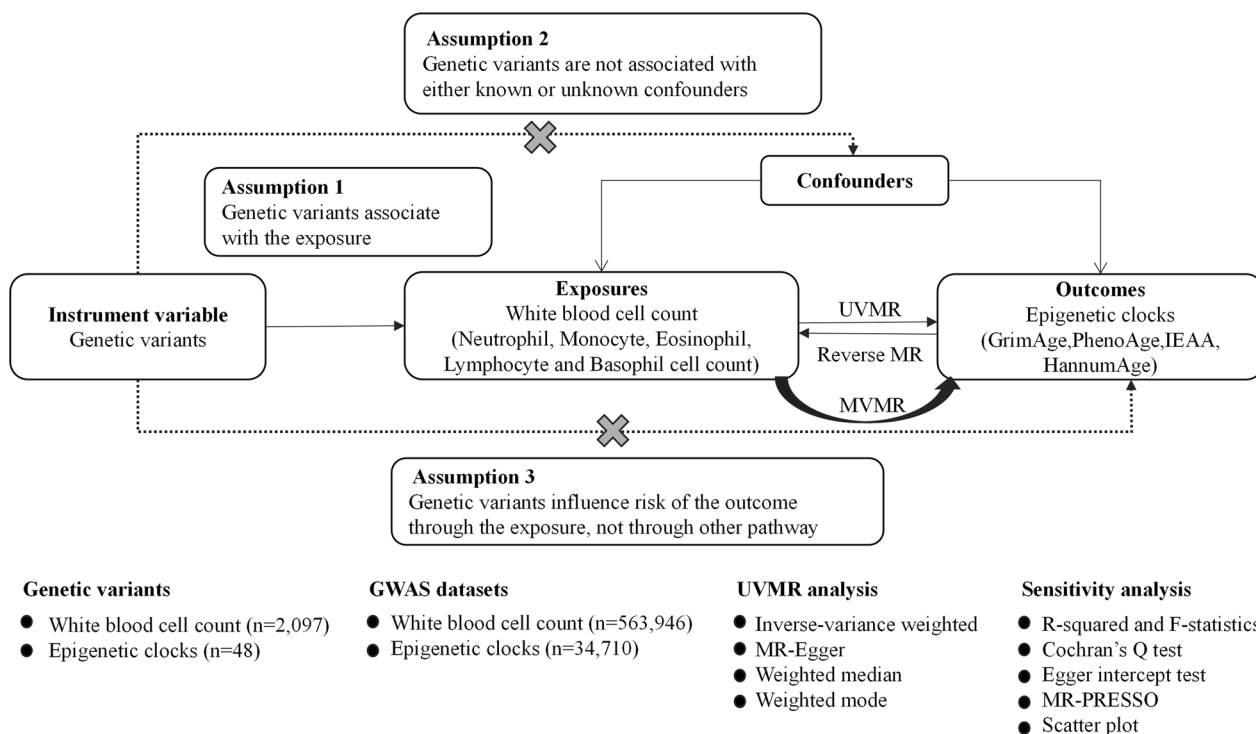


Fig. 1 Study design of this MR study. IEAA intrinsic epigenetic age acceleration, GWAS genome-wide association study, UVMR univariate MR, MVMR multivariate MR, MR-PRESSO Mendelian randomization pleiotropy residual sum and outlier

age acceleration (IEAA), and HannumAge) involving 34,710 participants were obtained from 28 cohorts through Edinburgh DataShare [21]. The GWAS analyses included in this research are all based on individuals of European heritage.

Instrumental variables selection

Single-nucleotide polymorphisms (SNPs) exhibiting strong associations with the exposure variable were identified using the GWAS significance criterion ($P < 5E-08$). To derive independent instrumental variables (IVs), we applied clumping procedures with the standard linkage disequilibrium (LD) parameters ($r^2 < 0.001$, distance > 10000 kb) utilizing the LD reference dataset from the 1000 Genomes Project. Following this step, SNPs that were palindromic or ambiguous were excluded to prevent potential misalignment issues during genotype imputation and to ensure the accuracy of our genetic association estimates. Additionally, SNPs showing significant association with epigenetic clocks ($P < 5E-05$) were discarded to avoid bias. Finally, we calculated the R -squared (R^2) and F -statistics to gauge the reliability of the instrumental variables, with F -statistics exceeding 10 considered indicative of robust instruments [26].

MR statistical analysis

In our MR analysis, the random-effects IVW method was utilized as the main method, presenting odds ratios (ORs) in relation to a standard deviation (SD) increment in risk factor levels [27]. The significance threshold for Bonferroni correction was set at $P = 0.05/N$, where N is the cumulative count of exposures assessed. The statistical treatments were implemented using R version 4.3.2 and TwoSampleMR package.

To ensure the reliability of our IVW MR analysis, we employed MR-Egger [28], weighted median [29], and mode-based [30] analyses. Specifically, we firstly employed the MR-Egger intercept to evaluate and adjust for potential horizontal pleiotropy (HP) among genetic variants [31]. We then applied Cochran's Q test ($P < 0.05$) to evaluate heterogeneity among the chosen IVs [32]. Moreover, the MR-PRESSO technique was implemented using the "MR-PRESSO" software package to detect and adjust for HP and outliers [33]. A scatter plot was generated to visually identify any outliers.

MVMR was then applied to independently ascertain the impact of each WBC count on epigenetic age acceleration.

IVW estimates were deemed to reflect causal associations solely if they were consistent in direction and statistical significance with at least one of the sensitivity analyses and displayed no evidence of HP ($P > 0.05$).

Result

UVMR analysis of the bidirectional causal relationship between white blood cells and epigenetic age acceleration

To investigate the causal effect of WBC count on epigenetic age acceleration, we employed the random-effects inverse-variance weighted (IVW) approach as our

primary analysis. The selection of SNPs was based on summarized GWAS datasets, with the involvement of these SNPs in each analytical step outlined in Additional File 1: Supplementary Tables 1–20. All the F -statistics associated with these SNPs exceeded 10, confirming their strength as IVs. Our analysis presented robust evidence for a causal effect of lymphocyte cell count on epigenetic markers such as GrimAge, HannumAge, and PhenoAge (GrimAge IVW β : -0.52 ; 95% CI $-0.68, -0.37$; $P = 2.51E-11$; HannumAge IVW β : -0.59 ; 95% CI $-0.75, -0.43$; $P = 2.08E-13$; PhenoAge IVW β : -0.76 ; 95% CI

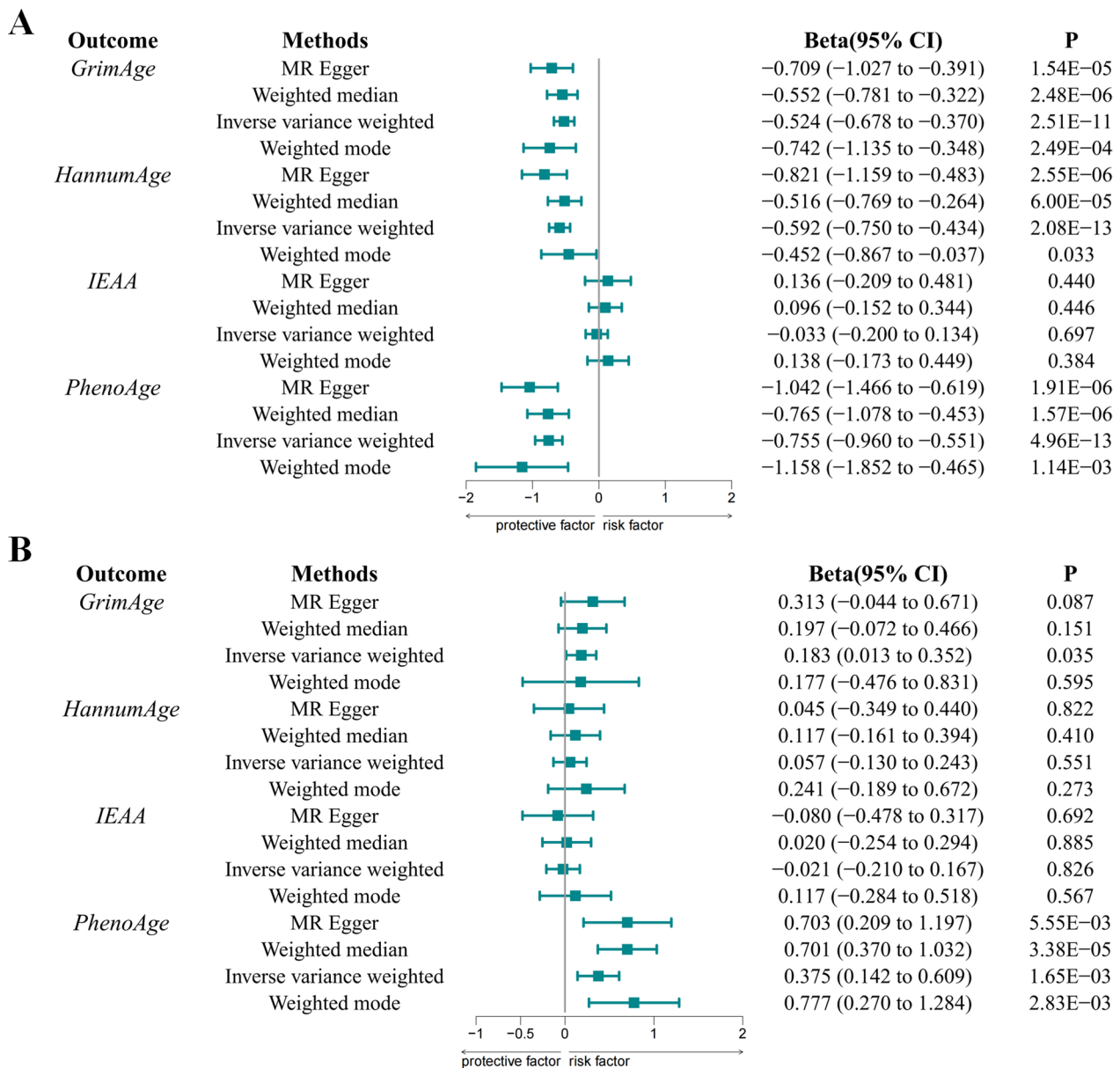


Fig. 2 IVW MR estimates for genetically predicted effects of lymphocyte cell count (A) and neutrophil cell count (B) on epigenetic clocks. MR Mendelian randomization, IEAA intrinsic epigenetic age acceleration

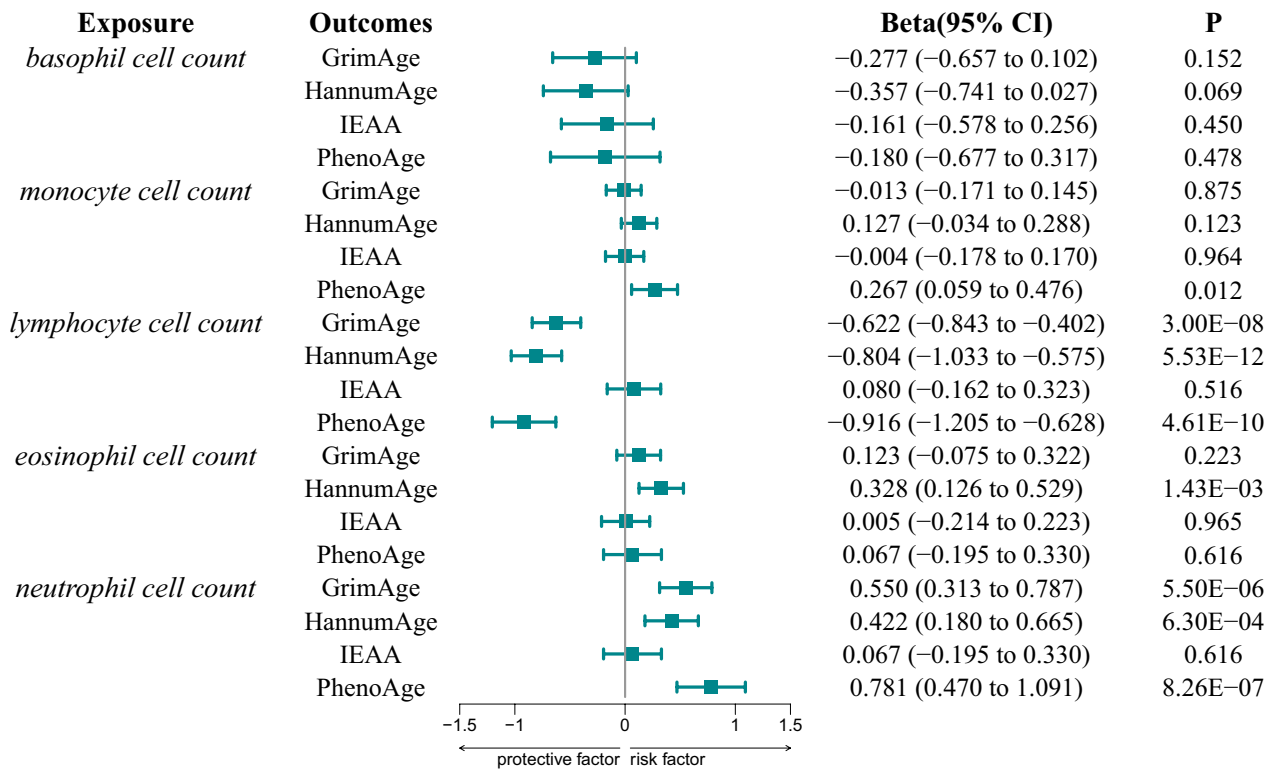


Fig. 3 MVMR analysis estimating effect of each white blood cell subtype on epigenetic age acceleration conditioning on other four WBC subtypes

-0.96, -0.55; $P=4.96E-13$). After adjusting for multiple testing with a significance threshold set at $P<(0.05/5)$, all P values from the IVW MR analysis and the accompanying sensitivity analyses were significant. Moreover, the directional consistency between the primary analyses and sensitivity analyses (MR-Egger, weighted median, and weighted mode) was confirmed. However, no significant association was found between lymphocyte cell count and IEAA (IVW β : -0.03; 95% CI -0.20, 0.13; $P=0.697$) as depicted in Fig. 2A and detailed in Additional File 1: Supplementary Table 21. Overall, a decrease of 1 SD in lymphocyte cell count corresponds to an increase of 0.52 years in GrimAge, 0.59 years in HannumAge, and 0.76 years in PhenoAge. Similarly, substantial evidence indicated that an increase of 1 SD in neutrophil cell count could result in an elevation of PhenoAge acceleration by 0.38 years (IVW β : 0.38; 95% CI 0.14, 0.61; $P=1.65E-03 < 0.01$). Moreover, the estimated effects observed in the primary analyses were in agreement with the outcomes of the sensitivity analyses. The causal effects identified in all analyses met the criteria for statistical significance, even after correcting for multiple testing using the Bonferroni correction ($P < 0.01$), as illustrated in Fig. 2B and detailed in Additional File 1: Supplementary Table 22. We did not find a causal effect of

basophil cell count, monocyte cell count, and eosinophil cell count on epigenetic clocks (Additional File 1: Supplementary Table 23–25).

While Cochran’s Q -statistic and MR-PRESSO analysis revealed notable heterogeneity ($P < 0.05$) (Additional File 1: Supplementary Tables 26, 27), the causal estimates remained reliable under the random-effects IVW method [34]. Furthermore, the Egger intercept test did not detect any HP (all $P > 0.05$) (Additional File 1: Supplementary Table 26), and scatter plots additionally confirmed the stability of these findings (Additional File 2).

In the reverse MR analysis, we identified 4 SNPs as IVs for GrimAge, 9 for HannumAge, 24 for IEAA, and 11 for PhenoAge (Additional File 1: Supplementary Tables 28–47). The IVW analyses revealed no major causal relationship between epigenetic clocks and WBC counts, with all P -values exceeding 0.0125 (Additional File 1: Supplementary Tables 48–51).

MVMR analysis of the causal relationship between white blood cells and epigenetic age acceleration

In our analysis, we employed MVMR to examine the causal effect of WBC counts on the acceleration of aging as measured by epigenetic clocks. The findings highlighted that lymphocyte counts maintain a significant

association with GrimAge, HannumAge, and PhenoAge (GrimAge β : - 0.62; 95%CI - 0.84, - 0.40; P = 3.00E-08; HannumAge β : - 0.80; 95% CI - 1.0, - 0.58; P = 5.53E-12; PhenoAge β : - 0.92; 95% CI - 1.21, - 0.63; P = 4.61E-10). Notably, we also observed that neutrophil cell count was significantly related to PhenoAge (β : 0.78; 95% CI 0.47, 1.09; P = 8.26E-07), as well as GrimAge and HannumAge (GrimAge β : 0.55; 95% CI 0.31, 0.79; P = 5.50E-06; HannumAge β : 0.42; 95% CI 0.18, 0.67; P = 6.30E-04) (Fig. 3, Additional File 1: Supplementary Table 52). All P values passed multiple testing correction (P = 0.05/5 = 0.01). Likewise, eosinophil cell count demonstrated a significant association with HannumAge (β : 0.33; 95% CI 0.13, 0.53; P = 1.43E-03 < 0.01) (Additional File 1: Supplementary Table 52).

Discussion

To the best of our knowledge, this study represents the first comprehensive exploration into the causal effect of WBC count on epigenetic clocks. By employing bidirectional UVMR and MVMR methods, we found that decrease of 1 SD in lymphocyte cell count or an increase in of 1 SD in neutrophil cell count significantly accelerates GrimAge, HannumAge, and PhenoAge. These causal effects persist even after adjusting for the other WBC subtypes, suggesting their effects are independent of other WBC types. Our results are in harmony with the observed shift from lymphoid to myeloid lineage in hematopoiesis [35] and corroborate findings in the literature, notably the study by McCartney et al., which identified potential causal impacts of lymphocyte cell count on the acceleration of PhenoAge, GrimAge, and HannumAge, but not on IEAA [21]. The IEAA clock, derived from Horvath's multi-tissue predictor, adjusts for DNA methylation-based estimates by factoring out influences from WBCs. Although we did not find significant causal associations between any WBC types and IEAA, this result actually supports the effectiveness of IEAA in mitigating the confounding effect of WBC counts. Through these findings, we aim to provide further empirical support for the use and interpretation of IEAA, thus enhancing its reliability in aging research. The association between aging and immune system, termed immunosenescence, has been well studied. Aging is associated with a decrease in naive immune cells and an increase in activated immune cells [36]. From age 20 to 70, the population of naive T cells (CD4+ and CD8+) decreases approximately 100-fold [37, 38]. A functional genomics analysis study has reported associations between epigenetic clocks and lymphocyte T and NK cells [39]. We infer that decline of native immune cells might lead to aging,

warranting further exploration. Additionally, an enrichment of basophil cell count was noted in the GWAS outcomes for IEAA and GrimAge in a prior study [21]. However, no significant relationship was found in our study. We did find a causal effect of eosinophil cell count on HannumAge. Moreover, our analysis revealed no significant causal relationships between epigenetic clocks and WBC count, indicating that aging does not result in notable alterations in WBC counts.

However, our study has several limitations. Firstly, although WBC counts possess characteristics of a stable trait to some extent, they vary over time, including fluctuations within one day, potentially leading to errors [40]. Secondly, our study results pertain primarily to European population. Further investigations are needed to examine other populations. Thirdly, as WBC play a crucial role in immune response and inflammatory processes, future studies should consider broadening the scope by including inflammation factors to comprehensively investigate their potential effects in these causal pathways.

In summary, our research provides additional evidence of a potential genetic causal link between WBC count and the acceleration of epigenetic age. Nevertheless, further research could investigate the impact of more specific cell subtypes or other biomarkers on epigenetic age.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13148-024-01717-8>.

Additional file 1.
Additional file 2.
Additional file 3.

Acknowledgements

We thank the Blood Cell Consortium (BCX) and McCartney et al. for making GWAS summary statistics data publicly available. We thank the editors and the reviewers for their helpful suggestions that improved this article.

Author contributions

M. Sun designed research and analyzed data; H. Yang conducted research, performed statistical analysis, and wrote the article; Y. Hu provided idea on the research; J. Fan, M. Duan, J. Ruan, S. Li, Y. Xu helped proofread the manuscript; Y. Han has primary responsible for final content; and all authors read and approved the final manuscript.

Funding

Not applicable.

Availability of data and materials

Summary statistics for epigenetic age acceleration measures of HannumAge, Intrinsic HorvathAge, PhenoAge and GrimAge are available in the Edinburgh DataShare (<https://datashareed.ac.uk/handle/10283/3645>). Summary statistics for white blood cell count can be openly accessed at the IEU OpenGWAS project (<https://gwas.mrcieu.ac.uk/>).

Declarations

Ethics approval and consent to participate

All GWAS data used for this study are publicly available via the original studies. And all participants provided informed consent, and each study obtained relevant ethical approval. The study only used publicly available data, and hence, no ethics approval was required.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Received: 22 March 2024 Accepted: 25 July 2024

Published online: 27 August 2024

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