Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

COVID-19 subgroups may slow down biological age acceleration

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Highlights

- Hospitalized COVID-19 may slow down GrimAge acceleration and PhenoAge acceleration.
- COVID-19 with severe respiratory may slow down GrimAge and PhenoAge acceleration.
- General COVID-19 subgroup only slowed down PhenoAge acceleration significantly.
- No significant casual effect of biological age acceleration on COVID-19 subgroups.

COVID-19 subgroups may slow down biological age acceleration

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COVID-19 subgroups may slow down biological age acceleration

Dear editor,

COVID-19 has profound health and socioeconomic impacts globally. Liu et al. suggested that older patients with COVID-19 had a higher mortality rate and were more likely to progress to severe disease[1]. Moreover, evidence indicates that chronological age is a major risk factor for COVID-19[2-4]. Cao et al. suggested that biological aging was associated with the risk of SARS-CoV-2 infection and severe COVID-19 development[5]. However, Franzen et al. reported that epigenetic clocks were not accelerated in COVID-19 patients[6]. A longitudinal study by Pang et al. showed that epigenetic clocks might be slowed down for approximately 2.06 years in young COVID-19 patients (age < 50)[7]. The inconsistency of the conclusions of previous studies intrigued us to continue to explore whether there exist potential causal links between epigenetic age acceleration and COVID-19. The causal relationships between epigenetic age acceleration and various COVID-19 subgroups, especially hospitalized COVID-19 and COVID-19 diagnosed with very severe respiratory disease, remain unknown.

To further clarify the relationship between chronological age and COVID-19 subgroups, we conducted two-sample bidirectional Mendelian randomization (MR) analyses using publicly available genome-wide association studies (GWAS). Our MR analyses calculated the summary statistics of four epigenetic age acceleration

measures[8] (N = 34,710) (i.e., GrimAge, HannumAge, Intrinsic HorvathAge, and PhenoAge). The four epigenetic clocks are based on DNA methylation levels at different CpG sites that capture distinctive features of biological aging[9]. HannumAge and Intrinsic HorvathAge are 'First-generation' epigenetic clocks[10]. HannumAge was trained on 71 age-related CpGs found in blood, while Intrinsic HorvathAge was based on 353 age-related CpGs found in several human tissues and cell types, and further adjustments were made for blood cell counts. GrimAge and PhenoAge are 'second-generation' epigenetic clocks[10]. The GrimAge measure combined data from 1,030 CpGs associated with smoking pack-years and seven plasma proteins, and the PhenoAge measure integrated data from 513 CpGs associated with mortality and nine clinical biomarkers. Though the four epigenetic clocks measure epigenetic age acceleration differently in terms of their CpGs components, they all have been shown to assess epigenetic age accurately[10]. The COVID-19 related datasets analyzed in our study include three subgroups: COVID-19 positive (COVID-19 vs control), hospitalized COVID-19 (hospitalized vs population), and COVID-19 diagnosed with severe respiratory disease (very severe respiratory confirmed vs population) (**Table S1**). All the datasets were obtained from the COVID-19 Host Genetics Initiative in 2020 and were available in EBI database (https://gwas.mrcieu.ac.uk/datasets/?gwas_id__icontains=ebi-a). The severe respiratory COVID-19 dataset was derived from a comparison between very severe respiratory failure patients secondary to COVID-19 vs control. COVID-19 with signs of severe respiratory distress is defined by WHO as severe COVID-19

(https://app.magicapp.org/#/guideline/j1WBYn). Hospitalized COVID-19 datasets were obtained by comparing laboratory-confirmed SARS-CoV-2 infected patients hospitalized with symptoms of COVID-19 vs control. All participants of GWAS datasets are European ancestry.

Leveraging three stages of MR analysis, we estimated the causal effect of COVID-19 subgroups on epigenetic age accelerations. In Stage 1, we selected independent COVID-19 genetic variants in each dataset with genome-wide significance ($P < 5 \times 10^{-8}$) as instruments to satisfy the assumption that the instruments chosen for MR analysis should be strongly associated with exposure. To test the instrumental variable bias, we calculated F-statistic and R^2 ($F = (R^2 \times (N - 2))/(1 - R^2)$, $(R^2 = 2\beta^2 \times MAF \times (1 - MAF))/2\beta^2 \times MAF \times (1 - MAF)) + 2N \times MAF \times (1 - MAF) \times SE^2$). MAF = effect allele frequency, β = effect estimate of the SNP in the exposure GWAS, SE = standard error, and N = sample size). All F-statistics of instruments were larger than 10, indicating that the probability of weak instrumental variable bias was minimal. In Stage 2, we extracted selected instrumental variants from four epigenetic age acceleration datasets. LD proxies ($r^2 > 0.8$) were allowed to replace the missing instrumental variants in epigenetic age acceleration datasets. Subsequently, we conducted inverse-variance weighted (IVW) MR and MR-Egger analyses. To satisfy the second and third assumptions of MR analysis, MR-Egger intercept test indicated the presence of potential pleiotropy. In Stage 3, we performed fixed effect meta-analysis to pool results across different COVID-19

subgroups, which has been applied in several studies to improve the precision of MR results. In fixed effect meta-analysis, I^2-statistic and p-value of the heterogeneity test depicted the heterogeneity across studies. The Chi-square test was used to test for subgroup differences. Furthermore, the reverse MR analysis and fixed effect meta-analysis were also performed. All statistical analyses were completed using R software version 4.1.1 with "TwoSampleMR" and "meta" R packages.

Meta-analyzed IVW MR results indicated significant causal effects between hospitalized COVID-19 and GrimAge acceleration (beta = -0.19, 95% CI -0.26 to -0.12, p = 4.68E-07), and PhenoAge acceleration (beta = -0.26, 95% CI -0.34 to -0.17, p = 8.86E-09). Interestingly, we found that COVID-19 diagnosed with very severe respiratory disease had the same casual effects as hospitalized COVID-19 (GrimAge IVW beta = -0.16, 95% CI -0.36 to -0.15, p = 7.59E-08; PhenoAge IVW beta = -0.22, 95% CI -0.30 to -0.15, p = 1.05E-09). Besides, COVID-19 only slowed PhenoAge acceleration (beta = -0.40, 95% CI -0.62 to -0.17, p = 7.06E-04) significantly (**Figure 1, Table 1, Table S2-S5, S13**). The threshold of statistically significant association between COVID-19 subgroups and epigenetic age accelerations was a Bonferroni correction (P < 0.05/4 = 1.25E-02). MR-Egger intercept test indicated no pleiotropy present (**Table S11**). Additionally, reverse MR analyses and fixed effect meta-analysis illustrated no significant casual effect of epigenetic clocks on three COVID-19 subgroups (**Figure S1, Table S6-S10, S14**). MR-Egger intercept test indicated no pleiotropy present (**Table S12**).

In conclusion, our research was initiated to further explore and investigate the conflicting views on the issue of the relationship between epigenetic aging and COVID-19 based on GWAS-based MR analysis and DNA methylation profile-based longitudinal analysis[6, 7]. Taken together, our findings provided evidence to support that hospitalized COVID-19 subgroup and COVID-19 diagnosed with very severe respiratory disease may slow down GrimAge acceleration and PhenoAge acceleration. The general COVID-19 positive subgroup only slowed down PhenoAge acceleration significantly. For the reverse direction of MR analysis, we found no significant casual effect of epigenetic clocks on three COVID-19 subgroups.

Conflicts of interest

The authors report no potential conflicts of interest.

Author contributions

Y.G. analyzed the data and drafted the manuscript and designed the study. Y.H. and Y.Z. helped proofread the manuscript. All authors have read and approved the final version of the manuscript.

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Table 1. P-value of fixed effect meta-analysis of the causal effect of COVID-19 subgroups on epigenetic clocks.

COVID-19 subgroups	GrimAge	HannumAge	Intrinsic HorvathAge	PhenoAge
COVID-19	**0.057**	**0.071**	**0.253**	7.06E-04
hospitalized COVID-19	4.68E-07	**0.036**	**0.723**	8.86E-09
COVID-19 with very severe respiratory confirmed	7.59E-08	**0.042**	**0.301**	1.05E-09

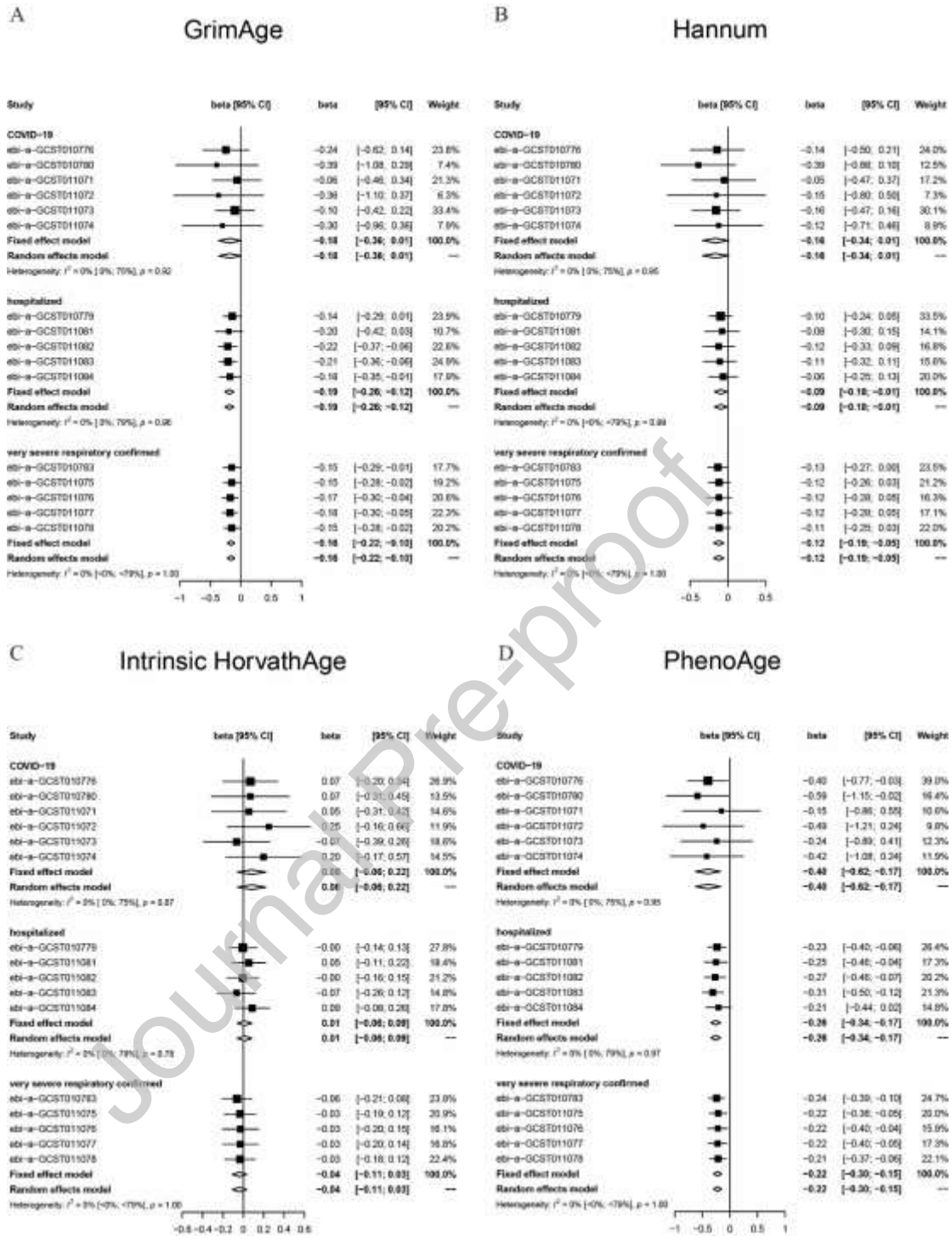

Figure 1. Fixed effect meta-analysis of inverse-variance weighted Mendelian randomization estimates for genetically predicted effects of COVID-19 subgroups on epigenetic clocks: GrimAge acceleration (A), HannumAge acceleration (B), Intrinsic HorvathAge acceleration (C) and PhenoAge acceleration (D).

Figure S1. Fixed effect meta-analysis of inverse-variance weighted Mendelian randomization estimates for genetically predicted effects of epigenetic clocks (GrimAge acceleration (A), HannumAge acceleration (B), Intrinsic HorvathAge acceleration (C) and PhenoAge acceleration (D)) on COVID-19 subgroups.

Supplementary file 1:

Table S1. Sources of GWAS summary datasets.

Table S2. Inverse-variance weighted Mendelian randomization analysis of the causal effect of COVID-19 subgroups on GrimAge acceleration

Table S3. Inverse-variance weighted Mendelian randomization analysis of the causal effect of COVID-19 subgroups on HannumAge acceleration

Table S4. Inverse-variance weighted Mendelian randomization analysis of the causal effect of COVID-19 subgroups on Intrinsic HorvathAge acceleration

Table S5. Inverse-variance weighted Mendelian randomization analysis of the causal effect of COVID-19 subgroups on Intrinsic PheoAge acceleration

Table S6. Inverse-variance weighted Mendelian randomization analysis of the causal effect of GrimAge acceleration on COVID-19 subgroups

Table S7. Inverse-variance weighted Mendelian randomization analysis of the causal effect of HannumAge acceleration on COVID-19 subgroups