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Prenatal stress and its relationship with inflammaging and cognitive performance in young adulthood

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Abstract

Prenatal stress has adverse effects on the offspring, which may be driven by inflammatory pathways. However, it is not clear whether prenatal stress might have time-specific effects on epigenetic signatures of chronic low-grade inflammation, accelerated epigenetic aging, and cognition in young adulthood. A follow-up of the ELSPAC prenatal birth cohort (n = 178) revealed that early prenatal stress (GW 1–20) was associated with chronic low-grade inflammation, premature epigenetic aging (GrimAge), and faster pace of aging (DunedinPACE) in young adulthood independently of sex, chronological age, BMI, cigarette smoking, recent stress, birth weight, maternal education, and later prenatal stress (GW 20–40). Moreover, the epigenetic inflammatory scores mediated the relationship between early prenatal stress and premature aging (GrimAge), as well as accelerated pace of aging (DunedinPACE). Importantly, faster pace of aging (DunedinPACE) mediated the relationship between early prenatal stress and lower IQ, suggesting early prenatal stress has long-term effects on inflammation, aging, and cognition.

INTRODUCTION

Maternal stress during pregnancy has been associated with altered functioning of the hypothalamic-pituitary-adrenal (HPA) axis and an increase of stress hormones, which may cross the placenta and directly impact the fetus (1, 2). According to the Developmental Origins of Health and Disease Hypothesis (DOHaD), these effects of early life adversity are long-term (3) and the exposure to a suboptimal environment in utero may alter the timing and rate of growth and development (4, 5), resulting in accelerated biological aging, such as acceleration of telomere attrition and establishment of DNA methylation patterns reflecting accelerated epigenetic aging (6). The telomeres shorten at each cell division and thus reflect the age of the cells. It has been demonstrated that exposure to stress during intrauterine life is linked with shorter telomere length in newborn cold blood (7–9), 3–5-year-old children (10), as well as young adults (11). Maternal stress during pregnancy was also associated with epigenetic age acceleration in the mother as well as the newborn (12). However, a recent systematic review and meta-analysis (6) concluded that while greater maternal stress (both greater perceived as well as greater lifetime trauma) is associated with shorter telomeres in the offspring, they concluded that findings on maternal stress and DNA methylation age acceleration are inconsistent. The DNA methylation age is complex and captures distinct health-related information. Therefore, more targeted research is needed to elucidate the specific pathways through which maternal stress may alter DNA methylation age and influence long-term outcomes in the offspring.

Prenatal stress has been associated not only with accelerated aging but also with inflammation, and a review of clinical and preclinical research on this topic (13) suggested that inflammation is a mediator of the relationship between psychosocial stress and offspring neuropsychiatric outcomes. Given the literature on close relationships between low-grade inflammation and aging, Franceschi et al. (14–16) introduced the concept of inflammaging. Further research reported relationships between epigenetic aging and inflammatory markers from blood (17, 18). However, the blood-based markers capture only

acute inflammation, and therefore the use of markers capturing a more sustained state of inflammation would be more appropriate.

A meta-analysis of epigenome-wide association studies (EWAS) of serum C-reactive protein (CRP), which is a sensitive marker of low-grade inflammation, reported differential methylation of 218 CpG sites in 8863 participants of European descent (19). Further large-scale meta-analysis of EWAS on the relationships between serum-based CRP and DNA methylation then used data from 22,774 multi-ethnic participants and found a total of 1,765 differentially methylated loci associated with CRP (20). These independently derived epigenetic signatures of low-grade inflammation might thus reflect a more sustained state of inflammation. Still, to the best of our knowledge, no study so far tested the relationships between prenatal stress and these epigenetic signatures of low-grade inflammation, and it is not clear whether prenatal stress might have long-term effects on inflammaging in the offspring.

Exposure to stress in utero has also been associated with negative cognitive outcomes such as impaired learning and memory in rats (21) or compromised language and general intellectual abilities in 2- and 5-year-old children (22, 23). Further research on prenatal stress and cognition pointed out the importance of stress timing. While the effects of prenatal stress on language skills in children were particularly detrimental if the stressor occurred during the first trimester (24), attention was particularly affected by stress during the third trimester (25).

Building on the literature reviewed above, it has been suggested that the effects of prenatal stress on a child's cognition may be mediated by inflammation (26) and the associated changes in neurotransmission, synaptic plasticity, and branching of dendrites (27). Further research tested the relationships between inflammatory markers and cognition and reported associations between higher tumor necrosis factor-alpha [TNF-α], interferon gamma [IFN-γ], and poor working memory in 4-year-old children (28) and between higher salivary C-reactive protein (CRP) and poor memory and executive function in adolescents (29).

The current study aimed to test the role of inflammaging in developmental programming of cognition and evaluate whether prenatal stress might be associated with more chronic low-grade inflammation and accelerated epigenetic aging in young adulthood and whether these might be in turn linked to worse cognitive skills. In addition, we aimed to determine whether these effects might be time-specific and thus whether stress during the first half of pregnancy might have more implications than stress during the second half of pregnancy. We hypothesized that prenatal stress, and particularly stress during the first half of pregnancy, will be associated with DNA methylation signatures of chronic low-grade inflammation and accelerated epigenetic aging, and that these changes will manifest as worse cognitive performance in young adulthood. Chronic low-grade inflammation was assessed using two independently derived epigenetic inflammatory scores (19, 20) and accelerated aging was assessed using 5 epigenetic clocks, spanning the first (30), second (31–33), and third generation (34) clocks.

METHODS

Participants

Participants were members of the European Longitudinal Study of Pregnancy and Childhood (ELSPAC) (35), a prenatal birth cohort of individuals born in South Moravia, Czech Republic, between 1991 and 1992, who also participated in its follow-up in young adulthood (Health Brain Age [HBA] study in the late 20s]). A total of 260 participants (53% men) had epigenetic data in their late 20s, and 178 of them (52% men) also had historical data regarding exposure to prenatal stress. All participants provided written informed consent to participate in the HBA study, including agreeing to our merging the data from HBA with their historical data from ELSPAC. Ethical approval for the HBA study was obtained from the ELSPAC ethics committee.

Procedures

Prenatal stress

Stressful life events were measured using a self-report questionnaire in (1) mid-pregnancy, to reflect stressful life events during the first half of pregnancy, and (2) 2 weeks after birth, to reflect stressful life events during the second half of pregnancy. This questionnaire included 40 questions on stressful events such as a breakup or divorce with the partner, consideration of abortion, violence, serious illness or death in the family, or financial difficulties, answered by a 5-point Likert scale.

Recent stress in young adults

Recent stress was assessed in the young adults using the Social Readjustment Rating Scale (SRRS (36)), a 43-item self-report questionnaire regarding stressful life events in the past year, such as divorce, marriage, death in the family, loss of work, or financial difficulties.

Buccal swabs and epigenetic analyses

Buccal swabs were collected from all participants of the HBA study in the late 20s. DNA methylation was assessed with the Illumina MethylationEPIC Platform. DNA methylation signature of chronic low-grade inflammation was calculated using two independently derived scores - the Ligthart et al (19) score based on 218 CpG sites, and the Wielscher et al (20) score based on 1,765 CpG sites. Epigenetic age was calculated using five epigenetic clocks, namely Horvath's epigenetic clock (30), based on 353 CpG sites, which we have also used in our previous work (37–39), the AltumAge clock (32), based on 20,318 CpG sites, and the CheekAge clock (31), based on 115,533 CpG sites, which we have used in our previous work (39), the GrimAge clock (33) based on 1,030 CpG sites, and the DunedinPACE clock (34), based on 173 CpG sites. The number of shared CpG sites between the two epigenetic inflammatory scores and the five epigenetic clocks is available in **Supplementary Table 1**.

Briefly, to process the raw MethylationEPIC data, the R package ChAMP (v2.21.1) was used (40). Beta mixture quantile normalization (BMIQ) (41) method was used to adjust the beta-values of type II design probes to a statistical distribution characteristic of type I probes. Batch effects were evaluated using singular value decomposition, and cell-type proportions were estimated using the EpiDISH (42) R package with reference methylation profiles. The exact preprocessing parameters and analysis-specific implementation of batch-effect handling are documented in the GitHub Repository (see https://github.com/RCX-Bioinformatics/inflammation_signatures for methods on calculation of epigenetic inflammatory scores; see https://github.com/RCX-Bioinformatics/epigenetic_ageing_clocks for the methods on calculation of epigenetic clocks). A comprehensive description of the epigenetic age estimation workflow is available in Marečková et al. (43).

For each of the epigenetic clocks, the epigenetic age gap was calculated as the DNA methylation age residualized for batch, chronological age, and cell heterogeneity (epithelial, immune, and fibroblast cells). DunedinPACE was also residualized for chronological age as recommended for mixed-age samples by Sugden et al. (44) as well as Whitman et al (45). For the static measures of epigenetic age, Horvath's clock, AltumAge, CheekAge, and GrimAge, positive values of the epigenetic age gap reflect accelerated aging, and negative values reflect decelerated aging. For DunedinPACE, which measures the pace of epigenetic aging, a value of 1 corresponds to a rate of 1 year of biological aging per 12 months of calendar time, and values > 1 indicate faster pace of aging (e.g., a value of 1.50 would indicate a pace of aging is 50% faster than the norm), and values < 1 indicate a slower pace of aging (e.g., a value of 0.60 would indicate a pace of aging 40% slower than the norm).

Cognitive performance

Cognitive performance was assessed using the following subtests from the Wechsler Adult Intelligence Scale, fourth edition (WAIS-IV (46)): picture completion, matrix reasoning, arithmetic, digit span, information, similarities, and digit-symbol coding. These subtests allowed us to assess participants' perceptual reasoning (picture completion, matrix reasoning), working memory (arithmetic, digit span), verbal comprehension (information, similarities), and processing speed (digit-symbol coding). Subsequently, full-scale IQ was calculated based on the scores for these seven WAIS-IV subtests according to Tam (47).

Covariates

Given the literature on the role of sex (48, 49), maternal education (50, 51), timing of prenatal stress (52, 53), birth weight (54), BMI (55), recent stress (56), and cigarette smoking (57) in the context of prenatal stress, inflammation, and cognitive outcomes, we included the following covariates: sex, chronological age, maternal education, stressful life events during the second half of pregnancy, birth weight, body mass index (BMI), recent stressful life events (in the past year), and cigarette smoking in the past month.

Statistical analyses

First, we performed a t-test to assess sex differences in the epigenetic inflammatory scores and epigenetic age gap measures and used a linear regression to assess the relationships between the epigenetic inflammatory scores and epigenetic aging. Sex was used as a covariate, and the false discovery rate (FDR) method was used to correct for the number of multiple comparisons.

Next, a set of linear regressions assessed the relationships between stress during the first half of pregnancy and the 7 epigenetic scores (2 epigenetic inflammatory scores, 5 epigenetic aging clocks). The covariates included sex, chronological age, BMI, cigarette smoking, recent stressful life events, maternal education, stressful life events during the second half of pregnancy, and birth weight. Again, the FDR method was used to correct for the number of multiple comparisons.

Subsequently, the PROCESS macro (58) for SPSS, version 31 (IBM SPSS Statistics, IBM Corp, Armonk NY), was used to perform a mediation analysis using bootstrapping and assess whether the epigenetic inflammatory scores might mediate the relationships between prenatal stress and epigenetic aging. Conditional indirect effects were assessed for significance using bootstrapped bias- corrected 95% confidence intervals constructed around indirect effect estimates, based on 10,000 bootstrapping iterations. For consistency with the analyses above, we also assessed whether these mediations remain significant when using sex, chronological age, body mass index (BMI), cigarette smoking, recent stressful life events, maternal education, stressful life events during the second half of pregnancy, and birth weight as covariates. In addition, a multiple regression evaluated the relative contributions of prenatal stress, birth weight, maternal education, recent stress, BMI, smoking, chronological age, and sex to the 7 epigenetic scores.

Finally, we evaluated whether the epigenetic scores associated with prenatal stress might also predict cognitive performance in young adulthood. Potential interactions with sex were considered, and the FDR method was again used to correct for the number of multiple comparisons. Subsequently, we used the PROCESS macro (58) for SPSS, version 31 (IBM SPSS Statistics, IBM Corp, Armonk NY), to perform a mediation analysis using bootstrapping and assess whether the cognitive performance-related measures of aging might mediate the relationships between prenatal stress and FSIQ. Conditional indirect effects were assessed for significance using bootstrapped bias- corrected 95% confidence intervals constructed around indirect effect estimates, based on 10,000 bootstrapping iterations.

RESULTS

Sample characteristics and sex differences in the epigenetic inflammatory scores and epigenetic aging clocks in the late 20s

As detailed in **Supplementary Table 2**, women had a lower epigenetic age gap in the late 20s than men, as calculated using Horvath's clock (t(176)=-2.69, p = 0.008), but experienced a faster pace of aging than

men in the late 20s, as calculated using the DunedinPACE measure (t(176) = 2.10, p = 0.037). There were no sex differences in the other epigenetic aging gaps or the epigenetic inflammatory scores.

Relationships between the epigenetic inflammatory scores and epigenetic aging clocks in the late 20s

As detailed in Table 1, the two independently derived epigenetic inflammatory scores were very strongly related (beta = 0.96, p < 0.001, Adj R^2 = 0.93, FDRp < 0.001; see Fig. 1A). Moreover, their relationships with the GrimAge gap (Ligthart score: beta = 0.52, p < 0.001, Adj R^2 = 0.26, FDRp < 0.001; Wielscher score: beta = 0.53, p < 0.001, Adj R^2 = 0.28, FDRp < 0.001; see **Fig. 1BC**) and DunedinPACE (Ligthart score: beta = 0.47, p < 0.001, Adj R^2 = 0.23, FDRp < 0.001; Wielscher score: beta = 0.46, p < 0.001, Adj R^2 = 0.22, FDRp < 0.001; see **Fig. 1EF**) were also strong. In contrast, neither of the epigenetic inflammatory scores was related to Horvath's age gap, nor were they related to the AltumAge gap (see Table 1 for statistical details), and the Ligthart score showed even a small negative relationship with the CheekAge gap (beta=-0.20, p = 0.01, Adj R^2 = 0.04, FDRp = 0.02; see Fig. 1D).

Table 1
– Relationships between the 2 epigenetic inflammatory scores and 5 epigenetic clocks in the late 20s. All models were adjusted for sex. Significant relationships surviving the FDR correction are highlighted in bold.

Predictor	Outcme	Std Beta	p-value	FDR p-value
Wielscher score	**GrimAge gap**	**0.528**	**0.000**	**0.000**
Wielscher score	**DunedinPACE**	**0.454**	**0.000**	**0.000**
Wielscher score	AltumAge gap	-0.004	0.959	0.959
Wielscher score	CheekAge gap	-0.129	0.089	0.139
Wielscher score	Horvath's clock	-0.025	0.741	0.815
Ligthart score	**GrimAge gap**	**0.516**	**0.000**	**0.000**
Ligthart score	**DunedinPACE**	**0.466**	**0.000**	**0.000**
Ligthart score	AltumAge gap	-0.058	0.448	0.547
Ligthart score	**CheekAge gap**	**-0.200**	**0.008**	**0.015**
Ligthart score	Horvath's age gap	-0.093	0.211	0.290
Ligthart score	**Wielscher score**	**0.963**	**0.000**	**0.000**

Prenatal stress and its relationship with epigenetic inflammatory scores and epigenetic aging clocks in the late 20s

The offspring of mothers who experienced more stress during the first half of pregnancy had higher chronic low-grade inflammation, as indicated by the two independently derived epigenetic inflammatory scores (Ligthart score: beta = 0.18, p = 0.04, FDRp = 0.04, Fig. 2A; Wielscher score: beta = 0.20, p = 0.02, FDRp = 0.04, Fig. 2B) and faster epigenetic aging, as indicated by the GrimAge gap (beta = 0.18, p = 0.04, FDRp = 0.04, Fig. 2C) and DunedinPACE (beta = 0.22, p = 0.01, FDRp = 0.04, Fig. 2D). These effects were significant independently of the offspring's sex, chronological age, cigarette smoking, BMI, recent stressful life events, birth weight, stress during the second half of pregnancy, and maternal education. There were no similar relationships between stress during the first half of pregnancy and Horvath's age gap (beta = 0.01, p = 0.86, FDRp = 0.19), AltumAge gap (beta=-0.03, p = 0.69, FDRp = 0.07), or the CheekAge gap (beta=-0.09, p = 0.33, FDRp = 0.19).

Do epigenetic inflammatory scores mediate the relationships between prenatal stress and premature epigenetic aging?

The relationship between prenatal stress and premature epigenetic aging in young adulthood, as estimated using the GrimAge gap, was mediated by the Ligthart (ab = 0.073, SE = 0.041, 95% CI [0.002; 0.162]; Fig. 3A) as well as the Wielscher epigenetic inflammatory score (ab = 0.076, SE = 0.042, 95% CI [0.002; 0.167]; Fig. 3B). And these mediations remained significant even when correcting for all the covariates (Ligthart: ab = 0.078, SE = 0.045, 95% CI [0.001; 0.176]; Wielscher: ab = 0.091, SE = 0.046, 95% CI [0.011; 0.188]).

Moreover, the relationship between prenatal stress and faster pace of aging in young adulthood, as estimated using the DunedinPACE, was mediated by the Ligthart (ab = 0.069, SE = 0.040, 95% CI [0.003; 0.158]; Fig. 3C) as well as the Wielscher epigenetic inflammatory score (ab = 0.067, SE = 0.037, 95% CI [0.003; 0.148]; Fig. 3D). And these mediations remained significant even when correcting for all the covariates (Ligthart: ab = 0.071, SE = 0.042, 95% CI [0.001; 0.164]; Wielscher: ab = 0.079, SE = 0.042, 95% CI [0.009; 0.171]).

Predictors of epigenetic inflammatory scores and epigenetic aging clocks in the late 20s

As revealed in **Supplementary Table 3**, the Ligthart and Wielscher epigenetic inflammatory scores were predicted by stress during the first half of pregnancy and cigarette smoking in young adulthood, together explaining 8% and 6% of variability in the Ligthart and Wielscher scores, respectively. The DunedinPACE of aging was predicted only by the stress during the first half of pregnancy, explaining 8% of variability in the pace of aging ($AdjR^2$=0.08). In contrast, the GrimAge gap was predicted not only by stress during the first half of pregnancy, but also by stress during the second half of pregnancy, birth weight, and cigarette smoking in young adulthood, together explaining 7% of variance in GrimAge gap ($AdjR^2$=0.07). The models for Horvath's age gap, AltumAge gap, and CheekAge gap did not reach significance (see **Table 3** for statistical details).

Relationship of the epigenetic inflammatory scores and epigenetic aging clocks with cognition in the late 20s

Faster pace of aging, as estimated using the DunedinPACE, predicted lower full-scale IQ (beta=-0.18, p = 0.01, FDRp = 0.04, Adj R^2 = 0.03; Fig. 4), and there were no interactions with sex (beta=-0.07, p = 0.34). In contrast, a similar relationship between GrimAge gap and FSIQ pointed in the same direction but did not reach significance (beta = -0.14, p = 0.08). There were no relationships between the epigenetic inflammatory scores and full-scale IQ (Ligthart: beta=-0.03, p = 0.73; Wielscher: beta=-0.08, p = 0.27).

Pace of aging mediates the association between prenatal stress and cognitive performance

Faster pace of aging, as estimated using the DunedinPACE, mediated the relationship between prenatal stress and FSIQ (ab=-0.551, SE = 0.360, 95% CI [-1.382; -0.007]; Fig. 5). This mediation remained significant even when correcting for sex and birthweight (ab=-0.499, SE = 0.333, 95% CI [-1.299; -0.001]).

DISCUSSION

We have conducted an epigenetics follow-up of the ELSPAC prenatal birth cohort and tested the effects of prenatal stress on inflammaging and cognitive performance in young adulthood. Using two independently derived epigenetic signatures of chronic low-grade inflammation and five epigenetic aging clocks, spanning the first (e.g. Horvath's clock), second (AltumAge, CheekAge, GrimAge), and third-generation clocks (DunedinPACE), we showed that greater levels of stress during the first half of pregnancy were associated with greater epigenetic signatures of chronic low-grade inflammation, premature epigenetic aging estimated using the GrimAge clock, and a faster pace of aging estimated using the DunedinPACE clock. All these relationships were independent of sex, chronological age, BMI, cigarette smoking, recent stress, birth weight, maternal education, and stressful life events during the second half of pregnancy. Moreover, we showed that the epigenetic inflammatory scores mediated the relationship between prenatal stress and premature epigenetic aging estimated using the GrimAge clock, as well as the accelerated pace of aging estimated using the DunedinPACE. Additional analyses revealed that the faster pace of aging, as estimated using the DunedinPACE, was associated with lower full-scale IQ independently of sex and that the pace of aging mediated the relationship between stress during the first half of pregnancy and full-scale IQ. Together, these findings suggest that stress during the first half of pregnancy might have a long-term effect on inflammaging and cognitive performance in the offspring.

Our findings substantially extend previous animal research linking prenatal stress to placental and fetal brain inflammation (59) as well as further research on the effects of prenatal stress on acute concentrations of IL-6 in childhood (60) and midlife (61), and reveal that prenatal stress has long-term effects on chronic low-grade inflammation. Moreover, it substantially extends the findings on maternal stress during pregnancy and pubertal timing (62), maternal stress during pregnancy and telomere length

(6), and prenatal maternal anxiety and pediatric buccal epigenetic (PedBE) clock in children (63) to epigenetic aging in young adulthood, and particularly to the GrimAge clock and pace of aging, as estimated using the DunedinPACE. Finally, our findings regarding the prenatal stress-related measures of aging and their relationship with cognition support and extend animal research, which showed that prenatal stress might exacerbate age-dependent cognitive deficits (64), and a meta-analysis reporting weak negative associations between prenatal stress and a child's intellectual development (65).

Our findings regarding the time-specificity of the effects of prenatal stress on epigenetic inflammatory scores and epigenetic aging also substantially extend the research of Levendosky et al. (66) who pointed out the importance of the timing of prenatal stress on offspring development, but in the context of infants' HPA axis reactivity and temperament. The particular importance of prenatal stress during the first versus the second half of pregnancy is also consistent with our previous research on prenatal stress and brain structure, and specifically gyrification patterns (67), volume of amygdala nuclei (68), and amygdala's structural covariance (69).

The fact that we found the associations between stress during the 1st half of pregnancy and both independently derived epigenetic signatures of chronic low-grade inflammation suggests these relationships are robust. While the number of CpG sites used to calculate the Ligthart and Wielscher scores was very different (218 vs. 1,765 CpG sites), most of the CpG sites used to calculate the Ligthart epigenetic inflammatory score are part of the Wielscher epigenetic inflammatory score, and these two epigenetic inflammatory scores were highly correlated. Interestingly, the GrimAge gap and DunedinPACE, which were also related to stress during the 1st half of pregnancy, were highly correlated with the two epigenetic inflammatory scores, even after correction for cell type heterogeneity, despite the very minor overlap of CpG sites. These findings support the inflammaging concept (14, 15) and suggest that stress during the first half of pregnancy has long-term effects on both inflammation and biological aging of the offspring.

While the CpG sites involved in the calculation of AltumAge included a relatively high number of CpG sites used to calculate Horvath's clock and the CheekAge, the CpG sites used to calculate Horvath's clock were very different from those used to calculate Ligthart's score, Wielscher score, DunedinPACE, and GrimAge. This might, at least in part, explain the correlations of Horvath's age gap with AltumAge gap and CheekAge gap ($p < 0.001$) but not the GrimAge gap, DunedinPACE, or either of the epigenetic inflammatory scores ($p > 0.21$), as well as the lack of relationships between stress during the first half of pregnancy and Horvath's age gap, CheekAge gap, and AltumAge gap ($p > 0.33$).

In addition, the multiple regressions revealed that while stress during the first half of pregnancy was the sole predictor of the pace of aging in young adulthood, calculated using the DunedinPACE algorithm, the epigenetic inflammatory scores were predicted by stress during the first half of pregnancy as well as cigarette smoking in young adulthood. The fact that the epigenetic age gap estimated using the GrimAge was predicted by stress during both the first and second half of pregnancy, as well as birth weight and cigarette smoking in young adulthood, suggests that this second-generation epigenetic clock reflects

more complex pathways, potentially capturing the effects of a wider range of exposures across the lifespan. Interestingly, the multiple regression also revealed that none of these measures were associated with recent stress in young adulthood, sex, BMI, or maternal education.

Importantly, our study suggests that stress during the first half of pregnancy and the associated changes in chronic low-grade inflammation and aging might also have implications for cognitive performance. We showed that a faster pace of aging in young adulthood was associated with worse cognitive skills in young adulthood and that the pace of aging mediated the relationship between prenatal stress and cognitive performance. Our study is limited by its observational character, which limits causal inferences. Future research should follow the participants in subsequent decades to reveal whether the relationships between prenatal stress and the pace of aging, as well as the relationships between the pace of aging and cognitive performance, strengthen as the participants reach older adulthood. While it is possible that the effects of prenatal stress on the pace of aging and cognition might be stable over the lifespan, it might be that they will become more pronounced with age, particularly in participants who experience lower engagement in mentally and socially stimulating activities, which are known as protective factors against mild cognitive impairment (MCI) and depression (70). Our findings are also limited by the fact that while both the Ligthart and the Wielscher epigenetic inflammatory scores as well as the GrimAge and the DunedinPACE were originally derived from blood tissue-based DNA, our study applied them to buccal swab-based DNA. However, similar approach has been used in previous work (71–73). Still, future research should replicate our findings in datasets allowing DNA extraction from blood tissue.

Overall, our epigenetic data and the relationships between the epigenetic inflammatory scores and the second- and third-generation epigenetic clocks strongly support the concept of inflammaging. Moreover, our longitudinal data reveal the long-term and time-specific effects of stress during the first half of pregnancy on inflammaging in the offspring in young adulthood. Our study also reveals that the faster pace of aging, which was associated with higher exposure to stress during the first half of pregnancy, might contribute to worse cognitive performance already in young adulthood, pointing out the importance of early preventive interventions targeting pregnant women.

Declarations

CONFLICT OF INTEREST

All authors declare no financial or any other conflicts of interest.

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