

Epigenetic Clock Deceleration and Maternal Parity: Associations With Increasing Grey Matter Volume of the Precuneus

Shota Nishitani

University of Fukui

Ryoko Kasaba

University of Fukui

Daiki Hiraoka

University of Fukui

Koji Shimada

University of Fukui

Takashi Fujisawa

University of Fukui

Akemi Tomoda (✉ atomoda@u-fukui.ac.jp)

University of Fukui

Research Article

Keywords: Epigenetic Clock, Precuneus, Maternal Parity

Posted Date: November 25th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-111892/v1>

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Abstract

Reproductive effort experiences, such as pregnancy, delivery, and interaction with their children, make female maternal brains optimised for child-rearing. However, extensive studies in non-human species revealed there is a trade-off between reproductive effort and life expectancy. In humans, large demographic studies have shown that this is the case for the most part, but molecular marker studies of aging remain controversial, and there are no studies evaluating the relationship between reproductive effort, aging, and brain structures simultaneously. We examined the associations among the reproductive efforts, DNA methylation age acceleration, and the regional grey matter of structural brain scans by voxel-based morphometry in 27-46-year-old mothers. We found that greater reproductive efforts, including the number of delivery and motherhood periods, were significantly associated with decelerated aging in mothers with 1-4 children. We also found that the precuneus grey matter volume was larger as age deceleration occurred and found a mediation effect of the greater left precuneus grey matter volume on the relationship between parity and age deceleration. Our findings suggest that mothers of early childhood children who have less than four children may benefit from aging via precuneus structural changes.

Introduction

In non-human species, the established theory (LHT: Life History Theory) is that the greater the reproductive effort, the more finite energy is expended; thus, females trade-off their life expectancy [1]. In humans, several population-based large demographic studies of more than 140,000 women born between 1820 and 1920 in a preindustrial population in Utah, North America, have shown that women who made a more reproductive effort also had a shorter life-span [2, 3]. These results are largely in line with LHT, but according to Bolund *et al.* (2016) [2], life expectancy is shortened, for four or more births, as per the theory, while deliveries of less than four, in contrast, extend life expectancy. Since our current monogamous society no longer has an average of nine births, which was common in this era, a more recent North America-wide large demographics study of more than 20,000 women was conducted [4]; this study better reflects the current average number of births. Shadyab *et al.* (2017) reported that the odds ratio of longevity in Caucasians was higher for mothers who gave birth more than once (2-4 children) compared to that of those who gave birth once or were nulliparous, even after adjusting for demographic characteristics, socioeconomic status (SES), lifestyle behaviours, reproductive factors, and health-related factors [4]. However, it was consistent with both the LHT and previous population demographics in most aspects, especially with regard to increasing parity; the association between parity and longevity was attenuated and certainly not higher in those with five or more children. Therefore, it may be true that having more than four children reduces life expectancy in women, but it may not be simply consistent with the LHT for births of less than four children. Dior *et al.* (2013) reported a U-shaped association, and not linear, between the number of children and all-cause mortality, with the lowest risk at 2-3 children [5].

Hence, recent studies using molecular metrics have attempted to provide biological evidence for the phenomenon that reproductive effort was affecting women's longevity. Telomere length was used as a

molecular metric to reflect longevity at initial study stages. The first study reported that mothers having more children had shorter telomere lengths [6]. In contrast, a study of the Mayan tribe in Guatemala reported that mothers who have more children have longer telomeres [7]. In addition to telomere length, epigenetic age acceleration, which is obtained by calculating the deviation of DNA methylation age (mAge) computed by chronological age [8], has recently attracted the attention as a novel aging biomarkers. Using this metric, Ryan CP *et al.* showed that in 397 young 20-22-year-old Filipino women, a higher number of pregnancies was associated with a greater age acceleration and shorter telomere length [9]. Kresovich JK *et al.* also used this metric retrospectively in more than 2,000 women aged 35-74, living in the US, and reported that more age acceleration was seen with more births [10]. However, the results of these studies alone cannot sufficiently explain why the influence of births of four or fewer children on age acceleration might be exceptionally non-linear. Moreover, the cause of this putative possibility might be difficult to elucidate in retrospective studies on generations that have already completed child-rearing. While the participants in Ryan *et al.*'s study were supposed to be rearing children, the mean age of childbearing in most developed countries is considerably higher (ex. 30.7 years old in Japan, 2016); therefore, while their results are applicable to the younger mothers, it remains uncertain whether they can be applied to the general maternal population at present.

In addition to the phenomenon where reproductive effort affects aging and longevity, it has been affirmed that reproductive effort, including the experiences of pregnancy, delivery, and parenting, makes female brain structures more maternal, thereby, optimised for parenting [11-13]. Hoekzema E *et al.* (2017) conducted a longitudinal study that followed 25 first-time mothers from time of conception to two years after delivery and found that at ten months postpartum, compared to pregnancy, the brain grey matter (GM) volume of the inferior and medial frontal gyrus, superior temporal sulcus, fusiform gyrus, precuneus and hippocampus, which involves in the theory-of-mind network, was reduced [14]. Zhang K *et al.* (2019) examined a similar longitudinal study and found that mothers showed changes in the GM and white matter volumes and cortical thickness of several of regions, including the superior and medial frontal gyrus, insula, limbic lobe, superior and middle temporal gyrus, and precentral gyrus, after two years of follow-up [15]. Luders E *et al.* (2020) conducted a similar longitudinal study. They reported no decline in areas at 4-6 weeks postpartum as compared to immediate postpartum period (1-2 days postpartum); however, there were increased GM volumes in regions including the pre and postcentral gyrus, thalamus, precuneus, contrary to Hoekzema E *et al.*'s results [16]. Thus, reproductive effort affects not only aging but also changes in brain structures. In contrast to the rest of the species, humans do not reach the end of their life shortly after menopause; they live nearly twice as long as their age at menopause in this modern era. Indeed, the grandmother hypothesis that explains the existence of menopause in human life history by identifying the adaptive value of extended kin networking has been proposed [17], and postmenopausal women have been found to have a great deal of biological and social activity [18, 19]. This is unique to humans and may be related to humans' high sociability due to their most evolved brain.

Therefore, we hypothesised that reproductive effort might refine the brain structures involved in sociability required for parenting and that this influences age acceleration and longevity. In the present study, we examined the association between reproductive effort, age acceleration measured using

salivary DNA, and brain structures evaluated by voxel-based morphometry (VBM) in 27-46-year-old Japanese mothers who first gave birth at an average age of 29.2 years and have 1-4 children.

Results

Reproductive effort and epigenetic age acceleration

As expected, the mAge strongly correlated with chronological age ($R^2 = 0.57$, $t = 8.2$, $P = 8.4e-11$) (Supplementary Figure S1). Age acceleration was significantly dependent on the number of delivery ($t = -2.2$, $df = 50$, $P < 0.05$), motherhood period ($t = -2.1$, $df = 50$, $P < 0.05$), and cumulative motherhood period ($t = -2.2$, $df = 50$, $P < 0.05$) but did not quite achieve the conventional levels of significance for parity ($t = -2.0$, $df = 50$, $P = 0.053$) (Figure 1). Additionally, neither of the other demographics such as age at first childbirth ($t = 0.9$, $df = 50$, $P = 0.37$), age at last childbirth ($t = -0.48$, $df = 50$, $P = 0.63$), exclusive breastfeeding diet ($t = -0.53$, $df = 49$, $P = 0.60$), household income ($t = 0.31$, $df = 49$, $P = 0.76$), or proportion of epithelial cells ($t = -0.40$, $df = 50$, $P = 0.69$) were associated with age acceleration. In a three-stage hierarchical multiple regression, chronological age was entered at stage one of the regression as a demographic variable and Parenting Stress Index (PSI) was entered at the second stage as a control variable. Next, reproductive effort was entered at stage three to observe their effects on age acceleration and gain further knowledge with regard to these relationships (Table 2). Thus at stage two, PSI contributed significantly to the regression model ($F[1, 48] = 10.50$, $P < 0.005$, $\Delta R^2 = 0.16$). Adding the numbers of delivery to the regression model explained an additional 5.2 % of the variation in age acceleration, and this change in R^2 was significant ($F[1,47] = 4.13$, $P < 0.05$). These variables accounted for approximately 20 % of the variance in age acceleration. Parity ($F[1,47] = 3.35$, $P = 0.07$), motherhood period ($F[1,47] = 3.69$, $P = 0.06$), and cumulative motherhood period ($F[1,47] = 3.89$, $P = 0.05$) showed considerable trends toward significance.

VBM and path analysis

The VBM results showed that age acceleration was negatively correlated with GM volume within a cluster in the left precuneus (Montreal Neurological Institute [MNI] coordinates: $x = -17$, $y = -39$, $z = 68$; cluster size = 801 voxels; $P = 0.03$, family-wise error [FWE] corrected cluster level; Figure 2). There was a significant indirect effect of the precuneus GM volume on parity and age acceleration (indirect effect = -1.45 , $SE = 0.73$, $95\%CI = [-3.09, -0.14]$; Figure 3). No other combinations were significant.

Discussion

This study examined the relationship between reproductive effort, age acceleration, and brain structure in mothers of early childhood children. Our results showed that the reproductive effort in terms of the number of delivery, motherhood period, and cumulative motherhood period was associated with age deceleration, but only the number of delivery remained significant when a hierarchical multiple regression analysis was carried out. Although previous studies have examined the relationship between age

acceleration or telomere length and the number of deliveries as a representative variable, this is the first time that age acceleration has been simultaneously examined in relation to the other reproductive effort indices adjusted for daily parenting stress. We also found that the precuneus GM volume increased as age deceleration occurred. We also observed a mediation effect of a greater left precuneus GM volume on the relationship between parity and age deceleration. This suggests that the precuneus, which is a central node in the human brain and supports complex cognition and behaviour, may be associated with age deceleration in child-rearing mothers and that 2-4 births compared to one birth leads to maternal brain changes in the left precuneus, contributing to age deceleration.

Our hypothesis with regard to the relationship between age acceleration and reproductive effort was that women with high numbers of births, more than four, would adhere to the LHT and have a shorter life-span, but that mothers would benefit over the course of their life-span from having two or more children in the modern era, when the number of births is generally lower. Our results were limited because we only included mothers who gave birth to 1-4 children, and we do not know how this would have affected women with more than four births. However, among mothers who gave birth to at least 1-4 children, our results seemed to be contradictory to the LHT [1]. In other words, our results suggest that mothers who gave birth to less than four children and have currently been rearing them have aged slowly, depending on the number of children. However, our findings are consistent with the previous large demographic studies showing that deliveries of less than four rather extend life expectancy [2, 4, 5]. We speculate that this is due to avoiding a downward trend in the birth-rate since just one offspring born to a couple would lead to a decline in population in a monogamous society. While childbearing certainly comes at the cost of the energy as per the LHT, giving birth to two or more children while having fewer than four may be the most efficient and balanced way to benefit the mother's life-span, even accounting for its trade-off.

One possible biological cause underlying this phenomenon could be due to maternal brain alterations. Human mothers' brains undergo dynamic structural and functional changes during pregnancy and the early postpartum period to facilitate their psychological and behavioural adaptation to parenting [11, 14-16]. This transition to the maternal brain seems to particularly enhance the functioning of the reward, social information, and emotion regulation circuits [20]. We identified that age deceleration associated with parity was linked to an increase in precuneus GM volume in mothers, which is similar to the findings of the study by Luders E *et al.* where an increase in precuneus GM volume was observed at 4-6 weeks postpartum compared to 1-2 days postpartum [16]. The precuneus is included in the social information circuit which involves empathy as well as self-monitoring and reflection and is a hub for the default mode network (DMN) [21]. In other words, becoming a mother of two or more children may have led to more complex social interactions, such as engaging in one-to-one relationships between the 1st born child and parents, as well as more complex relationship between the 1st child, 2nd child, and parents, which may have contributed to a greater precuneus GM volume than in the past. On the other hand, one of the main symptoms of PTSD is an elevated rumination characterised by repetitive, negative self-focused cognition, and it has been reported that a reduced functional connectivity between the isthmus cingulate and the left precuneus within the DMN is linked to this high level of rumination [22]. Lifetime trauma burden and suffering from both current and lifetime PTSD have been reported to cause GrimAge acceleration [23]. It

has also been found that patients with Alzheimer's disease (AD) and in the prodromal stage of AD have decreased DMN functions and precuneus GM volume compared to controls. Using post-mortem brains, a telomere length analysis of each brain region comprising the DMN showed that the precuneus telomere length was shorter in AD and prodromal stage of AD than in controls, whereas there were no differences in the frontal, inferior temporal, posterior cingulate gyrus or visual cortex [24]. Furthermore, this telomere length reduction in the precuneus was also correlated with cognitive task performance. These findings support our findings that age deceleration associated with parity was linked to an increase in precuneus GM volume in mothers. The sophistication of the social information and DMN functions involved in becoming a mother may have contributed to the age deceleration. However, this finding is not related to the number of births which accounts for the major reproductive effort, but rather to parity, whether a baby is born once or twice or more, and is not a model that is valid as the number of births increases.

Our results were partially inconsistent with previous studies. Ryan CP *et al.* reported age acceleration in mothers who had given birth to 1-5 children, in addition to nulliparous women [9]. However, their study participants were 20-22-years-old, which is relatively young compared to the average age of a first birth in modern developed countries. Some reports suggest that a higher age at first birth is associated with a longer telomere length [25] and longer life expectancy [4]. Thus, the association may be linear rather than U-shaped when the age at first birth is relatively young, as it was in this study. In addition, various environmental factors have been reported to influence age acceleration, most notably stress [23, 26]. Ryan CP *et al.* adjusted for SES but did not consider the effects of psychological aspects such as parental stress; therefore, an analysis adjusting for these effects may also be necessary. In our results, higher parental stress, and not only reproductive effort, was associated with a deceleration of aging. On the other hand, Kresovich JK *et al.* reported accelerated aging in mothers who gave birth to 1-4 or more children, in addition to nulliparous women [10]. However, their study was retrospective and included those who are currently out of the child-rearing phase (average age of 55 years when the blood samples were collected), which is different from the present study of mothers of early childhood children. Their results probably involve the presence or absence of menopause [6] and the higher genetic risks for developing breast cancer, which is a unique characteristic of this cohort population [27]. In contrast, Barha CK *et al.* found longer telomeres in mothers who gave birth to 1-6 babies [7]. Although this study did not measure age acceleration, telomere length results indicate that age deceleration may have occurred, perhaps similar to our findings. However, our studies were similar in the aspect that the DNA was saliva-derived. Our method of estimating mAge by using Horvath multi-tissue epigenetic clock should not be noticeably affected, even if the tissue from which the DNA was derived was different [8]. Therefore, the similarity in type of sample may not be particularly relevant. Additionally, the participants in their study were, on average, 39.4-years-old, premenopausal, and probably still rearing their children. This consistency in age and rearing young children may be related to the directional consistency of the two results. Although their results were linearly regressed, on closer inspection an inverted U-shaped approximation with a peak at 2-3 births appears to be more appropriate.

There are at least three potential limitations concerning our study's results. First, is the number of participants; we only had one participant with more than four children, and this limits the ability to

observe the U-shaped association. Second, there were no nulliparous participants. Shadyab AH *et al.* (2017) reported that mothers have a longer life expectancy than nulliparous women [4]. It is also known that changes in brain morphology and cognitive function occur when women become mothers [14-16]. Therefore, it was necessary to include nulliparous women to show the association between age acceleration and precuneus GM volume with brain maternalisation. Finally, we used saliva DNA samples for mAge and age acceleration calculation, but this limits the use of other newly developed blood DNA metrics, such as PhenoAge [28] and GrimAge [29] to predict life-span. However, Horvath's original epigenetic clock can be used for any type of tissues, and this enables a direct comparison with previous studies based on blood DNA [9, 10].

Despite these limitations, our results endorse the idea that reproductive effort in mothers might refine the brain structures involved in sociability required for parenting, and this influences age acceleration and longevity. Our results also suggest that the age deceleration associated with changes in the precuneus may be one of the phenomena linked to the transformation to a maternal brain.

Methods

Participants

A total of 58 biological mothers who were rearing at least one preschool-age child (57 from a previous study [30] and one newly enrolled) participated in this study. We excluded four mothers who delivered by caesarean sections for multiple births and two mothers of children in disabilities; therefore, finally 52 mothers were included (age range = 27–46 years; mean age = 34.8 years; standard deviation (SD) = 4.5 years). Detailed demographics are shown in Table 1. Of all participants, 12 had one child, 30 had two children, 9 had three children, and 1 had four children. The total age of the oldest child of the participants was 6.2-years-old (SD = 3.2) (this equals the total motherhood period until the day of the experiment). We added up the ages of each child to calculate an index, named the cumulative motherhood period, which reflected the efforts invested in parenting more appropriately for mothers rearing multiple children (Supplementary Figure S2). All participants had received at least 12 years of education. Almost all (94 %) were right-handed according to the FLANDERS handedness inventory [31, 32].

This study's protocol was approved by the Ethics Committee of the University of Fukui and was conducted following the Declaration of Helsinki. All participants provided written informed consent for participation in this study.

Questionnaires

The Japanese version of the PSI [33], which is an adaptation of the PSI [34], was used to evaluate the participants' parenting stress (comprising items on Child and Parent domains). The Beck Depression Inventory-II [35, 36] was used to measure participants' depressive symptoms.

DNA methylation

Saliva samples were collected using Oragene Discover OGR-500 kits (DNA Genotek Inc., Ottawa, ON, Canada). Saliva DNA was extracted using prepIT®•L2P reagent (DNA Genotek Inc.) and was quantified with Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific Inc., Pittsburgh, PA, USA) [37]. Five hundred ng of DNA was bisulfite-treated for cytosine to thymine conversion using the EZ DNA Methylation-Gold kit (Zymo Research, Irvine, CA, USA). DNA was then whole-genome amplified, fragmented, and hybridized to the HumanMethylationEPIC BeadChip (Illumina Inc., San Diego, CA, USA). The BeadChips were scanned using iSCAN (Illumina Inc.), and the methylation level (β value) was calculated for each queried CpG locus using the GenomeStudio Methylation Module software. A quality check was conducted based on the Psychiatric Genomics Consortium-EWAS quality control pipeline [38]. Samples with probe detection call rates < 90%, and those with an average intensity value of either < 50% of the experiment-wide sample mean or < 2,000 arbitrary units were excluded. Probes with detection $P > 0.001$ or those based on less than three beads were set to missing as were probes that cross-hybridized between autosomes and sex chromosomes [39]. CpG sites with missing data for > 10% of samples within cohorts were excluded from the analysis. After quality control, 804,979 probes were left for further analysis. Horvath's multi-tissue clock mAge was calculated based on the online calculator (<https://horvath.genetics.ucla.edu/html/dnamage/>). We regressed mAge on chronological age; the unstandardised residuals indicated epigenetic age acceleration. Probes containing single nucleotide polymorphisms (based on 1000 Genomes) within 10 base pairs of the target CpG were maintained in each dataset, but flagged and tracked throughout the analysis pipeline. This decision was based on the growing recognition that sequence variants can influence DNA methylation patterns throughout the genome [40]. Normalisation of probe distribution and background differences between Type I and Type II probes was conducted using Beta Mixture Quantile Normalization [39] after background correction. Following normalisation, batch effect removal, as implemented in the ComBat procedure of the SVA package in Bioconductor, was used to account for sources of technical variations including batch and positional effects, which can cause spurious associations [41]. As saliva contains a heterogeneous mixture of cell types that differ in proportion in each sample, using the EpiDISH method [42], we estimated the proportion of epithelial cells derived from salivary DNA and entered it as a covariate in our statistical models.

MRI acquisition and VBM

Image acquisition of 52 participants was performed using a GE Discovery MR 750 3-Tesla scanner (GE Healthcare, Milwaukee, WI). A T1-weighted anatomical dataset was obtained from each subject by a fast-spoiled gradient recalled imaging sequence (voxel size $1 \times 1 \times 1$ mm, TE = 1.99 ms, TR = 6.38 ms, flip angle = 11°). Image acquisition of the other participants was carried out using a GE Signa PET/MR 3-Tesla scanner (GE Healthcare, Milwaukee, WI). High-resolution structural whole-brain images were acquired using a 3D T1-weighted fast spoiled-gradient recalled imaging sequence (voxel size $1 \times 1 \times 1$ mm, TE = 3.24 ms, TR = 8.46 ms, flip angle = 11°). VBM data were analysed using the Statistical Parametric Mapping software (SPM12; <https://www.fil.ion.ucl.ac.uk/spm>) implemented in MATLAB 2014a (<https://www.mathworks.com>). The T1-weighted images were pre-processed using the VBM approach with modulation, where the images were first segmented into GM, white matter, cerebrospinal fluid, and

skull/scalp compartments. Using the iterative high-dimensional normalisation approach provided by Diffeomorphic Anatomical Registration through an Exponentiated Lie algebra algorithm [43], the segmented GM images were spatially normalised into the stereotaxic space of the MNI. The GM images had an isotropic voxel resolution of 1.5 mm^3 . Any volume change induced by normalisation was adjusted via a modulation algorithm. The normalised modulated GM images were spatially smoothed by a Gaussian kernel of 8-mm full-width-at-half-maximum.

Statistical analysis

We conducted linear regression models with four types of reproductive effort indices parity (primiparous or multiparous), number of delivery, motherhood period, and cumulative motherhood period as independent variables to predict age acceleration. To investigate how much variance in age acceleration is accounted for by reproductive effort in the presence of a range of potential confounders (Chronological age and PSI), a three-stage hierarchical multiple regression using the enter method was conducted. In the VBM analysis, chronological age, scanner, and total brain volume were included as covariates of no interest in the design matrix to regress out their effects. The resulting set of voxel values used for each contrast generated a statistical parametric map of the t -statistic, $\text{SPM}(t)$, which was transformed to a unit normal distribution ($\text{SPM}[Z]$). The statistical threshold was set to $P < 0.05$ with family-wise error correction for multiple comparisons at the cluster level (height threshold of $Z > 3.09$). Significant clusters were localised in the Automated Anatomical Labelling atlases implemented in the MRIcron software package (<https://www.nitrc.org/projects/mricron>).

We conducted multiple mediation analyses to assess whether the GM volume mediated the link between reproductive effort and age acceleration. We included chronological age as the covariate in the model. The indirect effects of each model were tested by bootstrapping confidence intervals using the lavaan package [44]. The model parameters were set to give bias-corrected 95% confidence intervals and to run two thousand bootstrap resamples. All statistical analyses were performed with R 3.6.1 and SPM 12.

Declarations

Acknowledgments: All phases of this study were supported by AMED under Grant Number JP20gk0110052 (AT and SN), JSPS KAKENHI Scientific Research (A) (19H00617 to AT), Scientific Research (C) (20K02700 to SN), Grant-in-Aid for “Creating a Safe and Secure Living Environment in the Changing Public and Private Spheres” from the Japan Science and Technology Corporation (JST)/Research Institute of Science and Technology for Society (RISTEX), Research grant from Japan-United States Brain Research Cooperative Program (AT) and Grant-in-Aid for Translational Research from the Life Science Innovation Center, University of Fukui (LSI20305 to SN). We thank the staff at the Research Center for Child Mental Development and Department of Child and Adolescent Psychological Medicine, University of Fukui Hospital for their clerical support.

Author contributions: S.N. and A.T. conceived and designed the project. S.N., R.K., D.H., K.S., and T.X.F. performed the experiments, collected, and analysed the data. S.N. drafted the manuscript.

Competing interests: The authors declare no competing interests.

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Tables

Table 1. Demographics of the participants

	(<i>n</i> = 52)
Age (years), <i>Mean (SD)</i>	35.4 (4.4)
Age (years) at first childbirth	29.2 (4.0)
Age (years) at last childbirth	32.2 (3.9)
Parity, <i>n (%)</i>	
Primiparous / Multiparous	12 (23.1) / 40 (76.9)
Number of children, <i>n (%)</i>	
One	12 (23.1)
Two	30 (57.7)
Three	9 (17.3)
Four	1 (1.9)
Motherhood period (age of oldest child) (years)	6.2 (3.2)
Cumulative motherhood period (years)	9.8 (6.0)
Exclusive breastfeeding diet, <i>n (%)</i>	30 (57.7)
Household Income (currency = JPY), <i>n (%)</i>	
Less than 3 million	3 (5.9)
3-5 million	25 (49.0)
5-10 million	21 (41.2)
More than 10 million	2 (3.9)
MRI scanner, <i>n (%)</i>	
Discovery MR 750 3T / Signa PET/MR 3T	31 (59.6) / 21 (40.4)
Proportion of Epithelial cells (%)	15.8 (5.7)
FLANDERS handedness inventory (right / mixed / left)	49 (94.2) / 1 (1.9) / 2 (3.9)
PSI (Total / Child / Adult)	191.5 (39.8) / 86.5 (18.6) / 105.1 (24.6)
BDI-II	11.9 (9.2)

PSI: Parenting Stress Index (Abidin 1995, Namara et al., 1999), BDI-II: The Beck Depression Inventory-II (Beck, Steer, & Brown, 1996; Kojima et al., 2002)

One participant's data was not available for Exclusive breastfeeding diet, Household Income, PSI and BDI-II (*n* = 51).

Table 2. A three-stage hierarchical multiple regression using the enter method

		<i>b</i>	<i>P</i>	<i>R</i> ²	ΔR^2
Step1	Chronological age	-0.07	0.53	-0.01	
Step2	Chronological age	0.01	0.95	0.14	0.15
	PSI	-0.04	0.00		
Step3	Chronological age	0.02	0.85	0.20	0.06
	PSI	-0.04	0.00		
	Number of delivery	-1.30	0.047		

PSI: Parenting Stress Index (Abidin 1995, Namara et al., 1999)

One participant's data was not available for PSI (*n* = 51).

Figures

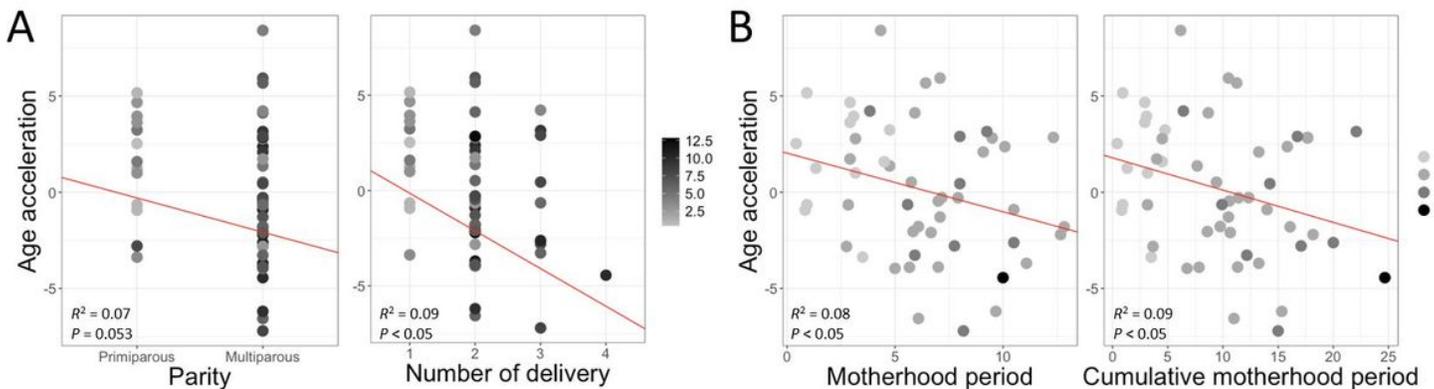


Figure 1

(A) Associations between parity (left), number of delivery (right), and age acceleration. The grey scale indicates motherhood periods (years). (B) Associations between motherhood period (left), cumulative motherhood period (right), and age acceleration. The grey scale indicates delivery number.

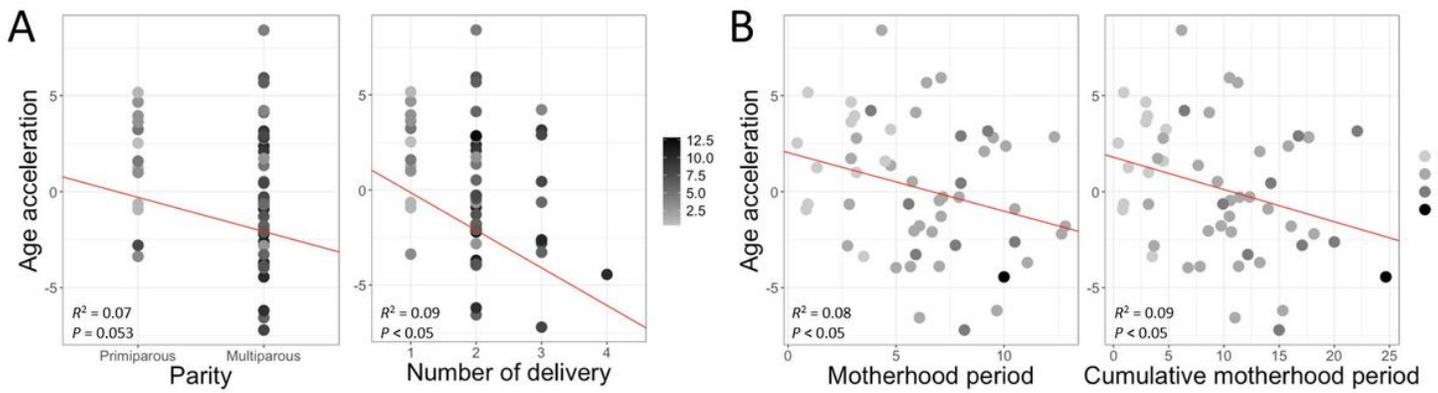


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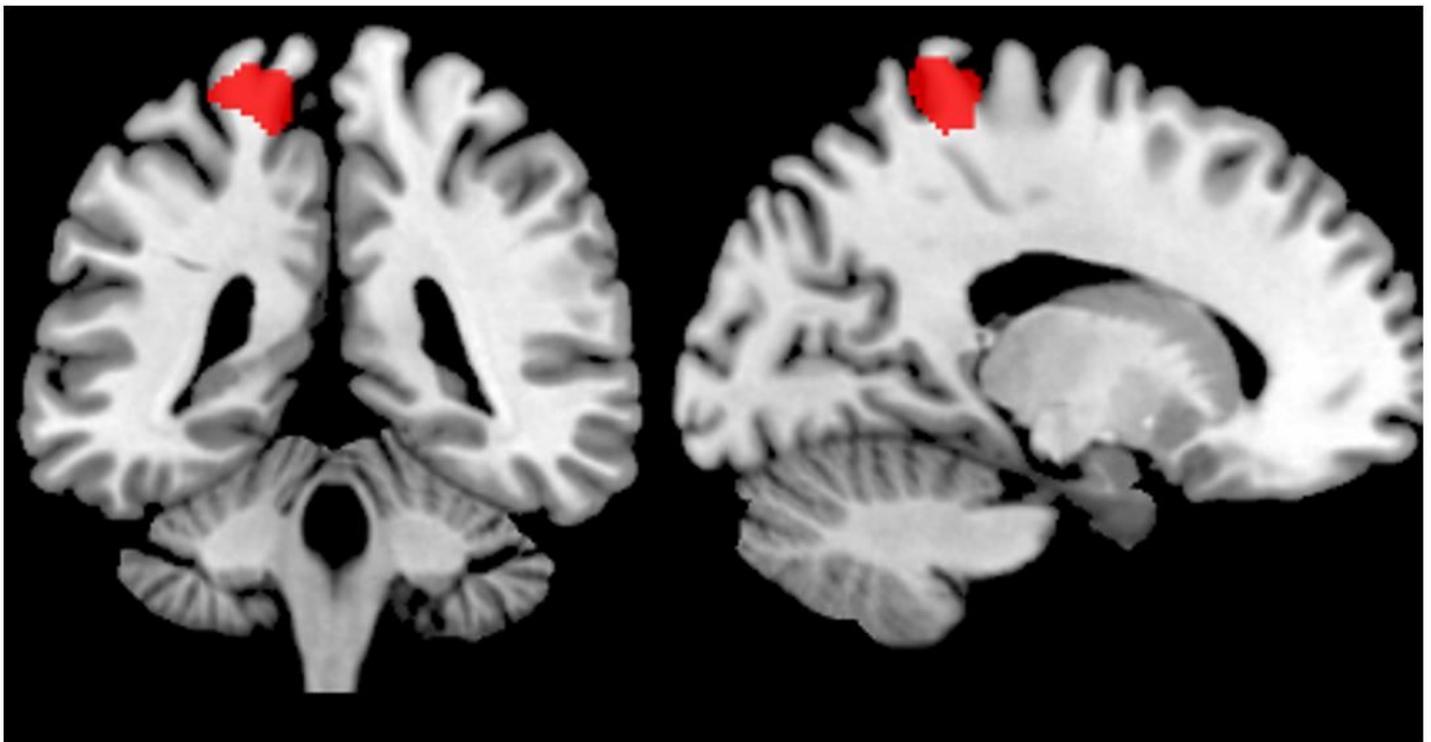


Figure 2

Regions of grey matter (GM) volume negatively associated with age acceleration (red; threshold $P < 0.05$, family-wise error corrected cluster level).

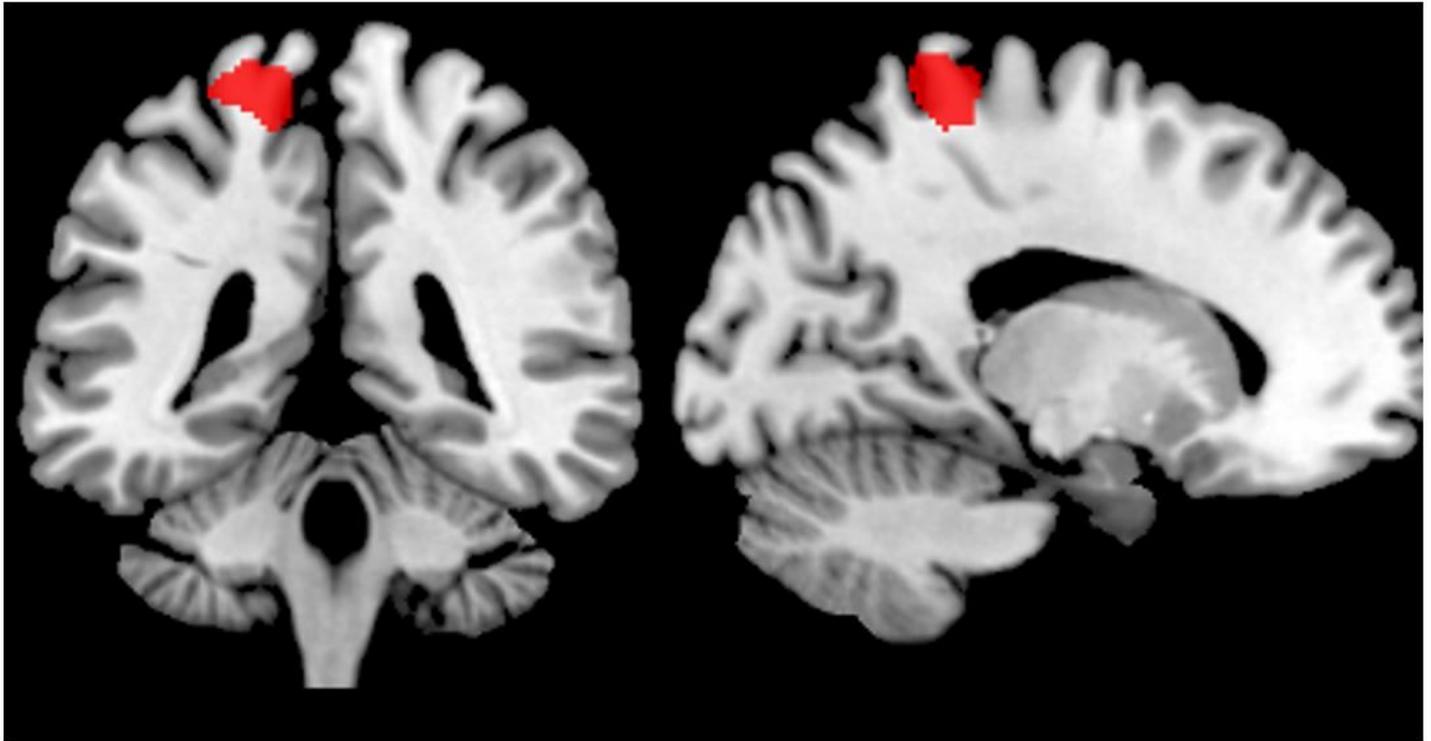
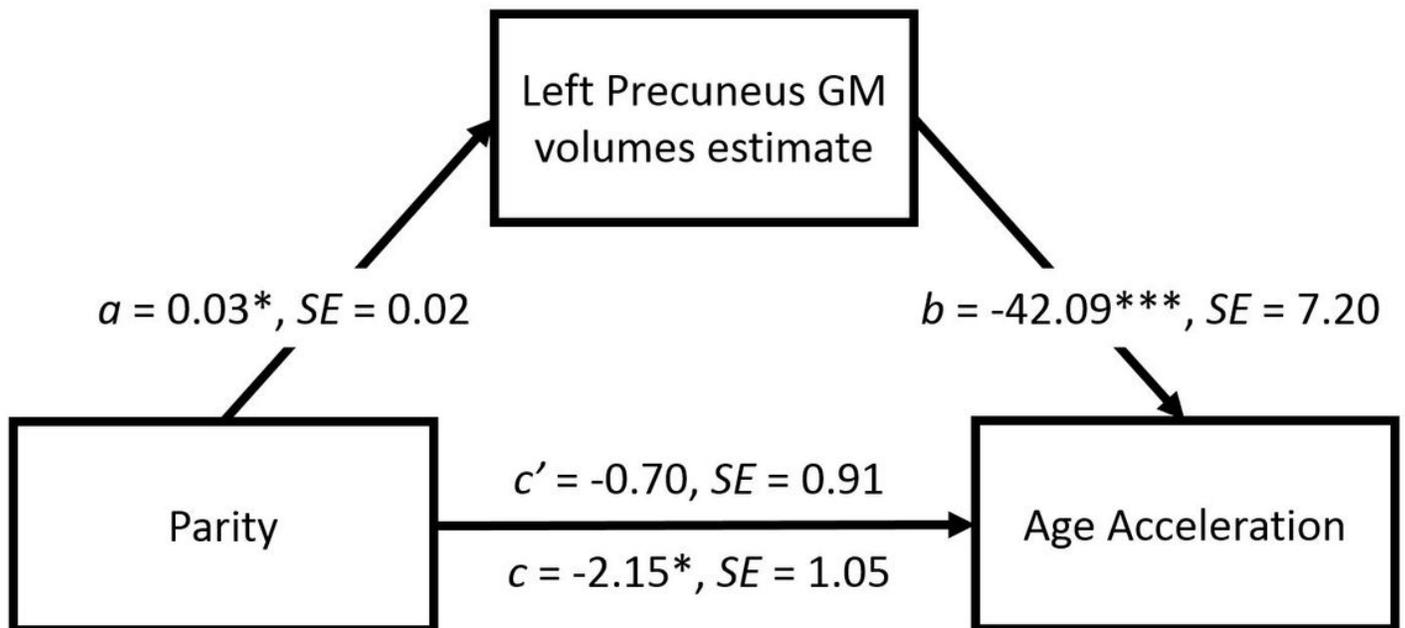


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Regions of grey matter (GM) volume negatively associated with age acceleration (red; threshold $P < 0.05$, family-wise error corrected cluster level).



indirect effect: $ab = -1.45$, $SE = 0.73$, $95\%CI = [-3.09, -0.14]$

*** $P < .001$, * $P < 0.05$

Figure 3

Path model for the mediation effect. Grey matter (GM) volume of the left precuneus was a mediator in the relationship between parity and age acceleration.

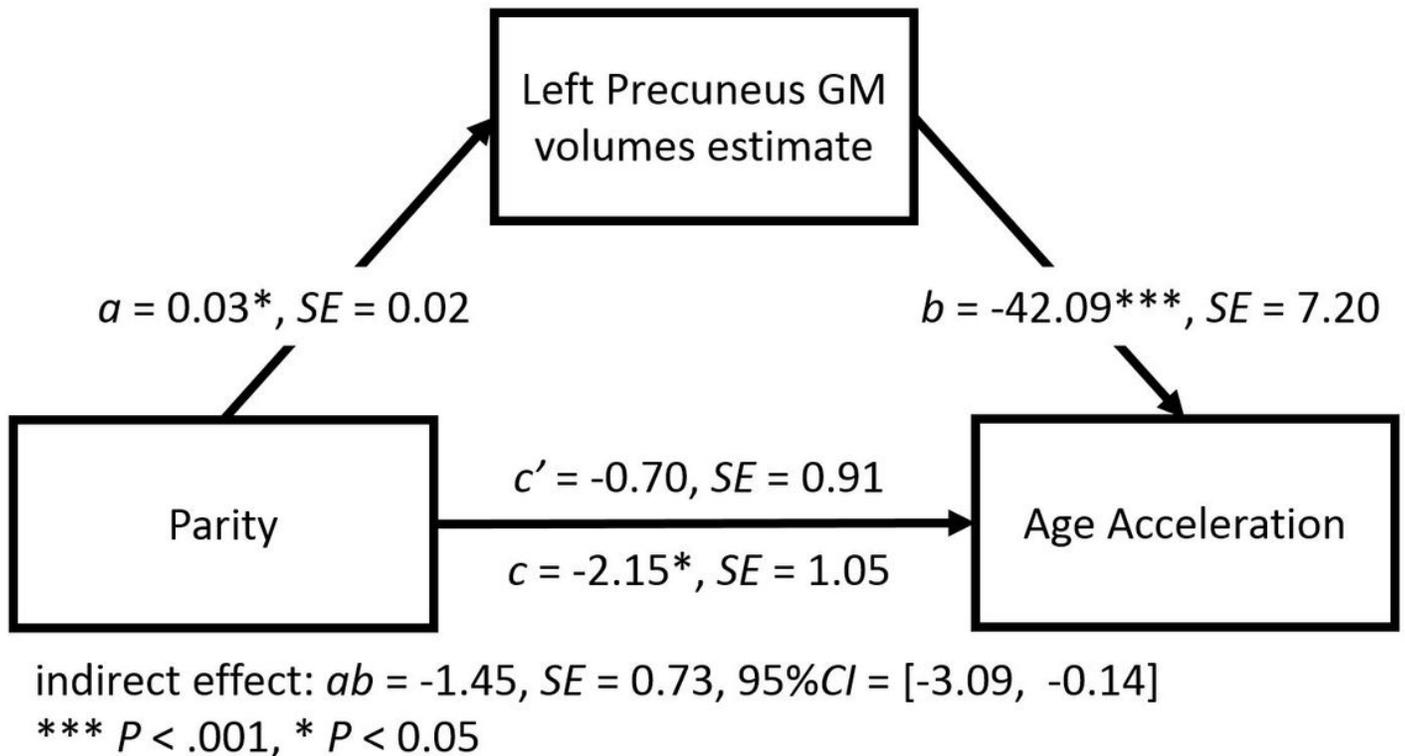


Figure 3

Path model for the mediation effect. Grey matter (GM) volume of the left precuneus was a mediator in the relationship between parity and age acceleration.

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