Cumulative estrogen exposure, APOE genotype, and women's brain aging - a population-based neuroimaging study

Ann-Marie de Lange*[1,2,3] ✉, Claudia Barth*[2] ✉, Tobias Kaufmann[2], Ivan I. Maximov[1,2], Dennis van der Meer[2,4], Ingrid Agartz[2,5,6], and Lars T. Westlye[1,2]

[1]Department of Psychology, University of Oslo, Oslo, Norway
[2]NORMENT, Institute of Clinical Medicine, University of Oslo, & Division of Mental Health and Addiction, Oslo University Hospital
[3]Department of Psychiatry, University of Oxford, Warneford Hospital, Oxford, UK
[4]School of Mental Health and Neuroscience, Faculty of Health, Medicine and Life Sciences, Maastricht University, The Netherlands
[5]Department of Psychiatry, Diakonhjemmet Hospital, Oslo, Norway
[6]Department of Clinical Neuroscience, Centre for Psychiatric Research, Karolinska Institutet, Stockholm, Sweden

Estrogen exposure may influence women's risk of Alzheimer's disease, but little is known about how it affects normal brain aging. Recent findings from the UK Biobank demonstrate less evidence of brain aging in women with a history of multiple childbirths. Here, we investigated the link between brain aging, estrogen exposure, and APOE genotype beyond the effects of parity in 16,854 UK Biobank women. Machine learning was used to predict brain age based on neuroimaging-derived measures, and the difference between an individual's predicted and chronological age was used as an estimate of brain aging. Cumulative estrogen exposure was estimated using an index including age at menarche and menopause, BMI, time since menopause, and duration of hormone replacement therapy. Endogenous hormone exposure was approximated by reproductive span, while exogenous exposure was estimated by usage, onset, and duration of hormone replacement therapy and oral contraceptives. Higher cumulative, endogenous, and exogenous estrogen exposure were each linked to *higher* brain age relative to chronological age. Earlier onset of hormone replacement therapy, particularly before menopause, was associated with less evident brain aging in APOE e4 carriers only, while higher circulating estradiol levels during menopause were linked to more evident brain aging in carriers and less evident brain aging in non-carriers. The results indicate that estrogen exposure and parity may differentially relate to women's brain aging, and that APOE e4-specific associations between estrogen and brain aging may be of importance for optimizing hormone replacement therapy regimes in perimenopausal women.

Women's health | Estrogens | Parity | Apolipoproteins E | Neuroimaging | Machine learning

Correspondence:
a.m.g.d.lange@psykologi.uio.no
claudia.barth@medisin.uio.no
**** both authors contributed equally to the paper***

1 Introduction

Women are at significantly greater risk of developing Alzheimer's disease (AD) or other types of dementia relative to men (1), and among women, a higher degree of lifetime exposure to estrogen has been linked to a lower risk of AD (2, 3). Estrogen may also have beneficial effects on cognition (4, 5), and studies have shown a link between endogenous estrogen exposure and less cognitive decline in older age (6, 7). However, other studies have found no association between endogenous exposure and dementia risk (8, 9) or cognitive performance (9, 10). Only a small number of studies have been population-based (8), and among those, findings are mixed. Results from the Guangzhou Biobank Cohort Study in China showed that longer reproductive span, i.e. higher endogenous exposure to estrogen, was associated with higher cognitive scores (11). Results from the Esprit study in France showed an association between longer reproductive span and better cognitive performance, but no relationship was observed between reproductive span and cognitive decline across four years (9). A recent meta-analysis found no link between endogenous estrogen exposure and incident dementia (8). Findings from the Rotterdam cohort showed that women with longer reproductive span had an *increased* risk of dementia, but the association was only evident in women carrying the apolipoprotein E type 4 (APOE e4) genotype (12). Carried by 14% of the worlds population (13), the APOE e4 allele is a known risk factor for AD: 91% of homozygous e4 carriers and 47% of heterozygous carriers have been shown to develop AD (14). The APOE-related risk of developing AD is modified by sex, with higher risk in women compared to men (15), and emerging evidence suggests that estrogen and APOE genotype may interact (16–18). For instance, Yaffe and colleagues (19) found that among non-carriers, hormone replacement therapy (HRT) use reduced the risk of cognitive impairment by almost half compared to never-users, while there was no such effect among carriers. Results from the Nurses' Health Study showed that HRT use was associated with worse rates of decline in general cognition, especially among women with an APOE e4 allele (20). While a number of studies indicates positive effects of HRT on cognition (9, 11, 21), other studies have shown associations between HRT use and worse rates of cognitive decline (12), as well as increased risk of dementia (20). Thus, results from HRT studies are inconclusive, and findings differ across observational studies and randomised trials (22, 23)

Despite its widespread use, exogenous estrogen exposure via oral contraceptives (OC) is understudied. Studies on OC use have mainly compared cognitive performance in users and non-users at high or low hormone states across the menstrual cycle in premenopausal women (24, 25). The results of these studies are inconclusive, largely due to small sample sizes with an overall mean of 24 OC users per study, including several studies with 10 or fewer participants (26). To the best of our knowledge, only one study has investigated the

impact of OC use on cognition later in life, using data from the Wisconsin Registry for Alzheimer's Prevention (27). The results from this study suggested a positive association between OC duration and cognitive performance. However, the sample of OC users (n = 227) and never-users (n = 34) was highly imbalanced, precluding the drawing of firm conclusions. Whether short or long-term use of OC modulates risk for developing AD is unknown.

While a number of studies have investigated the effects of estrogen and APOE genotype on dementia and AD risk, little is known about the influence on normal brain aging. Changes in hormones such as estradiol are known to influence brain plasticity (28, 29), and in premenopausal women, magnetic resonance imaging (MRI) studies have indicated modulating effects of endogenous estrogen fluctuations on brain structure across the menstrual cycle (30) and during pregnancy (31). While higher endogenous estrogen levels have been associated with larger hippocampal volumes during women's reproductive years (30, 32), results from the Rotterdam Scan Study showed that in menopausal women, higher endogenous estrogen levels were associated with *smaller* hippocampal volumes as well as poorer memory performance in menopausal women (33). Negative effects of exogenous estrogen levels have also been reported, and findings from the Women's Health Initiative Memory Study showed that conjugated estrogen, both alone and in combination with progestin, was associated with greater atrophy among women aged 65 years and older (34). In addition, conjugated estrogen administration has been linked to higher rates of ventricular expansion over 4 years in recently menopausal women (35). However, other MRI studies suggest a protective effect of HRT on gray matter (36), as well as white matter and ventricle size (37), and larger hippocampal volumes have been observed in users relative to non-users (38). Thus, the results from previous MRI studies are equivocal, and the majority of existing findings are based on relatively small sample sizes.

Cumulative estrogen exposure is often approximated by including information on age at menarche and menopause, duration of HRT use, postmenopausal weight, years since menopause, and parity (7). Parity represents a complex addition to the estimation given the role of immune factors in pregnancy such as the proliferation of regulatory T cells (39), which is suggested to protect against AD (40). In addition, estrogen levels rise up to 300-fold throughout pregnancy (41) and fall 100–1000 fold postnatally (42), often in relation to breastfeeding (8), of which the duration influences the number of total menstrual cycles within a woman's reproductive years (11, 43). Parous women may also have shorter menstrual cycles and lower levels of estradiol than nulliparous women (44, 45), indicating that age at first birth also influences lifetime estrogen exposure. Studies have demonstrated a protective effect of parity on brain aging (31, 46–48), and we recently showed lower brain age in parous compared to nulliparous women in the UK Biobank cohort (49). In the present paper, we investigate the association between estrogen exposure and brain aging beyond the effects of parity.

We analysed the association between endogenous as well as exogenous estrogen exposure and predicted brain age relative to chronological age (*brain age gap)* in 16,854 women with a mean age of 54.70 years $\pm$ 7.29 (standard deviation). Brain age prediction estimates an individual's apparent brain aging based on MRI-derived brain characteristics, and is becoming a widely applied marker of the neuroanatomical aging process (50–53). We estimated brain age using the XGBoost Python Package (54). To test for associations between estimates of estrogen exposure and structures and modalities (55–57), we included separate brain age models based on T1-weighted MRI measures (full T1w model, cortical thickness, cortical and subcortical volume) (51, 53, 58, 59), and diffusion-weighted MRI in a sub-sample of 9,829 women. Cumulative estrogen exposure was estimated by an index of cumulative estrogen exposure (ICEE) including age at menarche and menopause, time since menopause, body mass index (BMI), and duration of HRT use (7). Endogenous estrogen exposure was estimated by reproductive span (age at menopause – age at menarche), while exogenous exposure was estimated by usage, onset, and duration of HRT and OC use. To examine the effect of APOE e4 genotype on the association between estrogen exposure and brain age gap, we performed follow-up analyses including interaction terms between APOE e4 genotype and estimates of estrogen exposure for each of the measures (see Materials and Methods for details). In order to investigate the link between estrogen exposure and brain aging beyond the effects of parity (49), all analyses were corrected for number of childbirths.

2 Results

The root mean square errors (RMSE) for each brain age model are shown in Table 1. Figure 1, Figure 2, and Table 2 show an overview of the main results. p-values are reported before and after false discover rate (FDR) correction (60) (p_{corr}).

2.1 Index of cumulative estrogen exposure (ICEE): A multiple linear regression showed a positive association between ICEE and brain age gap based on the full T1w model, indicating that higher ICEE was linked to higher brain age relative to chronological age (n = 8,878). The inclusion of age at first birth and education as additional covariates yielded similar results for ICEE and brain age gap ($\beta = 0.04, SE = 0.02, t = 2.71, p = 6.72 \times 10^{-3}, p_{corr} = 8.06 \times 10^{-3}$, n = 7,068). Significant relationships were also found between ICEE and brain age gap based on cortical thickness, and cortical volume, and subcortical volume. No relationship was found between ICEE and brain age gap based on diffusion MRI.

2.2 Endogenous estrogen exposure:

2.2.1 Reproductive span: Reproductive span was calculated as age at menopause − age at menarche. 9,188 menopausal women had data on both variables and were included in the analysis. A multiple linear regression including number

Table 1. Number of MRI variables, RMSE values, and the correlations between predicted and chronological age for each of the brain age models.

Model	MRI variables	RMSE	Predicted age versus chronological age	Corrected predicted age versus chronological age
Full T1w model	1118	6.06	$r = 0.56, p =< 0.001, CI95\% = [0.55, 0.57]$	$r = 0.93, p =< 0.001, CI95\% = [0.93, 0.93]$
Thickness	126	6.02	$r = 0.57, p =< 0.001, CI95\% = [0.55, 0.57]$	$r = 0.93, p =< 0.001, CI95\% = [0.92, 0.93]$
Volume	360	6.45	$r = 0.47, p =< 0.001, CI95\% = [0.46, 0.48]$	$r = 0.95, p =< 0.001, CI95\% = [0.94, 0.95]$
Subcortical	25	6.02	$r = 0.56, p =< 0.001, CI95\% = [0.55, 0.57]$	$r = 0.92, p =< 0.001, CI95\% = [0.91, 0.92]$
Diffusion	170	5.55	$r = 0.66, p =< 0.001, CI95\% = [0.65, 0.67]$	$r = 0.93, p =< 0.001, CI95\% = [0.92, 0.93]$

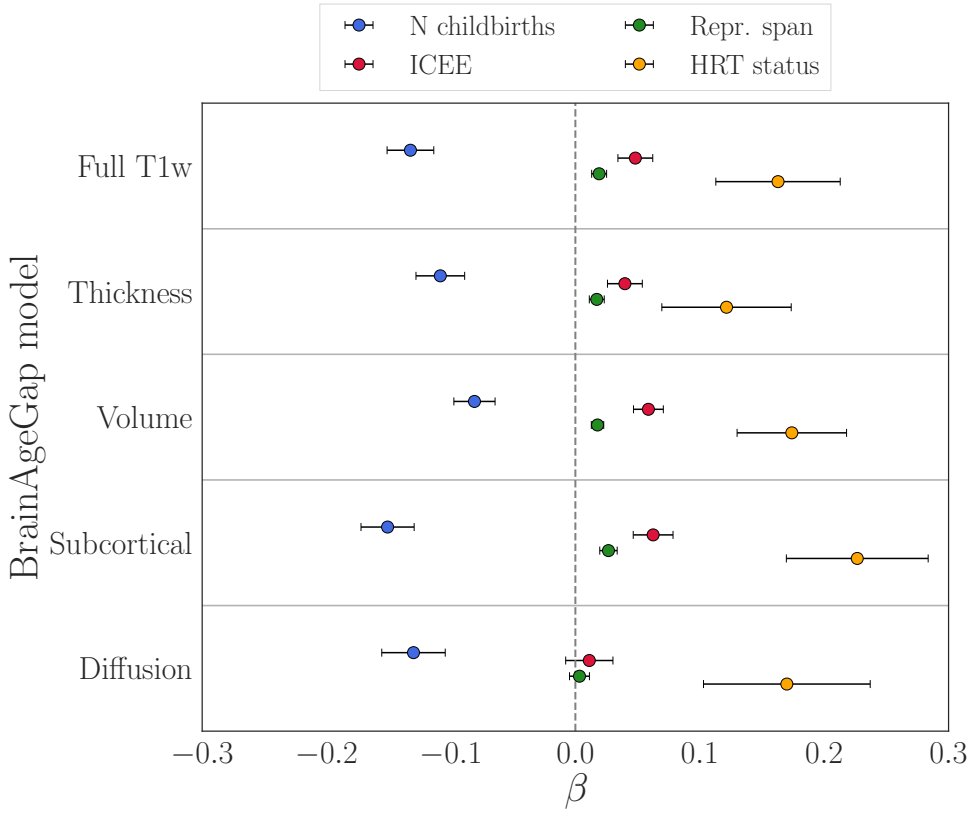

Fig. 1. Associations between estimates of hormone exposure and brain age gap. The points show the β values (slope) from multiple regression analyses on brain age gap and *number of births*, *index of cumulative estrogen exposure (ICEE)*, *reproductive span*, and *hormone replacement therapy (HRT) status*, for each of the brain age models (y axis). HRT status = 0 for never-users and 1 for current and former users. The error bars represent the standard error on the β. The ICEE, reproductive span, and HRT analyses were corrected for number of births. In addition, the analyses on reproductive span also included *ever used HRT and/or OC* as covariates, and the analysis on HRT status included *had hysterectomy and/or oophorectomy*.

of births, ever used HRT, and ever used OC as covariates showed a positive association between reproductive span and brain age gap based on the full T1w model, indicating that a longer reproductive span was linked to higher brain age relative to chronological age. When including age at first birth and education as covariates, the results were similar ($\beta = 0.02, SE = 6.65 \times 10^{-3}, t = 2.52, p = 0.01, p_{corr} = 0.01$, n = 7,323). Significant relationships were found between reproductive span and brain age gap based on cortical thickness, cortical volume, and subcortical volume. No significant relationship was found between reproductive span and brain age gap based on diffusion MRI. The associations between brain age gap and age at menarche and menopause are provided in the Supplementary Information (SI).

2.3 Exogenous estrogen exposure:

2.3.1 Hormone replacement therapy (HRT): A multiple linear regression including number of births, had hysterectomy and/or oophorectomy in the models showed an association between HRT status and brain age gap based on the full T1w model in pre-menopausal and menopausal women, with a lower brain age relative to chronological age in never-users (n = 11,139) compared to users (n = 5,546 with 1,182 still using). When including age at first birth and education in the model, the results were similar ($\beta = 0.22, SE = 0.06, t = 3.93, p = 8.57 \times 10^{-5}, p_{corr} = 4.57 \times 10^{-4}$, never-users = 8,414, n users = 4,505). HRT status also showed an association with brain age gap based on cortical thickness, cortical volume, subcortical volume, and diffusion (n for never-users = 6,461, n for users = 3,240), with a lower brain age relative

to chronological age in never-users compared to users.

Within the group of HRT users (n = 5,164), a positive relationship was found between ***age at HRT onset*** and brain age gap based on the full T1w model, indicating that starting HRT earlier may be beneficial (covariates: number of births, had hysterectomy, and/or oophorectomy). Positive relationships were found between age at HRT onset and brain age gap based on cortical volume and subcortical volume, as well as cortical thickness. No significant relationships were found between age at HRT onset and brain age gap based on diffusion MRI. No significant associations were found between ***duration of HRT use*** (age last used HRT - age started HRT) and brain age gap based on any of the brain age models (full T1w model: $\beta = 3.41 \times 10^{-3}, SE = 4.81 \times 10^{-3}, t = 0.71, p = 0.48, p_{corr} = 0.55$, n = 5,164; cortical thickness: $\beta = 4.32 \times 10^{-3}, SE = 4.97 \times 10^{-3}, t = 0.87, p = 0.39, p_{corr} = 0.47$; cortical volume: $\beta = 3.71 \times 10^{-4}, SE = 4.19 \times 10^{-3}, t = 0.09, p = 0.93, p_{corr} = 0.93$; subcortical volume: $\beta = 8.77 \times 10^{-3}, SE = 5.41 \times 10^{-3}, t = 1.62, p = 0.11, p_{corr} = 0.15$; diffusion: $\beta = 5.95 \times 10^{-3}, SE = 6.35 \times 10^{-3}, t = 0.94, p = 0.35, p_{corr} = 0.47$, n = 2,998, covariates: number of births, had hysterectomy and/or oophorectomy).

2.3.2 Oral contraceptives (OC): A linear regression showed no significant association between OC use and brain age gap based on the full T1w model in pre-menopausal and menopausal women, as shown in Table 2 (never-users = 2,213, users = 14,615 with 398 still using, covariate: numbers of births). After adjusting for education and age at first births, the result was similar ($\beta = -0.003, SE = 0.08, t = -0.04, p = 0.97, p_{corr} = 0.97$, never-users = 2,201, n users = 14,581). No relationships were found between OC use and brain age gap based on cortical volume, subcortical volume, or diffusion MRI (never-users = 1,279, n users = 8,501). An association was seen between OC use and brain age gap based on cortical thickness, with a lower brain age relative to chronological age in never-users compared to users ($\beta = 0.17, SE = 0.07, t = 2.55, p = 0.01, p_{corr} = 0.09$).

In OC users (n = 14,284), ***age started OC intake*** was not significantly associated with brain age gap based on any of the models (full T1w model: $\beta = 2.76 \times 10^{-3}, SE = 5.73 \times 10^{-3}, t = 0.48, p = 0.63, p_{corr} = 0.72$); cortical thickness: $\beta = 4.45 \times 10^{-3}, SE = 5.98 \times 10^{-3}, t = 0.74, p = 0.46, p_{corr} = 0.71$; cortical volume: $\beta = -5.82 \times 10^{-3}, SE = 5.07 \times 10^{-3}, t = -1.15, p = 0.25, p_{corr} = 0.47$; subcortical volume: $\beta = 4.42 \times 10^{-3}, SE = 6.53 \times 10^{-3}, t = 0.68, p = 0.49, p_{corr} = 0.71$; diffusion (n = 8,336): $\beta = -1.27 \times 10^{-3}, SE = 7.76 \times 10^{-3}, t = -0.16, p = 0.87, p_{corr} = 0.92$, covariate: number of births).

Within the group of OC users (n = 13,462), a positive relationship was found between ***duration of OC use*** and brain age gap based on the full T1w model ($\beta = 6.19 \times 10^{-3}, SE. = 3.08 \times 10^{-3}, t = 2.01, p = 0.04, p_{corr} = 0.15$, covariate: number of births), indicating higher brain age relative to chronological age in longer-term users. When including age at first birth and education as covariates, the association was less strong ($\beta = 6.19 \times 10^{-3}, SE = 3.58 \times 10^{-3}, t = 1.73, p = 0.08, p_{corr} = 0.24$, n = 10,654). No significant relationship was found between duration of OC use and brain age gap based on cortical thickness ($\beta = 4.99 \times 10^{-3}, SE = 3.21 \times 10^{-3}, t = 1.55, p = 0.12, p_{corr} = 0.29$). Positive relationships were found between duration of OC and brain age gap based on cortical volume ($\beta = 8.57 \times 10^{-3}, SE = 2.73 \times 10^{-3}, t = 3.15, p = 0.002, p_{corr} = 0.03$), subcortical volume ($\beta = 7.47 \times 10^{-3}, SE = 3.51 \times 10^{-3}, t = 2.13, p = 0.03, p_{corr} = 0.14$), and diffusion ($\beta = 9.83 \times 10^{-3}, SE = 4.18 \times 10^{-3}, t = 2.35, p = 0.02, p_{corr} = 0.11$, n = 7834).

2.4 APOE e4 genotype and estimates of hormone exposure: APOE e4 status showed no main associations with brain age gap based on any of the models (covariate: number of births, n carriers = 4,276, n non-carriers = 11,649). We found no effects of the interaction ***APOE e4 status * ICEE*** on any of the brain age gap models. Similarly, no effects were found for ***APOE e4 status * reproductive span***, and ***APOE e4 status * ever used HRT*** (see SI Table 1).

There was a effect of ***APOE e4 status * age for HRT onset*** on brain age gap based on cortical thickness ($\beta = 0.05, SE = 0.02, t = 2.66, p = 0.01, p_{corr} = 0.07$). Follow-up analyses showed that the relationship between age started HRT and brain age gap based on cortical thickness was confined to the carrier group (n = 1,227), as shown in Figure 2 ($\beta = 0.05, SE = 0.02, t = 2.96, p = 3.00 \times 10^{-3}, p_{corr} = 0.04$; for the non-carrier group (n = 3,646), $\beta = 0.01, SE = 0.01, t = 0.49, p = 0.63, p_{corr} = 0.80$). Within the group of carriers, there was no dose-dependent effect of the interaction age started HRT * carrier group (n with 1 e4 allele = 1,126, n with 2 e4 alleles = 101, $\beta = 0.05, SE = 0.07, t = 0.80, p = 0.43, p_{corr} = 0.73$).

In menopausal APOE e4 carriers, age started HRT relative to age at menopause (age at menopause – age started HRT) was associated with brain age gap based on the full T1w model ($\beta = -0.08, SE = 0.02, t = -3.34, p = 8.80 \times 10^{-4}, p_{corr} = 0.02$, n = 826, covariates: number of births, had hysterectomy and/or oophorectomy), indicating that HRT initiation before onset of menopause may have a beneficial effect on brain aging. Adjusting for age at first birth and education did not change the results ($\beta = -0.09, SE = 0.03, t = -3.39, p = 7.5 \times 10^{-4}, p_{corr} = 0.02$, n = 666). The effect was absent in non-carriers (full T1w model: $\beta = 6.33 \times 10^{-3}, SE = 0.01, t = 0.46, p = 0.65, p_{corr} = 0.80$, n = 2480), and not dose-dependent (n with 1 e4 allele = 758, n with 2 e4 alleles = 68, full T1w model: $\beta = -0.04, SE = 0.08, t = -0.48, p = 0.63, p_{corr} = 0.80$). In the carrier group, negative associations were also found between age for HRT onset relative to onset of menopause and brain age gap based on cortical thickness and cortical volume ($\beta = -0.06, SE = 0.02, t = -2.38, p = 0.02, p_{corr} = 0.13$ and $\beta = -0.04, SE = 0.02, t = -2.00, p = 0.05, p_{corr} = 0.21$, respectively), while no significant relationships were found with brain age gap based on subcortical volume or diffusion MRI ($\beta =$

Table 2. The associations between each estimate of hormone exposure and brain age gap. β = slope, SE = standard error. P-values are reported before and after false discovery rate (FDR)-correction (p_{corr}) (60)

Index of cumulative estrogen exposure (ICEE)					
Model	β	SE	t	p	p_{corr}
Full T1w model	0.05	0.01	3.43	6.09×10^{-3}	1.22×10^{-3}
Cortical thickness	0.04	0.01	2.74	6.08×10^{-3}	8.06×10^{-3}
Cortical volume	0.06	0.01	4.75	2.03×10^{-6}	1.22×10^{-5}
Subcortical volume	0.06	0.02	3.92	8.93×10^{-5}	2.68×10^{-4}
Diffusion	0.01	0.02	0.58	0.56	0.56
Reproductive span					
Model	β	SE	t	p	p_{corr}
Full T1w model	0.02	5.88×10^{-3}	3.25	1.17×10^{-3}	2.34×10^{-3}
Cortical thickness	0.02	6.05×10^{-3}	2.84	4.56×10^{-3}	6.83×10^{-3}
Cortical volume	0.02	5.16×10^{-3}	3.45	5.66×10^{-4}	1.70×10^{-3}
Subcortical volume	0.03	6.66×10^{-3}	4.00	6.45×10^{-5}	3.87×10^{-4}
Diffusion	3.30×10^{-3}	7.88×10^{-3}	0.42	0.67	0.68
Hormone replacement therapy (HRT) status					
Model	β	SE	t	p	p_{corr}
Full T1w model	0.16	0.05	3.27	1.07×10^{-3}	4.28×10^{-3}
Cortical thickness	0.12	0.05	2.34	0.02	0.03
Cortical volume	0.17	0.04	3.96	7.50×10^{-5}	4.57×10^{-4}
Subcortical volume	0.23	0.06	4.00	6.30×10^{-5}	4.57×10^{-4}
Diffusion	0.17	0.07	2.53	0.01	0.03
Age at HRT onset					
Model	β	SE	t	p	p_{corr}
Full T1w model	0.02	8.07×10^{-3}	2.79	5.3×10^{-3}	0.02
Cortical thickness	0.02	8.35×10^{-3}	2.06	0.04	0.06
Cortical volume	0.02	7.04×10^{-3}	2.53	0.01	0.03
Subcortical volume	0.02	9.08×10^{-3}	2.49	0.01	0.03
Diffusion	-1.29×10^{-3}	0.01	-0.12	0.91	0.93
Oral contraceptive (OC) status					
Model	β	SE	t	p	p_{corr}
Full T1w model	0.09	0.07	1.43	0.15	0.33
Cortical thickness	0.17	0.07	2.55	0.01	0.09
Cortical volume	0.03	0.06	0.60	0.55	0.72
Subcortical volume	0.04	0.07	0.48	0.63	0.73
Diffusion	0.07	0.09	0.79	0.43	0.71

$-0.03, SE = 0.03, t = -1.30, p = 0.20, p_{corr} = 0.55$, and $\beta = -0.03, SE = 0.03, t = -0.84, p = 0.40, p_{corr} = 0.72$, n = 464, respectively).

2.4.1 APOE e4 genotype and circulating estradiol: Significant cross-over interaction effects of ***APOE e4 status * circulating estradiol levels*** were found on brain age gap based on the full T1w model in menopausal women, as shown in Figure 2 ($\beta = 2.72 \times 10^{-3}, SE = 1.22 \times 10^{-3}, t = 2.24, p = 0.03, p_{corr} = 0.03$, n = 539, covariates: current HRT use, ever used HRT, length since menopause and number of births). When including age at first birth and education as covariates, the result showed a stronger association ($\beta = 7.78 \times 10^{-3}, SE = 1.75 \times 10^{-3}, t = 4.43, p = 1.25 \times 10^{-5}, p_{corr} = 6.39 \times 10^{-5}$, n = 411), with a nega-

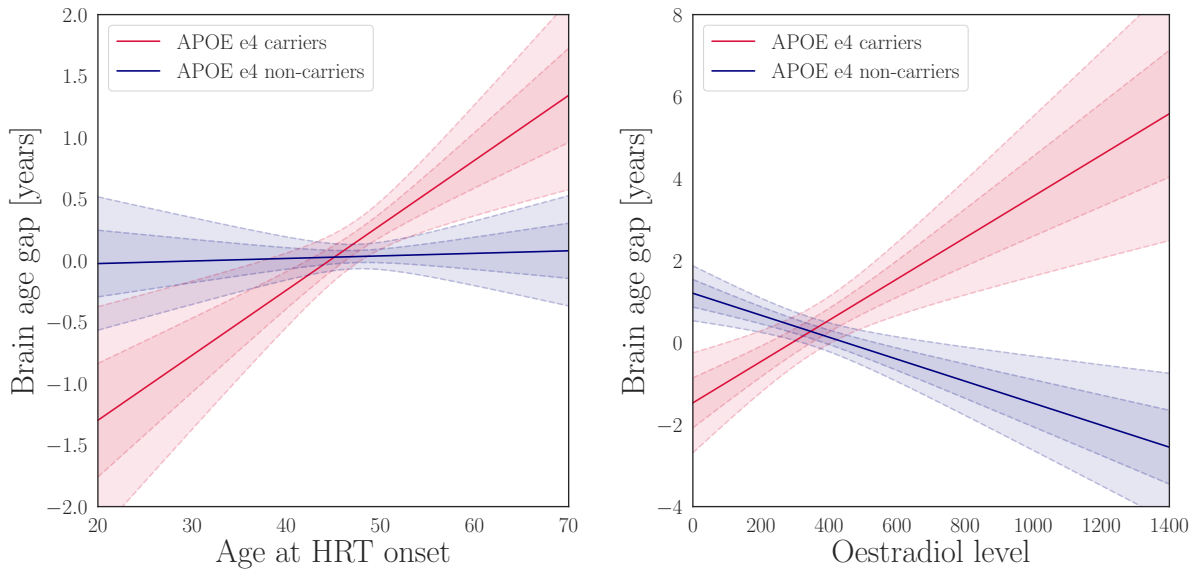

Fig. 2. APOE genotype interactions. Left plot: The lines show the β values (slope) from the regression analyses on thickness-based brain age gap and age started hormone replacement therapy (HRT), for the APOE e4 carriers (red) and non-carriers (blue). The fitted values are corrected for the effect of the covariates in the model (number of births, had hysterectomy, and/or oophorectomy). The shaded areas show the 68.3% (1 SD) and 95% (2 SD) confidence intervals for each fit. Right plot: The lines show the β values (slope) from the regression analyses on full T1w model brain age gap and oestradiol levels, for the APOE e4 carriers (red) and non-carriers (blue). The fitted values are corrected for the effect of the covariates in the model (number of births, current HRT use, ever used HRT, length since menopause, age at first birth, and education).

tive main effect of estradiol levels on brain age gap ($\beta = -2.74 \times 10^{-3}, SE = 8.46 \times 10^{-4}, t = -3.24, p = 2.60 \times 10^{-3}$). Based on this finding the subsequent analyses were also corrected for education and age at birth. Significant interactions were also found for the brain age model based on cortical thickness ($\beta = 6.08 \times 10^{-3}, SE = 1.82 \times 10^{-3}, t = 3.35, p = 8.93 \times 10^{-3}, p_{corr} = 2.60 \times 10^{-3}$), cortical volume ($\beta = 6.59 \times 10^{-3}, SE = 1.49 \times 10^{-3}, t = 4.42, p = 1.28 \times 10^{-5}, p_{corr} = 6.39 \times 10^{-5}$), and subcortical volume ($\beta = 5.47 \times 10^{-3}, SE = 1.93 \times 10^{-3}, t = 2.84, p = 4.82 \times 10^{-3}, p_{corr} = 6.88 \times 10^{-3}$). No effects were found on brain age gap based on diffusion ($\beta = 1.12 \times 10^{-3}, SE = 2.58 \times 10^{-3}, t = 0.47, p = 0.64, p_{corr} = 0.64$, n = 226). Follow-up analyses showed main effects of estradiol levels on brain age gap based on the full T1w model for both the APOE e4 carriers (n = 101; $\beta = 5.13 \times 10^{-3}, SE = 1.52 \times 10^{-3}, t = 3.37, p = 1.10 \times 10^{-3}, p_{corr} = 2.60 \times 10^{-3}$) and the non-carriers (n = 310; $\beta = -2.73 \times 10^{-3}, SE = 8.60 \times 10^{-4}, t = -3.17, p = 1.67 \times 10^{-3}, p_{corr} = 2.78 \times 10^{-3}$). The effect in carriers was not dose-dependent (full T1w model: $\beta = 3.10 \times 10^{-3}, SE = 4.89 \times 10^{-3}, t = 0.63, p = 0.53, p_{corr} = 0.59$, n with 1 e4 allele = 90, n with 2 e4 alleles = 11).

3 Discussion

The results show that higher cumulative estrogen exposure, higher endogenous exposure, and higher exogenous exposure were each linked to *higher* brain age relative to chronological age. In APOE e4 carriers, starting hormone replacement therapy earlier, particularly before onset of menopause, was associated with less evident brain aging. No relationship was seen between age at HRT onset and brain aging in non-carriers. Higher estrogen levels during menopause were associated with *higher* brain age gap in carriers and *lower* brain age gap in non-carriers. Two main conclusions can be drawn from the results: I) In light of our recent findings showing a link between parity and *lower* brain age relative to chronological age (49), estrogen exposure and parity may differentially relate to brain aging; II) The genotype-specific associations between age at onset and dosage of HRT and brain aging suggest that genetic factors are important to consider in clinical settings.

In accordance with studies showing negative effects of estrogen exposure on cognition (12) and risk of dementia (20), our results showed associations between estrogen exposure and more evident brain aging, also in HRT users compared to never-users. Effects of HRT have been conceptualized by two theories: the 'healthy cell bias of estrogen action hypothesis' (61) and the 'critical period hypothesis' (62). The first hypothesis states that neuronal viability and health before HRT initiation might be of importance for estrogen to exert its therapeutic effects. This mechanism might be relevant for HRT effects in women undergoing hysterectomy and/or oophorectomy based on medical indications (61). However, we did not find differences in brain age gap between women who underwent natural or surgical menopause (see SI). The 'critical period hypothesis' states that HRT may be neuroprotective if it is initiated near the time of cessation of ovarian function - approximately within 5 years of menopause (63, 64). Our results lend further support to this hypothesis, as we found that an earlier age at HRT initiation, particularly before menopause, was associated with less evident brain aging. However, this relationship was present in APOE e4 carriers only, indicating genotype-specific effects of HRT on brain aging. Our findings also showed that higher brain age rela-

tive to chronological age was linked to higher menopausal levels of estradiol in carriers and lower estradiol levels in non-carriers. Increased estradiol levels induced by estrogen therapy have been associated with less cognitive decline and reduced risk of developing AD in non-carriers, but not in carriers (19, 65). Thus, obtaining the APOE genotype may be of particular importance for optimizing timing and dosage of HRT in perimenopausal women.

Although millions of women worldwide use oral contraceptives (OC; (66)), and emerging evidence suggests effects of OC on aspects of brain structure and function in young adults (reviewed by (67)), its impact on brain aging is unknown. In the current study, neither OC use status nor age at OC initiation showed an association with brain age gap in pre-menopausal and menopausal women, but a positive association was found between duration of OC use and brain age gap based on cortical volume. While longitudinal studies are needed to draw conclusions, this finding could indicate that chronic, ovarian hormone suppression may lead to lower cortical volume later in life. This is contrary to previous findings suggesting an increase in gray matter volume after short-term OC use (68). However, evidence suggests that the direction of any impact of OC on brain structure likely depends on the drug formulation (67).

The opposite associations between estrogen exposure and brain aging versus number of childbirths and brain aging indicate that the inclusion of parity in approximations of estrogen exposure may complicate conclusions. Pregnancy involves additional factors that influence brain aging, such as immune regulations (39, 46, 69), and the pregnancy-induced increase in concentration of regulatory T cells may have implications for inflammatory susceptibility later in life (40). The *'pregnancy-compensation hypothesis'* (70) suggests that women's immune system ramps up throughout adulthood to prepare for a sequence of pregnancies, as from an evolutionary perspective and before birth control, the majority of women would have been pregnant for many of their adult years. According to Natri and colleagues, problems may arise when the 'expected' pregnancies fail to occur; without a frequent push-back from pregnancy-related mechanisms, the immune system can become overly elevated and start releasing auto-antibodies that attack healthy cells (70). Positive effects of parity on the brain may thus reflect mechanisms related to immune factors rather than estrogen fluctuations involved in pregnancy (40). The cessation of ovarian hormone function during menopause has been linked to altered inflammatory processes, increase in cytokine levels, and changes in T cell biology (reviewed by (71)). These processes might constitute a menopausal immune senescence that may increase the risk for AD, of which the pathogenesis is known to involve inflammatory processes (40, 72). Given the pregnancy-related proliferation of regulatory T cells, a first line of defence against inflammatory responses, one could speculate that an enduring elevation of T cells (73) might protect against menopause-related inflammation and lead to more favorable aging trajectories in parous women. However, prospective longitudinal studies are needed to investigate such hypotheses in depth.

To the best of our knowledge, the current study is the first comprehensive study of the associations between endogenous and exogenous hormone exposure, APOE genotype, and normal brain aging in a population-based cohort. Multidimensional large-scale studies are key to foster the understanding of who will undergo typical aging and who may be at risk for neurodegenerative diseases. Such knowledge is crucial to improve early interventions, treatment regimes, and subsequent outcomes for individuals at risk. Yet, the limitations of the presented work should be considered: First, the cross-sectional nature of the study does not enable causal inference, and longitudinal studies are needed to fully understand how estrogen exposure influences women's brain health across the lifespan. Secondly, the cohort data lacks details on HRT and OC formulation, administration, and dosage. While HRT commonly consists of either combined hormone treatment (estrogen plus progestin) or unopposed estrogen treatment (estrogen alone), combined OC mostly contains ethinylestradiol and varying levels of progestins. Different compound compositions and modes of administration may affect brain aging differently, and study-specific differences in compounds and usage may contribute to discrepancies in findings observed in the literature. In addition, duration of HRT/OC use was only available in years and not months or weeks, such that women who took it for less than a year were labelled as non-users. This lack of temporal resolution could have affected our results. The lack of information on breastfeeding, which is known to reduce cumulative exposure to endogenous estrogen, may also have influenced the precision of our approximations. Thirdly, genetic aging studies involve a bias towards survivors, and the number of APOE e4 carriers is assumed to decrease with increasing age (74). In the present study, age was negatively associated with number of carriers ($r = -0.03, p = 0.80 \times 10^{-4}, 95\% CI = [-0.05, -0.02]$), possibly influencing the genotype-related results. Further, oestradiol levels were only available for a subset of women, and within this subset, a relatively high proportion of women (80%) had oestradiol values in the lower range. The UK Biobank notes that this reflects the menopausal status of the participants at recruitment (with 25% being premenopausal (https://biobank.ctsu.ox.ac.uk/biomarker_issues.pdf), which is expected given the age range of the cohort. Hence, the presented results may not apply to populations beyond those represented in the UK Biobank (75). Finally, although the brain age model based on diffusion-weighted MRI was the most precise model in terms of age prediction (see Table 1), it only showed significant associations with HRT status and duration of OC, while the full T1w model showed associations with both endogenous and exogenous estrogen measures. While these differences may be genuine, they could also be due to the lower size of the diffusion sample. The inclusion of multiple brain age models can be informative in patient groups where tissue types are differently affected by disease (56, 57, 76–78), leading to varying brain age predic-

tions across models. However, such models could be more closely related in healthy samples (55) such as the current cohort, which aligns with the relatively consistent results observed across models (see Figure 1).

In conclusion, our findings indicate that I) estrogen exposure may relate to women's brain aging through different pathways than parity, and II) particularly exogenous estrogen exposure interacts with genotype, producing different associations between estrogen and brain aging in APOE e4 carriers and non-carriers. Future studies should emphasize longitudinal designs and genotype interactions to obtain a complete understanding of how estrogen exposure influences women's brain aging.

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4 Materials and Methods

4.1 Sample: The sample was drawn from the UK Biobank (www.ukbiobank.ac.uk), and included 16,854 pre-menopausal and menopausal women. For 9798 of them, diffusion-weighted imaging measures were available. Sample demographics are provided in Table 3.

4.2 Hormone exposure: Women who had missing data, or had responded 'do not know' or 'prefer not to answer' for any of the relevant variables, were excluded for each analysis. For relevant analyses, women with a reported age at menarche (n = 1) or age at OC start (n = 1) at 5 years were excluded. ICEE was approximated by including age at menarche and menopause, duration of hormone replacement therapy in years (HRT), body mass index (BMI), and time since menopause in years. The variables were first standardized and then either added (duration HRT, age at menopause, BMI) to or subtracted (age at menarche, time since menopause) from the index depending on their impact on endogenous estrogen (7). After removing women with missing data, 8,878 women were included in the ICEE analyses involving the main brain-age model, while 5,080 women were included in the analysis involving the diffusion model. Reproductive span was calculated as age at menopause — age at menarche. After removing missing data, 9,188 women were included in the analyses involving the full T1w model, while 52,89 women were included in the analysis involving the diffusion model. For HRT, 11,139 never-users and 5,546 users were included in the analyses involving the full T1w model, while 64,61 never-users and 3,240 users were included in the analysis involving the diffusion model. For OC, 2,213 never-users and 14,615 users were included in the analyses involving the full T1w model, while 1,279 never-users and 8,501 users were included in the analysis involving the diffusion model. Women who had never used HRT/OC were coded 0, while current and former users were coded 1. Multiple regression analyses were run to investigate the association between estimates of hormone exposure and brain age gap models. All analyses were corrected for number of childbirths. In addition, model-specific covariates were included to correct for potential confounds: (1) the reproductive span models included ever used HRT and ever used OC, and (2) the HRT models included had hysterectomy and/or oophorectomy. To test whether other known confounders such as age at first birth and education (49) could influence the results, additional models including these variables were run. The statistical analyses were conducted using R, version 3.5.2, and Python 3.

4.3 Genotyping: For genotyping, we used the UK Biobank version 3 imputed data, which has undergone extensive quality control procedures as described by the UK Biobank genetics team (79). The APOE e genotype was approximated based on the two APOE e single-nucleotide polymorphisms - rs7412 and rs429358 (80). Further information on the genotyping process is available in the UK Biobank documentation (www.ukbiobank.ac.uk/scientists-3/genetic-data), including detailed technical documentation (genotyping_workflow.pdf). APOE e4 status was labelled *carrier* for e3/e4 and e4/e4 combinations, and *non-carrier* for e2/e2, e2/e3 and e3/e3 combinations (81). The homozygous e2/e4 allele combination was removed due to its ambiguity with e1/e3 (82). To examine whether APOE e4 status influenced the observed associations between estrogen exposure and brain aging, we performed additional linear models including interaction terms for APOE e4 status and each estimates of hormone exposure that showed significant results. Covariates were the same as those included in the main analyses (provided in section 2.1 - 2.3). All the continuous variables (ICEE, reproductive span, age started HRT and OC, duration of HRT and OC use, and estradiol levels) were mean centered for the interaction analyses.

4.4 Hormone assay: Serum blood samples were taken at day of MRI scan. Estradiol was analyzed at the UK Biobank's purpose-build laboratory in Stockport, and measured by two step competitive analysis on a Unicel DXI 800 Access Immunoassay System (Beckman Coulter, UK, Ltd; analytical range: 73 - 17621 pmol/l). Further information on the immunoassay and quality control steps is available in the UK Biobank documentation (serum_biochemistry.pdf). To investigate whether APOE e4 status interacted with circulating estradiol levels in menopausal women, multiple linear regressions were run including an *APOE e4 status * circulating estradiol level* interaction term. The models were corrected for current HRT use, ever used HRT, length since menopause, and number of births.

4.5 MRI processing: An accurate overview of the data acquisition, protocol parameters, and image validation can be found in (83) and (84). Raw T1-weighted MRI data for all participants were processed using a harmonized analysis pipeline, including automated surface-based morphometry and subcortical segmentation as implemented in

Table 3. Demographics for the T1-weighted sample and the diffusion weighted sample. M ± SD = mean ± standard deviation. Ethnic background: W = white, B = black, M = mixed, A = Asian, C = Chinese, O = other. Educational qualification: U = university/college degree, A = A levels or equivalent, O = O levels/General Certificate of Secondary Education (GCSE) or equivalent, C = Certificate of Secondary Education (CSE) or equivalent, N = National Vocational Qualification (NVQ) or equivalent, P = professional qualification, e.g. nursing/teaching, Noa = none of the above. For the categories, see http://biobank.ctsu.ox.ac.uk/crystal/coding.cgi?id=100305 and http://biobank.ctsu.ox.ac.uk/crystal/coding.cgi?id=1001

DEMOGRAPHICS				
Sample	**N**	**Age (M ± SD)**	**Ethnic background %**	**Educational qualification %**
T1w	16854	54.70 (7.29)	W 97.39 | B 0.61 | M 0.52 | A 0.67 | C 0.33 | O 0.45	U 43.27 | A 13.97 | O 21.28 | C 4.14 | N 3.32 | P 5.75 | Noa 6.42
Diffusion	9829	54.68 (7.28)	W 97.55 | B 0.87 | M 0.54 | A 0.63 | C 0.35 | O 0.40	U 41.52 | A 13.77 | O 22.57 | C 4.30 | N 3.26 | P 5.90 | Noa 6.76

Table 4. Responses on the question *'Have you had your menopause?'* for the T1w sample and the diffusion sample. http://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=2724

MENOPAUSAL STATUS						
Sample	**N**	***'Yes'***	***'No'***	***'Not sure - had a hysterectomy'***	***'Not sure - other reason'***	***'Prefer not to answer'***
T1w	16854	50.97%	30.29%	10.50%	10.50%	0.08%
Diffusion	9829	53.44%	30.69%	10.73%	5.06%	0.07%

FreeSurfer 5.3 (58). In line with recent large-scale implementations (51, 53), we utilized a fine-grained cortical parcellation scheme (59) to extract cortical thickness, area, and volume for 180 regions of interest per hemisphere, in addition to the classic set of subcortical and cortical summary statistics from FreeSurfer (58). This yielded a total set of 1118 structural brain imaging features (360/360/360/38 for cortical thickness/area/volume, as well as cerebellar/subcortical and cortical summary statistics, respectively). The MRI variables were residualized with respect to scanning site, ethnic background, intracranial volume, and Freesurfer-derived Euler numbers (85) using linear models. To remove outliers, participants with Euler numbers of SD ± 4 were identified and excluded (n = 159). In addition, participants with SD ± 4 on the global MRI measures mean cortical or subcortical gray matter volume were excluded (n = 79 and n = 13, respectively), yielding a total of 16,854 participants with T1-weighted MRI data.

For the diffusion-weighted MRI data, a conventional Stejskal-Tanner monopolar spin-echo echo-planar imaging sequence was used with multiband factor 3. Diffusion weightings were 1000 and 2000 s/mm^2 and 50 non-coplanar diffusion directions per each diffusion shell. The spatial resolution was 2 mm^3 isotropic, and 5 AP vs 3 PA images with b = 0 s/mm^2 were acquired. All diffusion data were post-processed using an optimised diffusion pipeline (86) consisting of 6 steps: noise correction (87, 88), Gibbs-ringing correction (89), estimation of echo-planar imaging distortions, motion, eddy-current and susceptibility distortion corrections (90, 91), spatial smoothing using fslmaths from FSL package (92) with the Gaussian kernel 1mm^3, and diffusion metrics estimation. Diffusion maps of diffusion tensor imaging (DTI) and diffusion kurtosis imaging (DKI) derived metrics were estimated using Matlab R2017a (MathWorks, Natick, Massachusetts, USA) as proposed by Veraart and colleagues (93). The DTI metrics included mean diffusivity (MD), fractional anisotropy (FA), axial diffusivity, and radial diffusivity (94). The DKI metrics included mean kurtosis, axial kurtosis and radial kurtosis (95). White matter tract integrity included the metrics axonal water fraction, extra-axonal axial diffusivity and extra-axonal radial diffusivity (96). See (86) for details on the processing pipeline. Tract-based spatial statistics was used to extract diffusion metrics (97). Initially, all maps were aligned to the FMRI58FA template supplied by FSL, using non-linear transformation in FNIRT (98). Next, a mean FA image of 18600 subjects was obtained and thinned to create a mean FA skeleton. The maximal FA values for each subject were then projected onto the skeleton to minimize confounding effects due to partial voluming and any residual misalignments. Finally, all diffusion metrics were projected onto the subject-specific skeletons. For the brain age prediction, we used mean diffusion values across the skeleton and in regions of interests based on John Hopkins University atlases for white matter tracts (with 0 thresholding). To remove outliers, we excluded participants with Euler numbers of SD ± 4 (n = 89), and/or SD ± 4 on the full skeleton-based measures mean FA or mean MD (n = 58 and n = 9, respectively), yielding a total of 9,829 participants with diffusion-weighted MRI data.

4.6 Principal component analysis (PCA): A PCA run with z-transformed MRI variables $z = (x - \mu)/\sigma$, where x is an MRI variable of mean μ and standard deviation σ). The top 100 components were used in the subsequent analyses, explaining 56.48% of the total variance, as shown in Figure 3. As a cross check, the relationships between ICEE and brain age gap was re-analyzed with 200 components, explaining 70.61% of the total variance. With 200 components included, the association between ICEE and brain age gap was $\beta = 0.03, SE = 0.01, t = 2.42, p = 0.02$. As the results were consistent, 100 components were chosen to reduce computational time.

4.7 Brain age prediction: Brain age prediction estimates an individual's apparent brain aging based on structural brain characteristics derived from MRI. Subtracting chronological age from estimated brain age provides a measure of an individual's brain age gap; the difference between

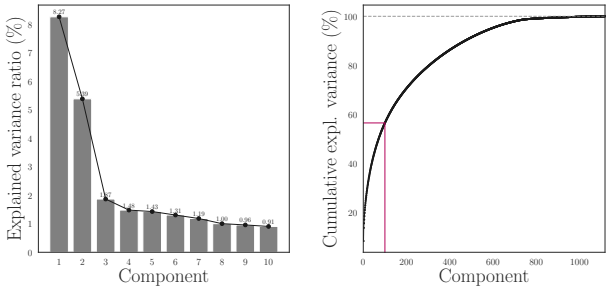

Fig. 3. Left: Cumulative explained variance for the PCA components based on 1118 z-transformed MRI variables used in the brain age analysis. Right: Explained variance ratio shown for the top 10 PCA components used in the brain age analysis.

their estimated brain age and their chronological age. For instance, if a 60 year old individual shows a brain age gap of -5 years, their typical aging pattern resembles the brain structure of a 55 year old individual, i.e. their brain is 'younger looking' than what is expected for their chronological age (50). The XGBRegressor model from XGBoost (https://xgboost.readthedocs.io/en/latest/python/index.html) was used to run the brain age prediction analysis with an algorithm that has been used in recent large-scale brain age studies (51, 52). Parameters were set to max depth = 3, number of estimators = 100, and learning rate = 0.1 (defaults). The predicted age based on the PCA components was estimated in a 10-fold cross validation with 10 repetitions per fold, assigning an estimated brain age to each individual. Brain age gap was calculated using estimated brain age - true age. Average RMSE and R^2 were calculated from the cross validation and compared to null distributions calculated from 10,000 permutations. The results are shown in Figure 4.

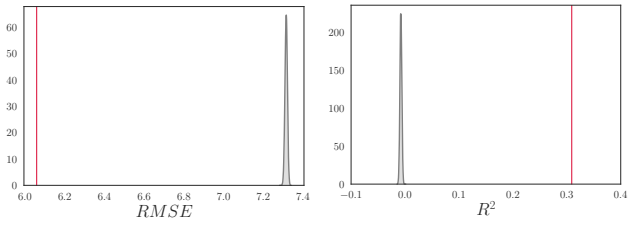

Fig. 4. Left: The mean $\pm$ SD root mean square error (RMSE) for the full T1w brain age model was 6.06 ± 0.09, based on a 10-fold cross validation with 10 repetitions per fold (red vertical line). The null distribution calculated from 10,000 permutations is shown in gray, with a mean $\pm$ SD of 7.32 ± 0.006. The number of permuted results from the null distribution that exceeded the mean from the cross validation was 0 ($p = 1.00 \times 10^{-4}$). Right: The mean $\pm$ SD R^2 for the brain age model was 0.31 ± 0.09, based on a 10-fold cross validation with 10 repetitions per fold (red vertical line). The null distribution calculated from 10,000 permutations is shown in gray, with a mean $\pm$ SD of -0.007 ± 0.002 ($p = 1.00 \times 10^{-4}$).

In order to adjust for a frequently observed bias leading to generally overestimated age predictions at low age and underestimated predictions at high age (52, 99), we employed the following regression:

$$\text{Predicted age} = A + B \times \text{True Age} + C \times \text{True Age}^2 \quad \textbf{(1)}$$

where the coefficients A, B and C parameterize the relationship between the true and predicted age. These coefficients were then used to remove the effect of the bias, as illustrated in Figure 5. To ensure that the bias correction was employed successfully, we tested the association between bias-corrected brain age delta based on the full T1w model and estimates of hormone exposure while controlling for chronological age. The test showed results consistent with the main findings: *ICEE*: $\beta = 0.05, SE = 0.01, t = 3.54, p = 3.98 \times 10^{-4}$; *reproductive span:* $\beta = 0.02, SE = 6.13 \times 10^{-3}, t = 2.84, p = 4.49 \times 10^{-3}$; *HRT status*: $\beta = 0.17, SE = 0.06, t = 3.03, p = 2.49 \times 10^{-3}$, *age at HRT onset*: $\beta = 0.03, SE = 8.71 \times 10^{-3}, t = 3.03, p = 2.46 \times 10^{-3}$; *duration of HRT use*: $\beta = 3.51 \times 10^{-3}, SE = 4.86 \times 10^{-3}, t = 0.72, p = 0.47$; *OC status*: $\beta = 0.11, SE = 0.07, t = 1.64, p = 0.10$; *Age at OC onset*: : $\beta = -4.21 \times 10^{-3}, SE = 6.31 \times 10^{-3}, t = -0.67, p = 0.50$; *duration of OC use*: $\beta = 7.78 \times 10^{-3}, SE = 3.11 \times 10^{-3}, t = 2.50, p = 0.01$

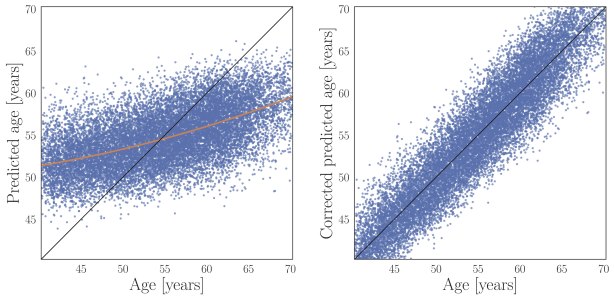

Fig. 5. left: Machine performance is biased towards the mean age, resulting in overestimated predictions at low age and underestimated predictions at high age. Right: After bias correction using Eq. 1, the predictions follow the expected dependence.

For the brain age models based on cortical thickness, cortical volume, and subcortical volume, and diffusion measures, the number of MRI variables, RMSE values, and the correlations between predicted and chronological age are shown in Table 1. The correlations between brain age gap estimated from the different models is shown in Figure 6.

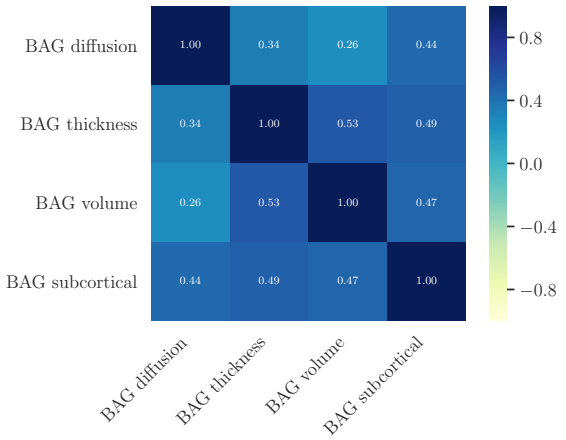

Fig. 6. The correlations between age corrected brain age gap estimated based on the different sub-models.

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