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Negative results

Are sex differences in cognitive impairment reflected in epigenetic age acceleration metrics?

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ABSTRACT

Sex differences are well-established in Alzheimer's disease (AD) frequency and pathogenesis, but are not mechanistically understood. Accelerated epigenetic age has been associated with both cognitive aging and AD pathophysiology, but has not been studied by sex in AD or related cognitive impairment. Using the ADNI cohort, we found that none of sex, cognitive impairment diagnosis, nor load of APOEε4 alleles (strongest genetic AD risk factor) were associated with epigenetic age acceleration (DNAmAge, Intrinsic DNAmAge, PhenoAge, or GrimAge), although females exhibit more accelerated epigenetic aging using the Skin & Blood clock in the transition from normal cognition to cognitive impairment than males.

Introduction

Biological sex and APOE genotype are associated with differences in Alzheimer's disease (AD) features including lifetime risk, AD neuropathology, and cognitive decline; these factors suggest that the molecular mechanisms underlying AD and its pathogenesis may operate in sex- or genotype-dependently (Alzheimer's Association, 2019). Recently, biomarkers have been developed to measure the biological age of a sample from genome-wide DNA methylation (DNAm) signatures, resulting in the creation of a metric called epigenetic age.

A well-known metric of epigenetic age is Horvath's pan-tissue "DNAmAge" clock (Horvath, 2013). The DNAmAge clock estimates biological age of samples in years, and positive deviations of epigenetic age from chronological age (epigenetic age acceleration) are postulated to reflect more rapid biological aging, which has been associated with disease phenotypes including markers of cognitive function in AD (Levine et al., 2015) and biological variables such as sex, with men exhibiting more accelerated epigenetic aging than women (Horvath, 2013). Intrinsic DNAmAge acceleration is a metric independent of age-related changes in relative cell type proportions, used particularly for blood (Chen et al., 2016).

Epigenetic clocks have also been developed that are more accurate for specific tissues of interest, such as Horvath et al.'s Skin and Blood clock (Horvath et al., 2018), or Zhang et al.'s blood clock (Zhang et al., 2019). The Zhang clock was designed for accurate chronological age estimation of samples independent of mortality

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Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

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Table 1
Basic demographic characteristics of 640 ADNI participants with available blood Illumina HumanMethylationEPIC microarray DNAm data

	Females (n = 284)	Males (n = 356)	*p* value*
Age	74.78 (±8.03)	76.31 (±7.32)	<0.0001
Baseline diagnosis			
CN	109 (38.38%)	108 (30.34%)	0.04
CI	175 (61.61%)	248 (69.66%)	
APOEε4 alleles			
0	169 (59.51%)	200 (56.18%)	0.42
1 or 2	115 (40.49%)	156 (43.82%)	

CN indicates cognitively normal, CI indicates broadly-defined cognitive impairment (early mild cognitive impairment [MCI], late MCI, and Alzheimer's disease cases).
*p values are from Wilcoxon rank sum tests for continuous variables and Fisher's exact tests for categorical.

or pathology (Zhang et al., 2019), while the Skin and Blood clock is similar to traditional epigenetic clocks and reflects biological aging of samples, as evidenced by accelerated measurements in cases of Hutchinson Gilford progeria (Horvath et al., 2018).

Recently, second generation epigenetic clocks (PhenoAge & GrimAge) have been constructed by training tissue-specific tools on relevant biomarkers of health in addition to chronological age. PhenoAge was developed first by constructing a score reflecting hazard of mortality based on 42 phenotypic markers in a longitudinal cohort, and then regressing whole blood DNAm levels onto the 9 most predictive markers plus chronogological age in order to predict phenotypic age and mortality risk (Levine et al., 2018). The GrimAge metric is based on estimating a mortality risk from blood DNAm-estimators of smoking pack-years and levels of health-associated plasma proteins (Lu et al., 2019). Time-to-death was regressed onto these estimators and linearly transformed to yield an age estimator that is strongly associated with cognitive aging, cancer, coronary heart disease, and time-to-death (Lu et al., 2019).

We hypothesized that sex differences observed in AD may be associated with sex-specific epigenetic age acceleration metrics (DNAmAge, Horvath's Skin & Blood clock, PhenoAge, and GrimAge) and used data from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database to examine associations between epigenetic age acceleration, cognitive impairment, sex, and biomarkers of AD risk.

Methods

Data were obtained from the ADNI database (adni.loni.usc.edu), a public-private partnership launched in 2003 led by Principal Investigator Michael W. Weiner, MD. ADNI's primary goal has been to test whether serial magnetic resonance imaging, positron emission tomography, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) to early AD. For up-to-date information, see www.adni-info.org.

Illumina Infinium HumanMethylationEPIC BeadChip data were downloaded for 1698 longitudinal blood samples from 640 unique ADNI participants (n = 284 females, n = 356 males). To increase power for analyses, diagnosis was categorized into 2 levels: normal cognition (CN) or cognitively impaired (CI) which was comprised of early mild cognitive impairment (MCI), late MCI and AD (Vasanthakumar et al., 2017). See Table 1 below and Supplementary Table 1 for more detailed demographic information.

All analyses were performed in R v 3.5.1 (R Core Team, 2018). DNAmAge was calculated with code from https://dnamage.genetics.ucla.edu/ (Horvath, 2013); Skin & Blood Age, PhenoAge and GrimAge were computed with the New DNA Methylation Age Calculator tool (https://dnamage.genetics.ucla.edu/). DNAmAge, Skin & Blood Age, PhenoAge, and GrimAge acceleration were calculated as the residuals of each metric regressed on chronological age, years of education, laboratory blood collection site, and microarray chip and row; intrinsic epigenetic age acceleration was calculated by regressing DNAmAge on the same covariates plus Houseman DNAm-estimated blood cell type proportions (Houseman et al., 2012). APOE genotype and cerebrospinal fluid (CSF) levels of tau were available in a subset of cases (n = 533, 46% female, see Supplementary Table 1). In biological analyses APOE genotype was collapsed into: (1) carriers of 1 or 2 ε4 alleles and (2) non-carriers of ε4 risk alleles.

Results

We first investigated whether sex and/or CI diagnosis were associated with epigenetic age acceleration metrics (DNAmAge, Intrinsic DNAmAge, Skin & Blood age, PhenoAge, or GrimAge) in ADNI blood samples using linear mixed effect models with fixed effects of patient sex, diagnosis, age, the interaction of sex and diagnosis, and by-individual random intercepts using the *lme4* package in R (Bates et al., 2015). p values were obtained by likelihood ratio tests comparing full models to models lacking the effect of interest, implemented for all fixed effects with the *afex* R package (Singmann et al., 2016), multiple comparisons were adjusted for using the Bonferroni method.

None of DNAmAge, Intrinsic DNAmAge, or PhenoAge acceleration metrics were significantly associated with sex, CI diagnosis, or their interaction; for full details and test statistics see Supplementary Table 2. A likelihood ratio test indicated that the GrimAge acceleration model including sex provided a better fit than the model without sex (χ^2 = 11.06, $p_{Bonferroni} < 0.05$). On average males exhibited +0.42 years faster GrimAge acceleration than females (B = 0.42, standard error = 0.31, t = 0.67). Similarly, the Skin & Blood Age acceleration model including an interaction term for sex and diagnosis provided a better fit than a model without the interaction term (χ^2 = 12.00, $p_{Bonferroni} < 0.05$, B = -1.64, standard error = 0.47, t = -3.47), whereby the slope of the transition from CI to CN is steeper in females than in males. Post-hoc within-sex analyses indicated that CI females have significantly higher Skin & Blood age acceleration than CN females, and that diagnosis is not significantly associated with Skin & Blood age acceleration in males, see Supplementary Table 3 and Supplementary Figure 2.

Based on the hypothesis that age acceleration may be more associated with levels of relevant biomarkers of AD, we ran a linear mixed effects model including sex, age, APOE genotype, CSF tau concentration and sex-APOE and sex-tau interaction terms (all fixed effects) on DNAmAge acceleration with by-individual random intercepts. Neither APOE allele load nor CSF tau were significantly associated with DNAmAge acceleration, nor did these associations differ by sex.

Discussion

We found no significant differences in DNAmAge, intrinsic DNAmAge, or PhenoAge acceleration by diagnosis or sex in ADNI participants, nor did we find a significant association between DNAmAge and CSF tau or APOE genotype. Males had significantly higher GrimAge acceleration than females, though the effect size was small (<1 year); which corroborates a previous report (Crimmins et al., 2021). A significant interaction between sex and diagnosis in Skin & Blood age acceleration indicated that females had accelerated Skin & Blood age in CI as compared to CN, while there was no effect of diagnosis on male Skin & Blood age

acceleration. For a more detailed discussion of these results, see the Supplementary Information.

To our knowledge, no other study has similarly tested whether epigenetic age acceleration metrics in the context of MCI or AD are sex-specific, and only 1 has probed MCI-associated epigenetic age acceleration in a peripheral tissue (blood), also with negative findings (Chouliaras et al., 2018). However, our negative results could arise from sample size limiting statistical power. Power simulations (n = 1000 simulations) indicated that this longitudinal cohort is 46.5% powered (95% confidence interval 43.4–49.7) to detect age acceleration differences of ±3.6 years between diagnosis groups, the median error of Horvath's DNAmAge clock. To detect effect sizes of ±1 year between diagnosis groups, our current study design is 23.4% powered (95% confidence interval 20.8–26.2); the effect of diagnosis on Skin & Blood age acceleration in our female-only post-hoc analysis was 1.13 years. A further discussion of statistical power is presented in the Supplementary Information. Nonetheless, our 640-participant longitudinal cohort advances the literature significantly, as it is much larger than the 48-participant cohort used in the only previous study (Chouliaras et al., 2018) of epigenetic age acceleration in peripheral tissues in the context of MCI, which did not consider sex. As larger cohorts with detailed clinical characterization become available, our results will benefit from future validation.

Finally, our finding of accelerated Skin & Blood age in females even when controlling for age may reflect the faster progression females exhibit in AD, with higher levels of CSF tau and more marked cognitive decline than males (Sohn et al., 2018). Future studies should determine whether similar sex differences in age acceleration are seen in each stepwise transition from CN to MCI to AD, and whether these sex differences extend to other tissues, ideally using the more accurate tissue-specific epigenetic clocks.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2021.09.022.

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