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RESEARCH ARTICLE

Associations between accelerated parental biologic age, autism spectrum disorder, social traits, and developmental and cognitive outcomes in their children

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Abstract

Parental age is a known risk factor for autism spectrum disorder (ASD), however, studies to identify the biologic changes underpinning this association are limited. In recent years, "epigenetic clock" algorithms have been developed to estimate biologic age and to evaluate how the epigenetic aging impacts health and disease. In this study, we examined the relationship between parental epigenetic aging and their child's prospective risk of ASD and autism related quantitative traits in the Early Autism Risk Longitudinal Investigation study. Estimates of epigenetic age were computed using three robust clock algorithms and DNA methylation measures from the Infinium HumanMethylation450k platform for maternal blood and paternal blood specimens collected during pregnancy. Epigenetic age acceleration was defined as the residual of regressing chronological age on epigenetic age while accounting for cell type proportions. Multinomial logistic regression and linear regression models were completed adjusting for potential confounders for both maternal epigenetic age acceleration ($n = 163$) and paternal epigenetic age acceleration ($n = 80$). We found accelerated epigenetic aging in mothers estimated by Hannum's clock was significantly associated with lower cognitive ability and function in offspring at 12 months, as measured by Mullen Scales of Early Learning scores ($\beta = -1.66$, 95% CI: −3.28, −0.04 for a one-unit increase). We also observed a marginal association between accelerated maternal epigenetic aging by Horvath's clock and increased odds of ASD in offspring at 36 months of age (aOR = 1.12, 95% CI: 0.99, 1.26). By contrast, fathers accelerated aging was marginally associated with decreased ASD risk in their offspring (aOR = 0.83, 95%

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CI: 0.68, 1.01). Our findings suggest epigenetic aging could play a role in parental age risks on child brain development.

Lay Summary

Parental age is a risk factor for ASD; however, little is known about possible biologic aging changes that contribute to this association. We found that mothers with faster epigenetic aging and fathers with slower epigenetic aging, relative to their chronologic age, were at increased odds of having a child with ASD and/or decreased early learning, at 3 years of age. These findings suggest epigenetic aging in parents may play a role in neurodevelopment and ASD.

KEYWORDS

age acceleration, autism-related traits, autism spectrum disorder, biologic age, DNA methylation, epigenetic age, parental age

INTRODUCTION

In the last two decades, molecular biomarkers that can capture disease onset, age-associated physiological decline, and death, have been developed to better understand biologic aging processes (Johnson, 2006). Compared with other types of biomarkers of biological age, such as telomere length, transcriptomic-based, and proteomic-based estimators (Jylhava et al., 2017), DNA methylation-based age estimators (Hannum et al., 2013; Horvath, 2013; Levine et al., 2018), referred to as "epigenetic clocks," have been shown to predict chronological age with very high accuracy including across tissues and ethnicities (Bormann et al., 2016; Zhao et al., 2016). More recently, PhenoAge and GrimAge clocks are designed to incorporate clinical and lifestyle factors, such as plasma proteins and cigarette smoking to predict physiologic dysregulation (Levine et al., 2018; Lu et al., 2019). At the individual level, predicted epigenetic age, that is, "biologic age," can be compared with actual chronologic age, whereas the difference between the chronologic age and predicted epigenetic age captures the age discordance of the individual. One type of discordances is epigenetic age acceleration, that is, being biologically older than ones chronologic age, which has been associated with a wide range of adverse health outcomes including neurological disorders and age-related outcomes such as all-cause mortality (Chen et al., 2016; Lu et al., 2019; Perna et al., 2016), cancer (Ambatipudi et al., 2017; Dugue et al., 2018; Durso et al., 2017; Levine et al., 2015; Perna et al., 2016), cardiovascular and metabolic diseases (Horvath et al., 2014; Horvath, Gurven, et al., 2016; Nevalainen et al., 2017; Perna et al., 2016; Roetker et al., 2018), and psychiatric and neurodegenerative disorders (Fries et al., 2017; Horvath, Langfelder, et al., 2016; Horvath & Ritz, 2015; Levine et al., 2015; Rosen et al., 2018). However, to our knowledge, no studies have investigated parental epigenetic age acceleration or deceleration with neurodevelopmental outcomes in their children despite advanced parental chronologic age being a known risk factor for several neurodevelopmental outcomes including autism spectrum disorder (ASD).

Parental chronologic age is an established risk factor for ASD (Lyall et al., 2017). Meta-analyses have shown increased risk of ASD independently associated with advanced maternal age and paternal age (Wu et al., 2017). In addition, combined effects of maternal age and paternal age on the risk for ASD in offspring have been reported (Idring et al., 2014; Sandin et al., 2016). Findings from a recent study of children at high familial risk for ASD observed younger chronologic age in parents was associated with increased risk of ASD and poorer cognitive ability (Lyall et al., 2020). Little is known about the biologic mechanisms underlying these associations but a number of mechanisms have been proposed including increased de novo mutation burden in older individuals (Girard et al., 2016; Leppa et al., 2016; Sebat et al., 2007; Virkud et al., 2009) and altered leukocyte telomere length (Lewis et al., 2020; Li et al., 2014; Nelson et al., 2015). Epigenetic changes have also been linked to chronologic age (Alisch et al., 2012; Bell et al., 2012; Bormann et al., 2016; Heyn et al., 2012; Martino et al., 2011) and to ASD (Andrews et al., 2018; Bakulski et al., 2021; Hannon et al., 2018; Ladd-Acosta et al., 2014), independently, but no studies, to our knowledge, have examined epigenetic aging as a potential aging-related mechanisms that may be relevant to ASD risk. One possible mechanism for the associations observed between older parental age and ASD is hypothesized to be DNA methylation changes associated with older parental age, but other aging factors could underly the parental age and ASD association, such as epigenetic aging as a marker of biological aging, in particular the deviation of epigenetic aging from chronological aging, may be an alternative marker for the aging processes that could be associated with ASD.

Previous studies have shown that parental age-ASD association may differ in families at low, general, or high familial risk. However, the mechanisms are not well understood. One hypothesis is that de novo mutation may not fully explain the parental age-ASD association and it is possible that de novo mutation plays a less role in the high-risk families (Gratten et al., 2016, Lyall et al., 2020). Given the differences in high familial and general

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population observed in other studies, it is important to better understand the biological risk factors associated with ASD in the high-risk population. To address these gaps in knowledge and to provide insights into whether the epigenetic aging process plays a role in ASD risks in the high-risk population, we tested the association between epigenetic age acceleration/deceleration in mothers and fathers during pregnancy, and ASD-related outcomes including clinical ASD diagnosis, related social communication traits, and cognitive and adaptive function in their children in early childhood.

METHODS

Study population

Participants for this analysis were enrolled through Early Autism Risk Longitudinal Investigation (EARLI), which has been previously described (Newschaffer et al., 2012). EARLI is a prospective study of ASD that utilizes a familial history design, that is, it enrolled pregnant women who had a previous child with ASD. The new baby sibling was at increased likelihood for ASD given the increased sibling recurrence of ASD (Newschaffer et al., 2012). Briefly, EARLI was implemented at four major metropolitan locations across the U.S. (Philadelphia, Baltimore, San Francisco Bay Area, and Sacramento), representing three distinct U.S. regions (Southeast Pennsylvania, Northeast Maryland, and Northern California). Recruitment methods varied by location to capitalize on unique resources at each study site. Enrolled mothers were seen at regular intervals during pregnancy (approximately once a trimester) and at birth to complete interviews that cover a wide range of exposure, medical, and demographic domains, as well as to collect biologic and environmental samples, including cord blood and placenta at birth. Infants were prospectively followed until 36 months of age. EARLI is racially, ethnically, and socioeconomically diverse. All infants included in this analyses were unrelated and were full siblings. The institutional review boards at organizations in the four study sites (Drexel University, Johns Hopkins University, University of California, Davis, and Kaiser Permanente Research) approved the EARLI study.

DNA methylation measurements and quality control

Maternal and paternal whole blood biosamples were collected using vacutainer EDTA tubes at study enrollment using a standardized protocol across all sites. The biosamples were shipped on the same day to Johns Hopkins Biological Repository for storage at −80°C. Genomic DNA was extracted using a Qiagen DNA Midi Kit (Qiagen Inc., Valencia, CA) and quantified using a NanoDrop spectrophotometer (ThermoFisher Scientific). DNA methylation was measured using the Illumina Infinium HumanMethylation450 BeadChip (Illumina, San Diego, CA). For each sample, 1 μg of genomic DNA was bisulfite treated using the EZ-96 DNA Methylation kit (Zymo Irvine, CA), as per the manufacturer's instructions. Biosamples were sent to the Johns Hopkins Genetic Resource Core Facility for processing.

Several sample- and probe-level quality control (QC) measures were applied (Aung et al., 2021). Samples were excluded if they were duplicates, had low overall array intensity, or a discrepancy between reported sex and empirically predicted sex. Crossreactive probes as well as probes that measured DNA methylation at known SNP positions and outside of CpG sites were removed. Probes with detection p-values > 0.01 in 10% of samples were removed from the analyses. A final count of 445,241 probes remained in the analysis. A total of 198 maternal DNA methylation samples and 93 paternal DNA methylation samples passed the QC procedures. DNA methylation data were then normalized using a modified beta-mixture quantile (BMIQ) function (Horvath, 2013; Teschendorff et al., 2013). While the original BMIQ is a within-sample normalization method to address probe type bias by modifying the type II distribution to match that of type I probes, Horvath modified this BMIQ procedure for a different purpose: the distribution of each given array is related to that of a "gold standard" array (defined here as the mean across all of the training datasets). Thus, Horvath's modification of the BMIQ method could be interpreted as a form of between sample normalization (Horvath, 2013). Finally, proportions of cell types, including B cells, $CD4^+$ T cells, $CD8^+$ T cells, eosinophils, monocytes, granulocytes, neutrophils, and natural killer cells, were empirically estimated using the estimateCellCounts function of the minfi R package and the Houseman reference panel (Aryee et al., 2014; Houseman et al., 2012).

Epigenetic age estimation

Parental epigenetic age was calculated using three existing epigenetic clock algorithms—Horvath's clock (Horvath, 2013), Hannum's clock (Hannum et al., 2013), and PhenoAge (Levine et al., 2018). Horvath's clock is based on 353 CpGs and has been robustly correlated with chronological age in multiple tissues, whereas the Hannum's clock is calculated from 71 CpGs and constructed on whole blood DNA methylation data. PhenoAge age estimators are developed based on 513 CpGs while taking into account 10 clinical characteristics (e.g., glucose, C-reactive protein levels, white blood cell counts). For each parental sample, a measure of epigenetic, that is, "biologic," age acceleration was computed as the residuals from regressing the respective epigenetic age on chronological age adjusted for cell type proportions.

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Positive epigenetic age indicates older biologic age compared with chronological age, or acceleration, and negative values indicate younger predicted biologic age relative to chronological age, or epigenetic age deceleration.

Outcome assessments

At 36 months, diagnostic category was determined as either meeting criteria for ASD, not meeting ASD criteria but having indications of nontypical neurodevelopment (Non-TD), or neuro-typical development (TD). ASD diagnosis was based on both a best estimate clinical diagnoses and evaluation of ASD features using the Autism Diagnostic Observational Schedule (ADOS) (Lord et al., 1989). To meet ASD criteria a child had to be at or above the ASD cutoff of the ADOS and also meet DSM-IV-TR criteria for Autistic Disorder or PDD-NOS based on expert clinical opinion. The Non-TD group was defined using the criteria defined by the Baby Siblings Research Consortium as used in prior EARLI publications: failing to meet criteria for ASD classification while also having two or more Mullen Scales of Early Learning (MSEL) subtest scores ≥1.5 SD below the mean (Ozonoff et al., 2014). TD were then children not meeting criteria for either ASD or non-TD.

To assess quantitative ASD-related phenotype, at 36 months we collected the 65-item, caregiver-report Social Responsiveness Scale (SRS), with higher raw score values indicating greater expression of the ASD-related phenotype. To assess other quantitative aspects of neurodevelopmental phenotype we used MSEL (Mullen, 1995), also used as described above in out categorical diagnostic algorithm, and Vineland Adaptive Behavior Rating Scales 2nd Edition (VABS-II) (Cicchetti, 1989). Both these care-giver report instruments were administered at 12 and 36 months. The MSEL assesses overall cognitive, language, and motor ability. The overall standardized MSEL (the early learning composite [ELC]) score shows high internal consistency, and convergent validity with other measures of IQ among children with and without ASD. The VABS-II score is a standardized measure of adaptive functioning from children aged 0 to 18 years and assesses the following functioning domains: communication, daily living skills, socialization, motor, and adaptive behaviors. Figure S1 presents total number of participants with each outcome available.

Covariate information

Maternal, paternal, and child characteristics, including maternal age, paternal age, race/ethnicity, maternal education, paternal education, annual family income, maternal prepregnancy body mass index (BMI), and child's sex were obtained through maternal-report questionnaires. Labor and delivery information, including birth weight and parity, were extracted from medical records by abstractors or physicians at each site. Genetic data were measured using the Illumina Omni5+exome array (Illumina, San Diego, CA) at the Johns Hopkins University Center for Inherited Disease Research. Ancestry principal components (PCs) were determined by merging the overlapping genotypes with 1000 genomes project (1000GP, version 5) data. Top five ancestry PCs were used for genetic ancestry adjustment in the analysis.

Statistical analyses

Descriptive analyses were conducted to examine maternal, paternal, and child characteristics by ASD status, distribution of epigenetic age acceleration/deceleration, and distribution of the quantitative traits. Pearson correlation coefficients between chronological age, estimated epigenetic age, and epigenetic age acceleration/deceleration across Horvath's clock, Hannum's clock, and PhenoAge were calculated. Adjusted multinomial logistic regression models were completed to evaluate the association between maternal and paternal epigenetic age acceleration and ASD diagnosis at 36 months (ASD, non-TD, and TD group as the reference). Multivariable linear regression models were used to explore the relationship between parental epigenetic age and quantitative traits adjusting for potential confounders. Potential confounders were selected based on prior knowledge and retained in the adjusted model if a 15% change in coefficient estimate was observed. The final primary models were adjusted for study site, child's sex, maternal or paternal education (high school or less, college, and graduate school or higher), and ancestry PCs. Additional models adjusted for maternal age, paternal age, parity, and maternal prepregnancy BMI were also evaluated in separate models. Results are presented as odds ratio (OR) or beta estimates with 95% confidence intervals (CI) per a one-unit increase (1-year) in maternal or paternal epigenetic age acceleration. In sensitivity analysis, we further assessed potential incremental increases in risk by examining strata of parental age as a categorical variable (<30, 30 to <35, and ≥35 for maternal age, and <30, 30 to <40, and ≥40 for paternal age) and epigenetic age as a binary variable (accelerated vs decelerated epigenetic age). We also further examined the associations with maternal or paternal epigenetic age acceleration in subgroups with both maternal and paternal samples available ($n = 80$ for maternal associations and $n = 78$ for paternal associations). A two-sided p-value less than 0.05 was considered statistically significant. All statistical analyses were performed using R 3.6 software. Data are available through the National Institute of Mental Health Data Archive under the collections for the EARLI network (1600).

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RESULTS

Biosamples from a total of 161 mothers and 80 fathers had high quality blood DNA methylation measures, chronologic age, and autism diagnosis and/or autism-relevant quantitative trait outcome data for their child (Figure S1). As shown in Table 1, mothers were predominantly White (66.4%), non-Hispanic (82.8%), and college educated or higher (88.6%). Paternal characteristics were similar across the maternal/paternal study population. The mean (SD) maternal age and paternal chronologic ages were 34 (5) years and 36 (6) years, respectively. Characteristics of the study group by ASD diagnosis are provided in Table S1. The distribution of child SRS raw scores in both maternal and paternal analytic samples showed a long tail suggests presence of more individuals with higher levels of ASD-related traits (Figure S2). MSEL-ELC scores ascertained in children at both 12-months and 36-months of age showed a slight shift to the left, that is, an increase in the proportion of lower cognitive abilities, in both parental samples. VABS scores (for which higher scores indicate stronger adaptive functioning) showed a relatively normal distribution.

Distributions of parental chronologic ages and estimated epigenetic ages are provided in Table S2. DNA methylation estimates of epigenetic age, using the Horvath and Hannum clock algorithms as well as PhenoAge, all showed significant high overall correlations with chronologic age for both the maternal and paternal samples (Figure 1, $p < 0.001$ and correlations ranging from 0.70 to 0.84). For example, the correlation between paternal chronologic age and Horvath epigenetic age is 0.84 ($p < 0.001$). PhenoAge consistently showed lower correlations with chronologic age than the Horvath- and Hannum-based epigenetic age estimates (Figure 1). After residualization, epigenetic age acceleration was not associated with chronological age (all $p > 0.05$).

For all three epigenetic age estimates, we computed epigenetic age acceleration in each parent sample and tested for associations with their child's ASD diagnosis at 36 months of age. As shown in Table 2, we observed 1.12 greater odds (95% CI: 0.99, 1.26; $p = 0.06$) of having a child with ASD for each 1-year increase in epigenetic age relative to chronologic age among mothers (accelerated epigenetic age), using the Horvath epigenetic clock estimates, compared with the TD group. The PhenoAge showed a similar direction and magnitude of risk effect as the Horvath clock but did not reach statistical significance. Marginal significant associations were observed between paternal accelerated epigenetic aging and decreased ASD risk in their child (aOR = 0.83, 95% CI: 0.68, 1.01; $p = 0.06$). Effect estimates for all three clocks suggest decreased ASD risk among fathers with accelerated biologic aging (Table 2). Accelerated aging in mothers and fathers was not associated with increased risk of non-TD in their child. These results were consistent even after further adjustment for maternal or paternal chronologic age (Table S3).

We also examined parental accelerated aging associations with autism-related quantitative traits as measured by the SRS (Table 3), MSEL (Table 4), and VABS (Table 5) scores. As shown in Table 3, maternal and paternal accelerated age was not significantly associated with SRS raw scores; however, the effect sizes and directions were generally consistent across clocks and crude and adjusted statistical models for Horvath's clock and Hannum's clock. Results were similar when examining SRS T-scores rather than total raw scores (data not shown) and after further adjustment for parental chronologic age for Horvath's clock and Hannum's clock (Table S4). We observed decreased child MSEL-ELC scores at 12 months (indicating poorer cognitive functioning) with maternal epigenetic age acceleration for all three clock algorithms, though only the result from Hannum's clock estimates reached statistical significance ($\beta = -1.66$, 95% CI: -3.28, -0.04, $p = 0.04$, Table 4). Similar associations were not observed between maternal accelerated aging and MSEL-ELC scores at 36 months. When we further adjusted for maternal and paternal chronologic age (Table S5), the associations of maternal epigenetic age acceleration and MSEL-ELC scores at 12 months were similar ($\beta = -1.69$, 95% CI: -3.32, -0.06, $p = 0.04$ in the model adjusted for maternal age; $\beta = -1.74$, 95% CI: -3.36, -0.12, $p = 0.04$ in the model adjusted for paternal age). Paternal epigenetic age acceleration was not significantly associated with child MSEL-ELC scores at 12 months or at 36 months. VABS scores showed no significant associations with either maternal or paternal epigenetic age acceleration (Table 5) including after further adjustment for parental chronologic ages (Table S6).

DISCUSSION

We examined whether epigenetic aging plays a role in parental age associations with ASD and autism-related quantitative traits. Maternal accelerated epigenetic age, that is, biological age older than one's chronologic age, as measured by Hannum's clock, was associated with decreased cognitive abilities as measured by the MSEL-ELC scores. Suggestive associations were also observed between accelerated maternal epigenetic age and increased risk for ASD and between accelerated paternal epigenetic age and decreased risk for ASD. Our results show that epigenetic aging in parents could play a role in child neurodevelopment and cognitive outcomes.

Maternal accelerated age associations with cognitive functioning were significant for estimates using the Hannum's clock but did not reach the same level of statistical significance or effect size in statistical models that used epigenetic aging measures from the Horvath's or PhenoAge clocks. Similarly, we only observed associations

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TABLE 1 Descriptive characteristics of the study population in the Early Autism Risk Longitudinal Investigation (EARLI) study

	Maternal age full study population ($N = 163$)	Paternal age full study population ($N = 80$)
Child characteristics, *N* (%)		
Child sex		
Male	88 (54.0)	44 (55.0)
Female	75 (46.0)	36 (45.0)
ASD diagnosis		
ASD	29/154 (18.8)	14/76 (18.4)
Non-typical	48/154 (31.2)	28/76 (36.8)
Typical	77/154 (50.0)	34/76 (44.7)
Quantitative traits		
SRS raw score, mean (SD)	36.9 (27.9)	33.9 (25.3)
MSEL-ELC score at 12 months, mean (SD)	201.7 (32.5)	207.6 (28.6)
MSEL-ELC score at 36 months, mean (SD)	97.8 (21.5)	98.1 (22.5)
VABS composite score at 36 months, mean (SD)	92.0 (13.2)	92.6 (11.9)
Parent characteristics, *N* (%)		
Maternal age, mean (SD)	34.0 (5.0)	34.2 (4.5)
Paternal age, mean (SD)	35.9 (6.2)	36.4 (6.2)
Maternal race		
White	105 (66.4)	54 (72.0)
Black	17 (10.8)	5 (6.7)
Other	36 (22.8)	16 (21.3)
Maternal ethnicity		
Non-Hispanic	135 (82.8)	68 (90.7)
Hispanic	28 (17.2)	7 (9.3)
Maternal education		
High school or less	19 (11.7)	7 (9.5)
Some college/college	97 (59.5)	39 (52.7)
Graduate school or higher	47 (28.8)	28 (37.8)
Paternal race		
White	104 (66.2)	56 (70.9)
Black	19 (12.1)	4 (5.1)
Other	34 (21.7)	19 (24.0)
Paternal ethnicity		
Non-Hispanic	130 (80.7)	67 (84.8)
Hispanic	31 (19.3)	12 (15.2)
Paternal education		
High school or less	36 (22.4)	7 (9.0)
Some college/college	88 (54.6)	44 (56.4)
Graduate school or higher	37 (23.0)	27 (34.6)
Study site, *N* (%)		
Drexel	41 (25.2)	28 (35.0)
Johns Hopkins	39 (23.9)	20 (25.0)
Kaiser Permanente	52 (31.9)	20 (25.0)
UC Davis	31 (19.0)	12 (15.0)

Note: The total sample size for each quantitative trait in the maternal age full study population: $N = 142$ for SRS, $N = 155$ for MSEL-ELC at 12 months, $N = 153$ for MSEL-ELC at 36 months, $N = 152$ for VABS; the total sample size for each quantitative trait in the paternal age full study population: $N = 66$ for SRS, $N = 77$ for MSEL-ECL at 12 months and 36 months, $N = 76$ for VABS.

Abbreviations: ASD, autism spectrum disorder; MSEL, Mullen Scales of Early Learning; MSEL-ELC, Mullen Scales of Early Learning early learning composite; SRS, Social Responsiveness Scale; VABS, Vineland Adaptive Behavior Rating Scales.

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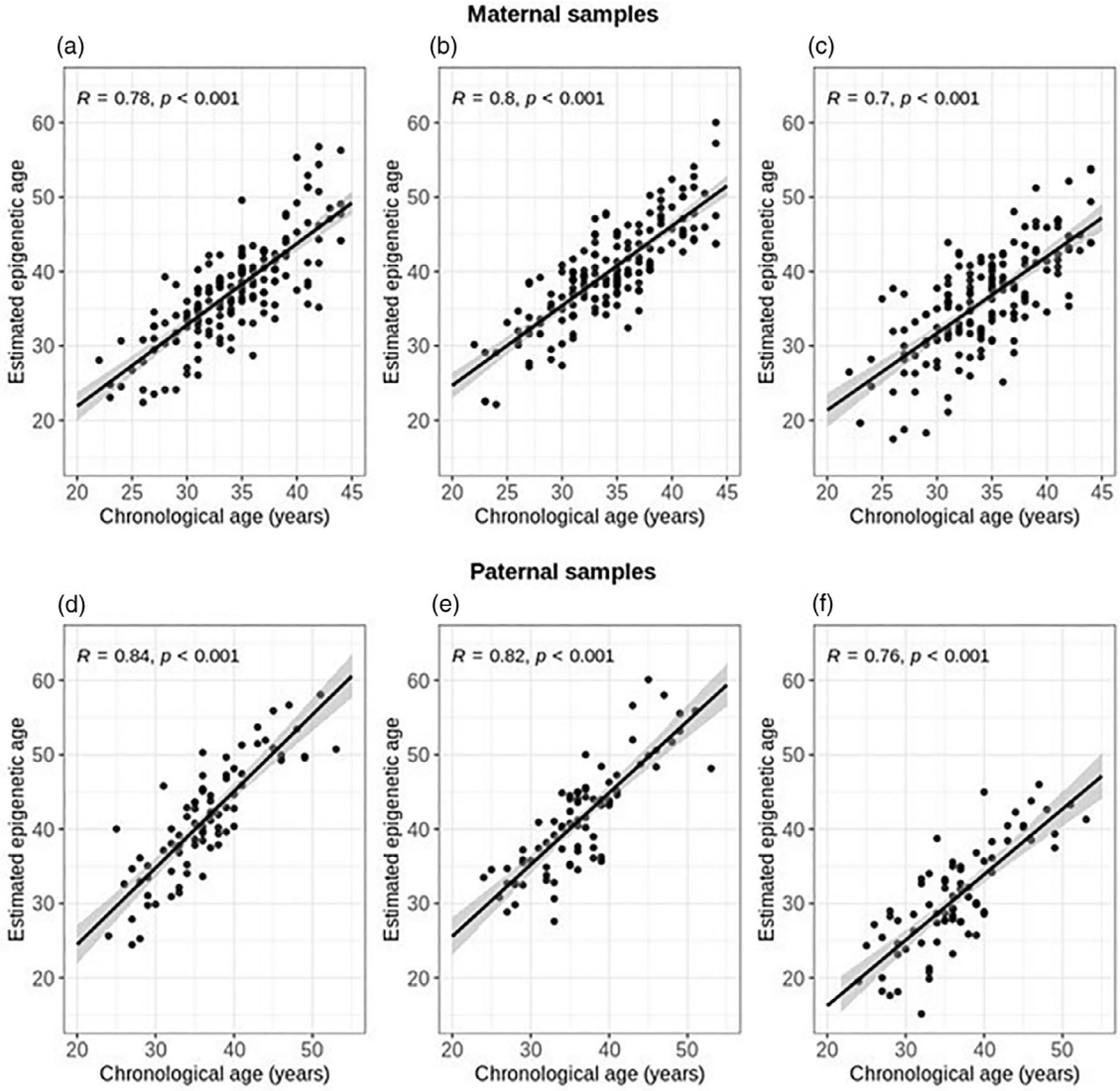

FIGURE 1 Correlations between parental chronologic age and epigenetic age. Maternal chronologic age compared with: (a) Horvath predicted epigenetic age estimates, (b) Hannum predicted epigenetic age estimates, (c) PhenoAge predicted epigenetic age estimates. Paternal chronologic age compared with: (d) Horvath predicted epigenetic age estimates, (e) Hannum predicted epigenetic age estimates, and (f) PhenoAge predicted epigenetic age estimates. Scatterplot shows regression line and 95% confidence intervals. *p*-value refers to the significance level of the spearman correlation.

between epigenetic aging measures from the Horvath's clock and risk of ASD but not with Hannum's or PhenoAge clocks. The lack of correspondence across the associations could be due to differences between the clocks or the different physiological processes captured by each clock. Specifically, the Horvath's clock is a multitissue clock that captures biologic aging across a diverse set of tissues and cell types (Horvath, 2013) whereas the Hannum clock measures biologic aging in a single tissue—whole blood. Unlike first-generation clocks, the Horvath's and Hannum's clocks that were derived on chronologic age alone, PhenoAge was built using clinical measures of health to predict overall health and lifespan (Levine et al., 2018). Growing evidence suggest that second-generation clocks including PhenoAge and GrimAge (Lu et al., 2019) clocks may be more sensitive to biological outcomes associated with lifestyle or clinical factors. Differences in our findings by clock algorithms suggest that detection of associations depends on clock methods.

In this study we examined cell-intrinsic aging, by adjusting for cell composition in our regression analyses, because we were specifically interested in evaluating the cellular aging process itself as opposed to age-related health changes that can cause shifts in blood cell composition and result in epigenetic aging differences (Horvath & Raj, 2018). We found cell-intrinsic accelerated aging in mothers whose child received an autism diagnosis at age 3. We did not observe significant differences in cell-intrinsic aging in fathers related to their child's neurodevelopmental outcomes but point estimates consistently indicated accelerated aging was associated with decreased risk for ASD and ASD-related quantitative traits measured

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TABLE 2 Crude and adjusted associations between parental epigenetic age acceleration and child ASD and nontypical development at 36 months of age

	Crude model		Adjusted model[a]	
	OR (95% CI)	***p*-value**	**OR (95% CI)**	***p*-value**
Maternal epigenetic age acceleration				
Horvath's clock				
TD	Ref		Ref	
non-TD	0.98 (0.89, 1.07)	0.60	0.94 (0.86, 1.06)	0.38
ASD	1.07 (0.97, 1.19)	0.19	1.12 (0.99, 1.26)	0.06
Hannum's clock				
TD	Ref		Ref	
non-TD	0.97 (0.86, 1.08)	0.55	0.95 (0.83, 1.10)	0.50
ASD	0.99 (0.86, 1.13)	0.84	0.95 (0.81, 1.17)	0.55
PhenoAge				
TD	Ref		Ref	
non-TD	0.98 (0.90, 1.07)	0.65	0.96 (0.86, 1.06)	0.39
ASD	1.03 (0.93, 1.14)	0.53	1.04 (0.93, 1.16)	0.50
Paternal epigenetic age acceleration				
Horvath's clock				
TD	Ref		Ref	
non-TD	1.00 (0.91, 1.10)	0.97	1.05 (0.90, 1.21)	0.54
ASD	0.97 (0.86, 1.09)	0.62	0.83 (0.68, 1.01)	0.06
Hannum's clock				
TD	Ref		Ref	
non-TD	1.03 (0.93, 1.14)	0.53	1.09 (0.93, 1.26)	0.29
ASD	1.00 (0.88, 1.15)	0.95	0.80 (0.63, 1.02)	0.07
PhenoAge				
TD	Ref		Ref	
non-TD	1.00 (0.90, 1.12)	0.98	1.02 (0.87, 1.20)	0.83
ASD	0.97 (0.85, 1.12)	0.72	0.84 (0.69, 1.02)	0.08

Note: Estimated Odds ratios (ORs) and 95% confidential intervals (CI) are shown per one unit increase in epigenetic age acceleration.
Abbreviations: ASD, autism spectrum disorder; Non-TD, nontypical development; TD, typical development.
[a]Adjusted for study site, child's sex, top five ancestry principal components (PCs), and maternal or paternal education.

TABLE 3 Crude and adjusted associations between parental epigenetic age acceleration and child Social Responsiveness Scale (SRS) total raw scores at 36 months of age

	Crude model		Adjusted model[a]	
	Beta (95% CI)	***p*-value**	**Beta (95% CI)**	***p*-value**
Maternal epigenetic age acceleration				
Horvath's clock	−0.71 (−1.80, 0.37)	0.19	−0.33 (−1.42, 0.76)	0.55
Hannum's clock	−0.70 (−2.05, 0.66)	0.31	−0.53 (−1.93, 0.88)	0.46
PhenoAge	0.03 (−1.05, 1.12)	0.95	0.42 (−0.66, 1.49)	0.44
Paternal epigenetic age acceleration				
Horvath's clock	−0.22 (−1.40, 0.95)	0.71	−0.61 (−1.86, 0.63)	0.33
Hannum's clock	−0.04 (−1.34, 1.26)	0.95	−0.79 (−2.12, 0.54)	0.24
PhenoAge	−0.02 (−1.3, 1.28)	0.98	−0.26 (−1.50, 0.99)	0.68

Note: Estimated beta coefficients and 95% confidential intervals (CI) are shown per one unit increase in epigenetic age acceleration.
[a]Adjusted for study site, child's sex, ancestry principal components (PCs), and maternal or paternal education.

via the SRS. Previous work suggests the mechanisms that are likely to differ for maternal and paternal chronologic age are complex (Grether et al., 2009; Lee & McGrath, 2015; Lyall et al., 2017). A hypothesized mechanism for maternal age acceleration with increased ASD risk is mediation by pregnancy or perinatal complications,

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TABLE 4 Crude and adjusted associations between parental epigenetic age acceleration and child Mullen Scales of Early Learning (MSEL) early learning composite (ELC) scores collected at 12 months and 36 months of age

	Crude model		Adjusted model[a]	
	Beta (95% CI)	***p*-value**	**Beta (95% CI)**	***p*-value**
MSEL-ELC scores at 12 months				
Maternal epigenetic age acceleration				
Horvath's clock	−0.35 (−1.58, 0.89)	0.58	−0.38 (−1.66, 0.90)	0.55
Hannum's clock	−1.19 (−2.74, 0.36)	0.13	−1.66 (−3.28, −0.04)	0.04
PhenoAge	−0.27 (−1.51, 0.98)	0.67	−0.49 (−1.76, 0.78)	0.45
Paternal epigenetic age acceleration				
Horvath's clock	0.57 (−0.63, 1.77)	0.35	1.05 (−0.20, 2.30)	0.10
Hannum's clock	−0.71 (−2.09, 0.67)	0.31	−0.17 (−1.57, 1.23)	0.80
PhenoAge	0.21 (−1.18, 1.60)	0.76	0.63 (−0.72, 1.97)	0.35
MSEL-ELC scores at 36 months				
Maternal epigenetic age acceleration				
Horvath's clock	0.15 (−0.66, 0.97)	0.71	0.17 (−0.06, 0.96)	0.67
Hannum's clock	0.34 (−0.73, 1.41)	0.53	0.31 (−0.74, 1.36)	0.56
PhenoAge	0.002 (−0.81, 0.81)	0.99	0.11 (−0.66, 0.89)	0.77
Paternal epigenetic age acceleration				
Horvath's clock	−0.05 (−1.02, 0.92)	0.92	0.43 (−0.50, 1.36)	0.36
Hannum's clock	−0.25 (−1.33, 0.82)	0.64	0.45 (−0.54, 1.44)	0.37
PhenoAge	−0.20 (−1.31, 0.92)	0.73	0.56 (−0.43, 1.56)	0.26

Note: Estimated beta coefficients and 95% confidential intervals (CI) are shown per one unit increase in epigenetic age acceleration.
Abbreviations: ELC, early learning composite; MSEL, Mullen Scales of Early Learning.
[a]Adjusted for study site, child's sex, top five ancestry principal components (PCs), and maternal or paternal education.

TABLE 5 Associations between parental epigenetic age acceleration and child Vineland Adaptive Behaviors Scale (VABS) scores at 36 months of age

	Crude model		Adjusted model[a]	
	Beta (95% CI)	***p*-value**	**Beta (95% CI)**	***p*-value**
Maternal epigenetic age acceleration				
Horvath's clock	0.05 (−0.47, 0.57)	0.85	0.02 (−0.53, 0.56)	0.96
Hannum's clock	0.36 (−0.30, 1.02)	0.28	0.15 (−0.56, 0.86)	0.68
PhenoAge	0.06 (−0.45, 0.58)	0.81	0.03 (−0.51, 0.57)	0.91
Paternal epigenetic age acceleration				
Horvath's clock	0.12 (−0.64, 0.40)	0.64	0.14 (−0.43, 0.71)	0.63
Hannum's clock	−0.19 (−0.76, 0.38)	0.51	0.08 (−0.53, 0.69)	0.79
PhenoAge	0.02 (−0.55, 0.59)	0.94	0.28 (−0.31, 0.86)	0.34

Note: Estimated beta coefficients and 95% confidential intervals (CI) are shown per one unit increase in epigenetic age acceleration.
[a]Adjusted for study site, child's sex, top 5 ancestry principal components (PCs), maternal or paternal education.

whereas an association of paternal age acceleration with decreased ASD risk could be explained by socio-economic status (SES) related factors. It is possible that extrinsic or environmental factors may also influence age-related shifts in parental blood cell composition and be related to child neurodevelopment. Understanding the impact of environmental and genetic factors on cell composition and biologic age, and their potential impact on child ASD risk and cognition is an important area of future investigation.

Our study was carried out in a population of children at increased likelihood for ASD due to having an older sibling with autism. Previous work suggests that the associations between parental age and autism and ASD-related outcomes may differ in the presence of familial risk. A recent study drawn from the ASD-enriched risk (ASD-ER) cohort reported that younger parental ages were associated with increased risk of autism and decreased MSEL scores when both parents were under the age of 30 (Lyall et al., 2020). Mechanisms underlying

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these relationships at different familial risk are not yet understood. One potential mechanism underlying the parental age and ASD association is the increased rates of de novo mutation with advanced parental age (Girard et al., 2016). It has been suggested that de novo mutation may play a lesser role in the high-risk families (Lyall et al., 2020). Our study observed that the discordance of aging measured by the deviation between chronologic age and epigenetic age of mothers was associated with autism and cognitive functioning in high familial risk setting. Given the differences in findings by familial risk in parental aging associations with ASD, future work should seek to investigate the discordance of aging and potential mechanisms in independent samples in general populations stratified by high and low familiar risk.

The sample size of our study was relatively small, limiting the ability to perform stratified analyses in subgroups. We cannot rule out the potential for selection bias given the differences in the SES-related characteristics of the maternal full study population and the paternal full study population. However, in our sensitivity analyses of the subgroup with overlapping maternal and paternal observations, we observed overall consistency in the direction of associations for both maternal and paternal epigenetic age acceleration with ASD outcomes. Our study population is relatively highly educated with increased resources and supports to the child, which could introduce residual confounding or mediation by SES-related factors. In addition, due to the small sample size, we were not able to examine the joint effect or combined effect of maternal and paternal epigenetic age acceleration on the outcome measures. Finally, we examined the associations across different clocks and different outcome measures, which could increase the potential for chance finding. Given the nature of the correlated measures of the exposures and outcomes in our study, we cannot assume that all tests are truly independent of each other, therefore we did not perform multiple testing and presented results at nominal levels.

We provide the first evidence showing that the epigenetic aging in mothers as measured by Hannum's clock is associated cognitive function assessed by MSEL scores in their children. In addition, our results suggest the biologic mechanisms related epigenetic aging associations with ASD risk may differ between mothers and fathers. Future studies need to be conducted to evaluate whether our results are specific to families with a history of ASD or whether they reflect biologic processes involved in autism risk, more broadly.

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CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data are available through the National Institute of Mental Health Data Archive (NDA) under the collections for the EARLI network (1600). https://nda.nih.gov/edit_collection.html?id=1600.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Drexel University: Johns Hopkins University: University of California, Davis: Kaiser Permanente Research. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

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