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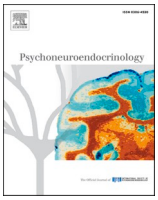

Incredible years parenting program buffers prospective association between parent-reported harsh parenting and epigenetic age deceleration in children with externalizing behavior

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ABSTRACT

Harsh parenting has been shown to increase the risk of physical and mental health problems in later life. To improve our understanding of these risks and how they can be mitigated, we investigated associations of harsh parenting with a clinically relevant biomarker, epigenetic age deviation (EAD), using data from a randomized-control trial of the Incredible Years (IY) parenting program. This study included 281 children aged 4–8 years who were screened for heightened externalizing behavior and whose parents were randomly allocated to either IY or care-as-usual (CAU). Parents reported on their own parenting practices and their child's externalizing behavior at baseline and at a follow-up assessment approximately three years later. Epigenetic age, based on the Pediatric Buccal Epigenetic (PedBE) clock, was estimated from child DNA methylation derived from saliva collected at the follow-up assessment. PedBE clock estimates were regressed on chronological age as a measure of EAD. Moderation analyses using multiple regression revealed that harsher parenting at baseline predicted epigenetic age *deceleration* in children that received CAU ($b = -.21$, 95% CI[-0.37, −0.05]), but no association was found in children whose parents were allocated to IY ($b = -.02$, 95% CI [-0.13, 0.19]). These results highlight a prospective association between harsh parenting and children's EAD and indicate a potential ameliorating effect of preventive intervention. Future work is needed to replicate these findings and understand individual differences in children's responses to harsh parenting in relation to epigenetic aging.

1. Introduction

Exposure to harsh parenting (e.g., shouting, threatening, and slapping) is associated with poorer physical health outcomes (Brody et al., 2014) and an increased risk for mental health problems (Kingsbury et al., 2020). In particular, harsh parenting can contribute to a cycle of coercive parent-child interactions that precipitates child externalizing behavior (Patterson, 1982), which is a core predictor of school drop-out, substance abuse, and criminality (Kim-Cohen et al., 2003). Nonetheless, a large population study in the Netherlands showed that almost 75% of parents yell or scream at their child during discipline situations, while approximately 15% of parents use physical punishment (Jansen et al., 2012, Sari et al., 2022). It is therefore vital that we not only better understand the mechanisms by which harsh parenting influences later health, but also investigate ways in which the detrimental effects of harsh parenting can be mitigated through preventive intervention.

Epigenetic age deviation (EAD) is a clinically relevant biomarker that might be influenced by harsh parenting. Epigenetic age is estimated based on measures of DNA methylation and can deviate from chronological age towards *acceleration* or *deceleration*. These deviations predict a number of adverse health outcomes. In particular, epigenetic age *acceleration* is robustly associated with increased risk for cardiovascular disease, diabetes, lung disease, cancer, mortality and, less consistently, with depression and schizophrenia (for a review see Oblak et al., 2021). On the other hand, epigenetic age *deceleration* has been linked to pediatric critical illness (Verlinden et al., 2023), colorectal cancer risk (Wang

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et al., 2020), and post-traumatic stress disorder (PTSD; Boks et al., 2015; Verhoeven et al., 2018).

Importantly, emerging evidence supports the theory that a harsh parenting environment contributes to epigenetic aging. For example, significant meta-analytic associations have been reported between childhood maltreatment and accelerated epigenetic age in adulthood (Wolf et al., 2018). Similarly, a study in children and adolescents found that threat-related experiences, including physical and emotional abuse, were also associated with accelerated epigenetic age based on the multi-tissue Horvath clock (Sumner et al., 2019). More recently, a method has been developed to reliably estimate epigenetic age in children based on buccal cell DNA methylation (Pediatric-Buccal-Epigenetic clock, PedBE clock; McEwen et al., 2020). Child maltreatment also predicted epigenetic age acceleration based on PedBE estimates, although only in children diagnosed with an internalizing disorder (Dammering et al., 2021).

Interestingly, both the Horvath clock and the PedBE clock are shown to be enriched for glucocorticoid-responsive CpG sites (Dammering et al., 2021; Zannas et al., 2015). Its posited that exposure to early adversity, such as harsh parenting, leads to chronic activation of the stress system and long-term dysregulation of glucocorticoid levels (Esposito et al., 2016; Koss and Gunnar, 2018; McEwen and Wingfield, 2003). Potentially, these perturbations in glucocorticoid levels due the environmental stress of harsh parenting could induce DNA methylation changes at age-related CpG loci. Thus, it is possible that EADs could arise from exposure to harsh parenting. If this is the case, EAD could be a marker linking harsh parenting to health outcomes.

However, despite the prevalence of harsh parenting and its adverse effects on child health, it has not yet been investigated whether typical variation in harsh parenting –as opposed to more severe maltreatment –is associated with epigenetic aging. Moreover, DNA methylation is dynamic and there are preliminary indications that it can be altered by parenting interventions (Hoye et al., 2019). Behavioral parenting interventions, such as Incredible Years BASIC, reduce harsh parenting and increase parenting behaviors that encourage children's desirable behavior in a more consistent and positive manner, thus creating a more predictable and less stressful environment for the child. Theoretically, by lowering environmental stress, parenting programs could potentially regulate children's glucocorticoid levels. In turn, changes in glucocorticoid levels could influence methylation levels at epigenetic clock CpG loci or prevent further detrimental effects on DNA methylation at those sites, which could occur if there is not adequate change in the parenting environment. Therefore, it is reasonable to question whether potential links between harsh parenting and epigenetic aging can be mitigated through early intervention. As proof-of-principle, one study found that a prospective association of parental depressive symptoms with later accelerated epigenetic aging in their offspring was ameliorated in young adults whose parents had previously participated in a family-centered intervention (Brody et al., 2016). Moreover, further analyses revealed that reductions in harsh parenting mediated the protective effect of the intervention on epigenetic aging in children of depressive parents. However, the study did not consider the possible prospective associations of harsh parenting as a main risk exposure on epigenetic aging or independently of parental mental health.

In the current study, we used data from a randomized controlled trial (RCT) to prospectively investigate whether harsh parenting predicts epigenetic age acceleration, and whether this association is mitigated by an established parenting intervention (i.e., Incredible Years, IY). Our sample comprised children with above average parent-reported externalizing behavior, which had several advantages: Children with externalizing behavior experience harsher parenting (Gershoff, 2002; Patterson, 1982); epigenetic aging effects may be more pronounced in children with emerging psychopathology (Dammering et al., 2021); IY is proven effective in reducing harsh parenting in parents of children with above-average externalizing behavior (Weeland et al., 2017). We predicted that harsher parenting at the start of the RCT would associate with epigenetic age acceleration at the final follow-up (FU; approximately 3 years later) in the control group, but that this association would be attenuated in the intervention group. This hypothesis was based on the reasoning that IY is shown to reduce harsh parenting and increase positive parenting behaviors (Leijten et al., 2018), thus reducing environmental stress on the child and, in turn, potentially inducing DNA methylation changes at glucocorticoid-responsive clock loci or halting further detrimental DNA methylation changes at the loci that could occur without intervention. The PedBE clock was selected as our primary measure of EAD because of the commonly available clocks it matches most closely to our study sample; specifically, it was trained in pediatric samples and in buccal cells, which are in high proportions in saliva, and can detect EADs in clinical samples (Kling et al., 2020; McEwen et al., 2020). However, to supplement the wider literature, we also conducted sensitivity analyses using the established multi-tissue Horvath clock (Horvath, 2013). Additionally, we explored whether child sex had a moderating effect on the results given prior evidence of sex-specific associations of epigenetic aging with both early life stress and mental health outcomes (McGill et al., 2022). The results can contribute to our growing understanding about the biological links between parenting and later health and inform how plausible it is that our current behavioral parenting interventions can influence biological markers of risk.

2. Materials and methods

2.1. Study population

Participants were 281 parent—child dyads from the longitudinal Observational Randomized Controlled Trial of Childhood Differential Susceptibility (ORCHIDS; Chhangur et al., 2012); METC UMCU, protocol number 11–320/K; ERB FMG-UvA, record 2015-CDE-6392), in which the IY program was compared to CAU. Families of children aged 4–8 years were recruited through municipalities in the Netherlands based on screening for above-average externalizing behavior through parent reports (i.e., scores >75th percentile on the Intensity Scale of the Eyberg Child Behavior Inventory; ECBI, Eyberg and Pincus, 1999), and were randomized to one of the two trial arms (IY or CAU). One participating parent per family completed the assessments at each wave, although the other parent was invited to take part in IY if they wished. Of the original study sample (N = 396), 296 children provided saliva samples on parental consent at the final follow-up assessment of the trial (2.5 years post-intervention and approximately 3 years after baseline). This resulted in genomic and DNA methylation data for 289 children after quality control (see Figure S1 for participant flow). A further eight participants were excluded from the analyses due to missing data for parenting behavior at baseline (N = 3) or follow-up (N = 5). Characteristics of the final current study sample (N = 281) are shown in Table 1 alongside characteristics per trial arm (IY vs CAU).

2.2. DNA methylation and genetic covariates

Genome-wide DNA methylation was assessed using the Infinium EPIC 850k array and genotyping was performed with Infinium iSelect GSA (see supplementary information for details) using bisulphite-converted genomic DNA derived from saliva collected by passive drool. Cell type proportions were estimated in the *meffil* package (Min et al., 2018) in R using the Houseman algorithm (Houseman et al., 2012) and buccal cell proportion was included as a covariate in the statistical models to control for cell heterogeneity. To account for population stratification, we performed principal component analysis on the genetic data and included the first two genetic principal components (gPCs) as covariates in the statistical models.

Table 1
Sample characteristics.

	Whole sample (N = 281)	IY (n = 135)	CAU (n = 146)	Test statistic[a.]
	M (*SD*)	*M* (*SD*)	*M* (*SD*)	
Harsh parenting (PPI)				
Baseline	2.74 (0.58)	2.81 (0.58)	2.67 (0.57)	U = 8558, p =.057
FU	2.42 (0.56)	2.39 (0.53)	2.45 (0.59)	U = 10398, p =.425
Δ harsh parenting	-0.32	-0.42	-0.22	F(1278) = 6.01, p =.015
Externalizing behavior (ECBI)				
Baseline	132.80 (19.14)	135.43 (20.63)	132.80 (17.36)	U = 8409, p =.034
FU	115.47 (25.32)	113.75 (25.04)	117.07 (25.56)	U = 10642, p =.248
Δ externalizing behavior	-17.33	-21.68	-15.73	F(1278) = 5.89, p =.016
Child age at FU	9.97 (1.32)	9.98 (1.37)	9.96 (1.27)	U = 9763, p =.893
Parent age at FU	41.80 (4.58)	41.81 (4.40)	41.79 (4.76)	U = 9963, p =.875
Child sex (% female)	47	44	51	$\chi2$ = 1.36, p =.244
Parent sex (% female)	92	92	93	$\chi2$ = 0.03, p =.852
Child ancestry (n)				$\chi2$ = 1.01, 5 =.908
European	211	103	108	
Asian	4	2	2	
African	6	2	4	
South American	5	3	2	
Mixed	55	25	30	

Note. IY = Incredible Years, CAU = Care-As-Usual, FU = Follow-Up (~3 years after baseline), PPI = Parenting Practices Interview (completed by parent), ECBI = Eyberg Child Behavior Inventory (completed by parent).
[a.] Test of difference between the IY group and CAU group for each variable.

2.3. Epigenetic age deviation (EAD)

Epigenetic age was estimated based on the Pediatric-Buccal-Epigenetic clock (PedBE; (McEwen et al., 2020) from Noob-method normalized beta methylation values using the *Methylclock* package in R (Pelegí-Sisó et al., 2021). Of the 94 CpG loci that consist the PedBE clock, 92 loci were available in the current dataset to compute the PedBE age estimates, which were moderately correlated with child age (r =.54, p <.001; Figure S2). Separate linear regression models were used to regress chronological age on the PedBE age estimates and the resulting residuals provided the measure of EAD. Horvath age estimates were also moderately correlated with child age in the current sample (r =.57, p <.001; Figure S3). There was no significant correlation between EAD calculated based on the PedBE and Horvath clocks (r =.09, p = 0.09; Figure S3).

2.4. Intervention status

After a baseline assessment, parent—child dyads were randomized to either the Incredible Years BASIC intervention (IY; Webster-Stratton, 2001) or to a care-as-usual (CAU) control condition. IY is a collaborative, social learning-based parenting program that reduces child conduct problems. Parents took part in 14 weekly two-hour group sessions during which they were taught child-led play and reinforcement skills, effective limit-setting, and nonviolent discipline techniques. In the current study sample, 16 of the 135 parents allocated to IY did not attend any sessions despite taking part in the assessments, and the remaining parents attended on average 10 sessions. The CAU group did not receive any intervention as part of the trial, but for ethical reasons were permitted to seek outside support themselves to manage their children's behavior or address other issues. We used an intention-to-treat approach during analysis to preserve the randomization. There were no significant baseline differences between participants allocated to the IY and CAU groups in terms of harsh parenting, parent age and sex, or child age, sex, and ancestry (Table 1 and Figure S4), however levels of parent-reported externalizing behavior were higher at baseline in the IY than the CAU group, and thus baseline ECBI scores were included as a covariate in the statistical models.

2.5. Harsh parenting

Harsh parenting was measured by parent report using the *physical punishment* scale (6 items) and *harsh and inconsistent discipline* scale (15 item) of the 74-item Dutch version of the Parenting Practices Interview (Webster-Stratton et al., 2001). Parents responded to statements about the frequency of their responses in different parenting situations (e.g. how often do you "raise your voice" when your child misbehaves), which were rated on a 7-point scale from 'never' to 'always'. A composite mean score ranging 1–7 was computed using items from both scales, with higher scores representing a higher frequency of harsh parenting practices. Reliability of the composite score was acceptable at baseline (α =.78) and at FU (α =.79).

2.6. Child externalizing behavior

Child externalizing behavior was measured by parent-report using the sum score of the ECBI, which assesses the frequency of the child's externalizing behavior with 36-items (e.g., "has temper tantrums", "acts defiant when told to do something") rated on a 7-point scale (1 = never, to 7 = always). The ECBI was designed as a brief screening instrument that can accurately discriminate between children with and without a diagnosis of a conduct-related disorder (Rich and Eyberg, 2001). Reliability was good at baseline (α =.85) and excellent at FU (α =.92).

2.7. Statistical analyses

All analyses were performed in R version 4.2.1 and an alpha level of.05 was used to assess statistical significance. Scores for harsh parenting and child externalizing behavior were mean centered to reduce possible multicollinearity in the regression models, which could occur due to the interaction term and correlations of the same variables across timepoints, as well as to aid interpretation (Iacobucci et al., 2017). A multiple linear regression was used to test whether harsh parenting at baseline predicts PedBE-derived EAD at FU (approximately 3 years after baseline). To assess whether the association would differ based on whether the family was allocated to IY or CAU, we included an interaction term of condition by baseline harsh parenting in the model. Covariates were child sex and age at saliva collection, buccal cell proportion, gPCs, and baseline child externalizing behavior. We also explored potential sex differences in the results by repeating the model including a three-way interaction of child sex with baseline harsh parenting and condition. Additionally, we repeated all models controlling for 1) harsh parenting at FU and 2) externalizing behavior at FU, to test whether prospective associations of harsh parenting with EAD were independent of current levels of harsh parenting and externalizing behavior. Finally, all analyses were repeated with the Horvath-derived EAD measures. Visual inspection of histograms of the residuals, QQ-plots of the standardized residuals, and scatter plots of the standardized residuals against fitted values confirmed all models met the assumptions of normality of residuals, linearity, and homoscedasticity. The assumptions of no multicollinearity (variance inflation factor < 5) and no influential cases (Cook's distance < 1) were also met. In line with recommendations, we did not conduct a power analysis because the study was a secondary analysis on already collected data and post-hoc power calculations are potentially misleading (Perugini et al., 2018).

3. Results

At baseline, parents' composite scores ranged 1.29–4.52, with 9.25% of parents reporting using physical discipline "sometimes" or more frequently and 96.80% of parents reporting using harsh and inconsistent discipline "sometimes" or more frequently. There was no statistical difference between the IY and CAU group in terms of harsh parenting at baseline (Table 1). An ANCOVA revealed that harsh parenting was significantly lower in the IY group than the CAU group at follow-up, while controlling for baseline levels of harsh parenting, $F(1278) = 6.01$, $p = .015$. Additionally, levels of parent-reported child externalizing behavior were significantly lower after IY than CAU, while controlling for baseline levels of externalizing behavior, $F(1278) = 5.89$, $p = .016$. In other words, as expected, there was a greater reduction in parent-reported harsh parenting and child externalizing behavior in the IY group compared to the CAU group. The proportion of participating mothers and fathers did not differ between groups (Table 1) and there were no associations of parent sex with harsh parenting and child internalizing scores. However, there were small but significant associations of parent with buccal cell proportion and EAD (PedBE) estimates, with both being higher in mothers than fathers (Figure S4). Furthermore, no group differences (IY versus CAU) were found in EAD at follow-up based on the PedBe clock, $F(1273) = 0.91$, p =.340, or Horvath clock, $F(1273) = 0.004$, p =.994, while controlling for baseline parent-reported child externalizing behavior, child age and sex, and technical covariates.

As shown in Table 2, we found a significant but negative main effect of harsh parenting at baseline on EAD at FU (i.e., approximately 3 years later), $b = -0.21$, $p = .010$, indicating epigenetic age *deceleration* in children whose parents use more harsh parenting. Overall, the model explained 1.9% more variance in EAD than a covariate only model, which was statistically significant ($F = 2.95$, $p = .033$). Furthermore, there was a significant interaction effect between condition and baseline harsh parenting on EAD, $b = 0.24$, 95% CI [0.02, 0.46], $p = .036$. Post-hoc simple slope analyses revealed that harsher parenting significantly predicted epigenetic age *deceleration* in the CAU group ($b = -0.21$, 95% CI[-0.37, −0.05]), but not in the IY group ($b = -0.02$, 95% CI [-0.13, 0.19]), as shown in Fig. 1. This indicates that in the CAU group every unit increase in harsh parenting significantly predicted a.21 units decrease in EAD, which ranged from −1.90–2.15 in the CAU group. Whereas, in the IY group every unit increase in harsh parenting predicted a −0.02 decrease in EAD, which ranged −1.67–1.69, which was not statistically significant. Additionally, while girls had higher EAD than boys at FU, we did not find any significant interaction effect with child sex ($b = 0.18$, $p = .444$).

As a follow-up analysis, we tested whether residualized change in parent-reported harsh parenting from baseline to follow-up statistically predicted EAD, controlling for child sex, age, and baseline conduct problems as well as technical covariates. The regression model indicated a small increase in EAD with a reduction in harsh parenting, but this association was not statistically significant ($b = -0.05$, 95% CI [-0.18, 0.09], $p = .496$). This would suggest that the between-group difference in the association between harsh parenting and EAD was not primarily driven by the significant, greater reduction in harsh parenting seen in the IY group compared to the CAU group.

Table 2
Regression results for the association of harsh parenting with EAD as moderated by condition.

	EAD PedBE		
Predictors	*b*	95% CI	*p*
Intercept	-1.64	[-2.18, −1.09]	<.001
Harsh parenting (baseline)	-0.21	[-0.37, −0.05]	.010
Condition (IY)	0.10	[-0.03, 0.23]	.129
ECBI (baseline)	<0.01	[-0.00, 0.01]	.197
Buccal cells	2.84	[2.41, 3.27]	<.001
Age at FU	0.02	[-0.02, 0.07]	.326
gPC1	-0.65	[-1.76, 0.45]	.244
gPC2	0.29	[-0.78, 1.37]	.590
Child sex (female)	0.14	[0.01, 0.27]	.032
Harsh parenting (baseline) × condition	0.24	[0.02, 0.46]	.036
Observations	281		
R^2 / R^2 adjusted	.409 /.390		

Note. IY = Incredible Years, ECBI = Eyberg Child Behavior Inventory, gPC = genetic principal component

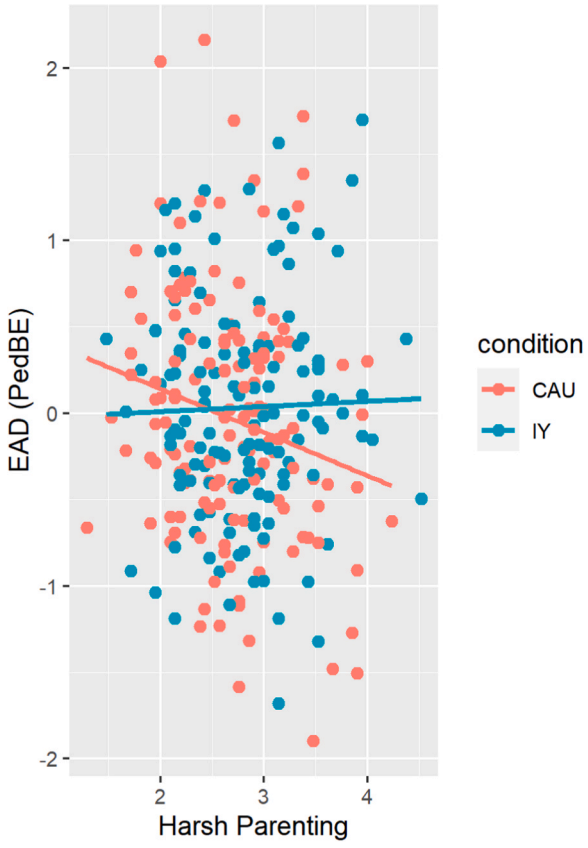

Fig. 1. Association of harsh parenting with epigenetic age deviation as moderated by condition. *Note.* Simple slopes graph showing the moderating effect of condition (Incredible Years, IY, versus care-as-usual CAU) on the association of harsh parenting at baseline with epigenetic age deviation (EAD) at follow-up rived from pediatric buccal epigenetic (PedBE) clock age estimates (interaction term: $b = 0.24$, 95% CI [0.02, 0.46], $p = .036$).

Sensitivity analyses showed that the results did not meaningfully differ when harsh parenting at FU (see Table S1) or child externalizing behavior at FU (see Table S2) were included as covariates. This suggests that the prospective association of harsh parenting at baseline with EAD in the CAU group, and the lack of an association in the IY group, was not driven by current levels of harsh parenting or child externalizing behavior.

In further sensitivity analyses using Horvath-derived estimates of EAD, we did not find a significant effect of baseline harsh parenting on EAD and there was no significant interaction (see Table S3 and Figure S5).

4. Discussion

In this study, using data from a RCT, we found that the IY parenting program moderated a propective association between parent-reported harsh parenting and PedBE-derived epigenetic age deviations, based on PedBE clock estimates, in children with parent-reported above-average externalizing behavior. Contrary to our hypothesis that harsher parenting would predict *accelerated* epigenetic age, we found that harsh

parenting was prospectively associated with epigenetic age *deceleration* approximately three years later in children randomized to care-as-usual. However, there was no association of harsh parenting with EAD for children whose parents were randomized to IY, indicating a potential buffering effect of the parenting program.

Prior research has linked early life stress to epigenetic age *acceleration* (Dammering et al., 2021; Sumner et al., 2019; Wolf et al., 2018), whereas in the current study we found epigenetic age *deceleration* in children exposed to harsher parenting reported by parents. However, epigenetic age *deceleration* estimated from cord blood at birth has been linked to prenatal stress during pregnancy, including maternal depression and post-traumatic stress disorder (Appleton et al., 2022; Koen et al., 2021). Harsher parenting is more often seen in parents with mental health problems and other psychosocial risk factors (Eamon, 2001; Serbin and Karp, 2004) Therefore, one possibility is that our results reflect the remnant effects of prenatal stress, or the continued effects of family psychosocial stress, on epigenetic age in children who were not allocated to the preventive intervention. However, we were not able to test this possibility in the current cohort due to a lack of prenatal and perinatal measures.

As an alternative explanation for the results, epigenetic age *deceleration* may have been seen in children exposed to harsher parenting in the current study because the sample comprised children with heightened parent-reported externalizing behavior. It is possible that the association of environmental stress, such as harsh parenting, with epigenetic aging may differ based on individual characteristics. For example, two studies have shown that traumatic stress is linked to epigenetic age *acceleration* in individuals without PTSD, but epigenetic age *deceleration* in individuals with PTSD (Boks et al., 2015; Verhoeven et al., 2018). Notably, externalizing behavior has previously been linked with demethylation at the glucocorticoid receptor gene (Heinrich et al., 2015) and blunted stress responses (Ruttle et al., 2011) – a profile opposite to that reported in individuals exposed to early adversity in studies based on community samples (Wadji et al., 2021). Moreover, both exposure to harsh parenting and child externalizing behavior have been linked to delayed neurodevelopment (Jarvers et al., 2022; Suffren et al., 2022), thus it is conceivable that together they may also contribute to slower biological aging during development. Aside from phenotypic characteristics, it has also previously been reported that harsh parenting has differential effects on adolescent health depending on genotype (Brody et al., 2014). As such, future research should consider how interactions of environmental exposures with children's phenotypic and genotypic characteristics contribute to epigenetic aging and other biomarkers of health risks in larger longitudinal studies.

In terms of clinical relevance, it is important to note that EAD in both directions has been linked to negative health outcomes, therefore mitigating the effects of environmental exposures on EAD could have health benefits. Alongside links with pediatric illness and certain adult cancers (Verlinden et al., 2023; Wang et al., 2020), epigenetic age *deceleration* has been linked to lower birth weight (Knight et al., 2016), which is an important predictor of adverse child outcomes, including externalizing-related disorders (Mathewson et al., 2017). However, because of considerable methodological heterogeneity between studies, it is not yet possible to conclude whether the deviations in epigenetic age seen in children who experienced harsher parenting in the CAU are large enough to have clinical relevance. Harmonization across studies and a broadening of the literature is required for EAD to become a more informative as a biomarker. Moreover, while EAD may increase risk for some health conditions, it is possible that EAD has an adaptive function for some individuals under certain environmental conditions. Future research is needed that tracks deviations in epigenetic aging over the lifespan alongside environmental exposures, cognitive and behavioral development, and health outcomes.

Our results indicated that IY buffered the prospective association of parent-reported harsh parenting with children's epigenetic age deceleration. The RCT design coupled with an intention-to-treat analysis allows us to infer that the between-group differences in this association resulted from the intervention (McCoy, 2017). However, without a baseline assessment of EAD, we cannot ascertain in which group changes in epigenetic age occurred. It is possible that epigenetic age deceleration emerged during the trial in children exposed to harsher parenting in the CAU group, but not for those in the IY group because they received early intervention. Alternatively, epigenetic age deceleration may have been present at baseline for children with harsher parents across both groups, but may have been improved by the intervention for children in the IY group. In the sensitivity and follow-up analyses, we found that the results did not change when controlling for parent-reported harsh parenting or externalizing behavior at the follow-up assessment, and that residualized change in harsh parenting did not significantly predict EAD. This suggests that the buffering effects of IY were not primarily driven by reductions in parent-reported harsh parenting or child externalizing behavior from baseline to follow-up, at least as measured by parent report. It may be that IY leads to an inherently less stressful environment for the child through its wider effects. For example, IY also promotes positive parenting techniques, which have been shown to buffer the effects of early adversity on children's stress system (Brown et al., 2020).

Notably, the results did not replicate when using Horvath clock age estimates to calculate EAD. It is possible that the difference in results reflects the different ways these clocks were trained and, therefore, the different CpG loci they include. The Horvath clock was trained on a large, diverse sample of individuals of all ages and using cross-tissue methylation estimates, with the aim of accurately estimating chronological age (Horvath, 2013). In contrast, the PedBE was trained on a more modest sample of typically developing children using methylation estimates from buccal cells, with the aim of more accurately estimating epigenetic age in children and how deviations from chronological age might relate to disease (McEwen et al., 2020). As the current study sample comprised children showing emerging symptoms, it is intuitive that the deviations in epigenetic age might be captured by the PedBE, but not the Horvath clock. Specifically, the difference in results may reflect the ability of the PedBE clock to detect EAD in children, and thus associations between EAD and the environment, while the Horvath clock may partial out environmental noise in favor of more accurate age estimates across tissues, as reflected in the null results. Indeed, this exemplifies the importance in future research of selecting an epigenetic clock based on the study sample and aims, and being cautious over whether results can be generalized across epigenetic clocks. Also of note for future research, a novel PC-based method may bolster reliability of methylation-based clocks (Higgins-Chen et al., 2022), however the method is not yet available for the PedBE clock.

A strength of this study was the implementation of a proven-effective parenting program that allowed us to not only study the association between parent-reported harsh parenting and children's epigenetic aging, but also whether this association can be mitigated, which has been largely overlooked in the epigenetic literature (Overbeek et al., 2020). Moreover, the RCT design, combined with intention-to-treat analyses, reduced the influence of characteristic differences in between-group comparisons. Furthermore, the relatively longer follow-up assessment allowed for the study of a prospective rather than cross-sectional association of parent-reported harsh parenting with epigenetic aging. On the other hand, it is worth noting that the associations found between parent-reported harsh parenting and epigenetic age deceleration were correlational, and thus we must be cautious not to infer causality. Also, harsh parenting was based on parent reports, which may be subject to bias and differ between parents based on levels of social-desirability. That said, there is some evidence that parenting self-report measures are able to capture reported child maltreatment better than observation (Bennett et al., 2006) and that there is less discrepancy between self-reported and observed harsh parenting in higher social economic samples (Herbers et al., 2017). Additionally, self-reports provide a measure of parenting practices over time in the

home, compared to observations that are subject to less bias but take place in an unnatural setting where socially desirable behavior may be higher due to direct observation. Moreover, the randomized design coupled with intention-to-treat analysis should have mitigated individual differences between parents, such as their levels of socially desirable behavior, in the moderation analysis (McCoy, 2017). As mentioned, DNA methylation measures were not available at baseline, which means it was not possible to assess changes in epigenetic aging in each group or more rigorously test the direction of the association of parent-reported harsh parenting with epigenetic aging. Finally, although the sample size was relatively large for an intervention study, conclusions based on the results should be made tentatively until the results are replicated in an independent cohort.

5. Conclusions

The current study highlights a prospective association of parent-reported harsh parenting in early childhood with epigenetic age *deceleration* in children with above-average parent-reported externalizing behavior, and suggests that this association may be mitigated by an established parenting program. These results add to the accumulating evidence that early life stressors are likely to contribute towards biological aging and support the possibility that preventive intervention may be able to ameliorate such effects. Future research should utilize pre- and post-test DNA methylation measures in randomized control trial settings to better understand the possible pathways between harsh parenting and epigenetic aging and illuminate the causal influence of preventive interventions. Moreover, the current research highlights the need to carefully consider how contributions of parenting-related stressors to clinically relevant biomarkers, such as EAD, may differ depending on the characteristics of the child.

CRediT authorship contribution statement

Geertjan Overbeek: Writing – review & editing, Supervision, Project administration, Funding acquisition. **Patty Leijten:** Writing – review & editing, Supervision. **Nicole Creasey:** Writing – review & editing, Writing – original draft, Visualization, Software, Resources, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Marieke S. Tollenaar:** Writing – review & editing, Supervision.

Declaration of Competing Interest

None

Data Availability

*Data and the analytic code necessary to reproduce the analyses are available from Nicole Creasey (*n.creasey@erasmusmc.nl) and *Geertjan Overbeek (*g.overbeek@uva.nl*) on reasonable request.* The materials necessary to attempt to replicate the findings presented here are not publicly accessible.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2024.107043.

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