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Children's internalizing behavior development is heterogeneously associated with the pace of epigenetic aging

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ABSTRACT

Background: Internalizing behaviors are an indicator of children's psychological and emotional development, predicting future mental disorders. Recent studies have identified associations between DNA methylation (DNAm) and internalizing behaviors. This prospective study aimed at exploring the associations between pace of biological aging and the developmental trajectories of internalizing behaviors.

Methods: Participants were children from the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort (N = 974). Measures of DNA methylation were collected at birth, age 7 and ages 15–17. The pace of aging was estimated using the DunedinPoAm algorithm (PoAm). Internalizing behaviors reported by caregivers between ages 4 and 16 using the Strengths and Difficulties Questionnaire. To explore heterogeneity in the association between PoAm and internalizing behaviors we use Poisson quantile regression in cross-section heterogeneity and longitudinal latent class analysis over the childhood and adolescence.

Results: Internalizing behavior trajectories were identified: low-risk, childhood limited, late onset and early onset (persistent). Accelerated aging at birth was negatively associated with internalizing behaviors in early childhood but positively correlated during adolescence. Higher PoAm at birth increased chance of low-risk profile, while decreasing likelihood of childhood limited trajectory. PoAm at age 15 was negatively associated with childhood limited profile and positively linked to late onset trajectories. Associations were larger at higher values of internalizing symptoms.

Conclusions: The heterogeneity in the association between biological age acceleration and internalizing behaviors suggests a complex dynamic relationship, particularly in children with high or increased risk of adverse mental health outcomes.

1. Introduction

Mental disorders are among the leading causes of non-transmissible, chronic diseases and health disparities in adults (Prince et al., 2007), children, and adolescents worldwide (Baranne and Falissard, 2018; Kieling et al., 2011). Internalizing behaviors (or internalizing symptoms) are an early indicator of children's psychological and emotional development, predicting future mental disorders such as anxiety and depression (Liu et al., 2011). Adverse life experiences during childhood increase the risk for internalizing symptoms, although the precise biological and psychological pathways remain unclear (Barker, Walton et al., 2018). Evidence from longitudinal studies suggests that internalizing behaviors in 11-year-old children are prospectively associated with early substance use (age 14 years), and anti-social behavior and mental disorders in adulthood (Althoff et al., 2010; King et al., 2004).

Recent advances in epigenetics helped identified associations between children's emotional development and biological developmental pathways, particularly DNA methylation, an epigenetic process that regulates gene expression (Czamara et al., 2021; Khulan et al., 2014; Parade et al., 2021). For example, Barker, Cecil et al. (2018) showed that a methylation index for low-grade inflammation risk is correlated with internalizing behaviors during childhood.

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In the last decades, epigenetic clocks have been developed as global indices of DNA methylation to estimate biological age acceleration (AA) from chronological age (Horvath and Raj, 2018; Marioni et al., 2019). We define AA as individuals who are biologically *older,* relative to their chronological age. To address the biological mechanisms underpinning AA, a new generation of epigenetic clocks have been designed involving multiple biological and clinical biomarkers of ageing rather than chronological age alone (Belsky et al., 2018; Levine et al., 2018). Belsky et al. (2020) proposed a novel method for quantifying accelerated aging (hereafter, the PoAm algorithm standing for Dunedin (P)ace (o)f (A)ging (m)ethylation) using longitudinal data over a wide array of biological markers tracking organ integrity in young adults (from age 26–38 years). Unlike the earlier epigenetic clocks measuring biological age in years, PoAm calculates relative age acceleration (or deceleration), with respect to the reference population. PoAm is strongly associated with cognitive functioning and physical decline in adults from the Dunedin cohort at age 45. PoAm has been recently validated in a pediatric sample, showing positive associations between economic disadvantage and accelerated aging, in a sample of U.S. children and adolescents (Raffington et al., 2021). Moreover, in E-Risk longitudinal data, Belsky et al. (2020) found significant associations between early childhood adversity (i.e. poverty and victimization) and AA at age 18.

Based on the evidence from Simpkin et al. (2017), Barker, Cecil et al. (2018) and Ellis et al. (2019), DNAm is associated to multiple biological processes, including cortisol levels and the timing of physical development, both associated with the emergence of internalizing behaviors. Moreover, the potential effects of DNAm on psychopathology risks can be both cumulative and time-sensitive, varying significantly in the population (Barker, Walton et al., 2018). Therefore, as noted by Barker, Walton et al. (2018), environmental exposures imprinted in the pace of epigenetic aging are likely to be linked dynamically with the developmental trajectories of internalizing behaviors, due to both the timing of exposure and cumulative hazard. Recent studies have established direct associations between AA and higher internalizing symptoms in children, using the first-generation clocks in cross-sectional data (Sumner et al., 2019; Tollenaar et al., 2021). Using Horvath's (2013) clock at a single time point (age 6), Tollenaar et al. (2021) showed that epigenetically older children were more likely to exhibit high internalizing symptoms at age 30 months. Also, AA was positively associated with internalizing behaviors between the ages of 8 and 10 years. Using the same epigenetic clock, Sumner et al. (2019) found that early experiences of high stress (e.g. violence) were associated with accelerated epigenetic aging in children, which in turn was positively linked to depressive behaviors and early pubertal stage during adolescence. Recently, emotion regulation skills, such as self-control, have been shown to be prospectively associated in a similar manner with a deceleration in ageing, using the PoAm clock (Richmond-Rakerd et al., 2021). While prior research had established associations between DNAm and internalizing symptoms during childhood, these studies do not account for developmental trajectories. As shown in Barker and Maughan (2009) and Barker, Walton et al. (2018), the developmental trajectory of internalizing behaviors in childhood and adolescence is both highly dynamic and heterogeneous in any given population. Similarly, environmental exposures are imprinted in the DNAm along the life course, which in turn can shape developmental trajectories, increasing (or decreasing) psychopathology risks (Barker, Walton et al., 2018). Therefore, relying in a longitudinal cohort with repeated measurements is key to understand the dynamic heterogeneity between DNAm and internalizing behaviors.

The current study extends the literature by investigating the heterogeneity of cross-sectional and longitudinal associations between the epigenetic pace of aging and internalizing behavior trajectories from birth to age 17 years, accounting for concurrent environmental stressors and demographic characteristics. We believe understanding the range of associations between AA and internalizing behaviors in the population is key to determine at-risk groups and the severity of environmental risks that are imprinted in DNAm. We explore the degree of heterogeneity in the association between PoAm and internalizing symptoms in cross-section at different quantiles of the internalizing sub-scale score for given ages, as well as the impact of PoAm on latent trajectories of internalizing behaviors during childhood and adolescence.

2. Methods

2.1. Participants

The Avon Longitudinal Study of Parents and Children (ALSPAC) survey, also known as "The Children of the 90 s", is an ongoing population study to understand the environmental influences on different aspects of childhood development. The study website contains details of all the data that is available through a fully searchable data dictionary and variable search tool. (http://www.bristol.ac.uk/alspac/). For detailed cohort information see (Fraser et al., 2013). A brief cohort description is provided in Supplemental Appendix A, Section A. Informed consent was obtained from all participants. Consent for biological samples was collected in accordance with the Human Tissue Act (2004).

We restricted our analytical sample to the ALSPAC subgroup of families that provided epigenetic data (1018 mother-child pairs) at birth for mothers and newborns, for the children only at age 7, and then again for mother-child dyads at child age 15–17. We excluded observations where it was not possible to compute PoAm, or those where caregivers did not provide information in the behavioral questionnaire to build measures for internalizing behaviors. Our final analytic dataset, excluding 44 removed samples, consisted of 974 participants with epigenetic profiles and behavioral questionnaire data from at least one time point.

2.2. DNA methylation

DNA methylation values were obtained from the ALSPAC consortium. The procedure for collecting and storing biological samples has been described elsewhere (Alfano et al., 2019; Sharp et al., 2015). Briefly, following DNA extraction from peripheral venous blood, bisulphite modification was performed with the Zymo EZ DNA MethylationTM kit (Zymo, Irvine, CA, USA). DNA methylation levels were measured using the Infinium HumanMethylation450 BeadChip (Relton et al., 2015). Initial data processing by the ALSPAC consortium was performed in R (version 3.2.4) with the *meffil* library (https://github.com/perishky/meffil). Quality control included functional normalization and correction for cellular heterogeneity using the Houseman procedure, leaving 4854 individual samples for subsequent analysis. Full description to the quality control procedure is available in Supplemental Appendix A. In our analytical sample, we used the ALSPAC technical guide to account for population stratification, excluding duplicates and individuals with non-Caucasian or missing ethnicity (261 observations).

2.3. Internalizing behaviors

The child mental health measure used in the present study was derived from the internalizing sub-scale of the Strengths and Difficulties Questionnaire (SDQ). The SDQ is a behavioral-screening questionnaire for children, consisting of 25 questions capturing dimensions of social and emotional development (Goodman, 1997). A full list of the items included in the SDQ questionnaire appears in Supplemental Appendix A, Section B. Ratings of the child's SDQ were provided by the main caregiver (typically the mother) when children were approximately 4, 6, 8, 9, 11, 13 and 16 years old. The 25 items in the SDQ questionnaire correspond to five sub-scales addressing emotional symptoms, peer symptoms, conduct symptoms, hyperactivity/inattention and pro-social behavior. For each sub-scale (ranging from 0 to 10 points), a reference cut-off point of five has been suggested for at-risk groups (Durbeej et al., 2019). The internalizing sub-scale is the sum of the questions related to

the emotional and peer symptoms sub-domains, as defined by (Goodman, 2001) and (Goodman et al., 2010). We only focus on internalizing (and not externalizing) symptoms, as they provide a proxy for psychopathology risks in adult life (Barker, Walton et al., 2018).

2.4. Data analyses

Pre-processed Infinium array methylation beta values were used to calculate the Dunedin PoAm pace of biological aging (Belsky et al. 2020) using the library DunedinPoAm38 (https://github.com/danbelsky/DunedinPoAm38) in R (version 4.0.2.). The PoAm algorithm is based on 46 CpG sites, obtained from fitting DNAm data with elastic-net over a standardized index which summarizes 18 different biomarkers at multiple time points during adulthood (ages 26–38). The PoAm epigenetic clock tracks multiple biological processes in a longitudinal sample to estimate acceleration (or deceleration) in organ systems integrity, while most available clocks are based in cross-section comparisons, focusing mostly on predicting chronological age and mortality. The PoAm has been recently contrasted in a sample of children, showing significant associations with several risk factors and biological markers (Raffington et al., 2021).

For comparison, we also calculated the clocks from Horvath (2013), Hannum et al. (2013) and Levine et al. (2018), using the R libraries wateRmelon, limma and minfi (Pidsley et al., 2013). We also estimated competing gestational DNAm clocks (Knight, Lee, Bohlin, Mayne) at birth using the *methylclock* library in R (Pelegí-Sisó et al., 2021).

To examine heterogeneity in the trajectories of internalizing behaviors over time, we conducted a longitudinal latent class analysis of the internalizing symptoms score. Longitudinal latent class models allow to infer multiple subpopulation trajectories from a sample of longitudinal data. For a detailed description of the estimation methods see Marcoulides and Schumacker (2001). The adjusted Bayesian information criteria (BIC) and entropy index were used to determine the number of subgroups (van der Nest et al., 2020).

We estimated mean associations and heterogeneity in cross-section using Poisson regression, as shown to be consistent even when the model is misspecified, providing reliable estimates without imposing additional distribution assumptions (Wooldridge, 1999). Model selection included a wide set of covariates that could be expected to be linked to internalizing behaviors, including family history, household demographics and socio-economic characteristics, child stimulation and diet, and other aspects of birth and early life. We distinguished between baseline covariates determined at or before birth (parental education, gestational age, parity, sex, maternal age at birth, tobacco exposure, and diet quality index), environmental covariates associated with exposure before age 4 (parenting score and breastfeeding), and longitudinal covariates that were measured prior to the SDQ subscale in each period (financial difficulties, income and maternal depression). In our sensitivity analysis we also included polygenic risk scores (PGS) for depression and non-cognitive skills based on previous work by Demange et al., 2021, Turley et al., (2018), and computed as described in Menta et al. (2021). Supplemental Appendix A, Sections C and D contain the description of the covariate selection process and model specification.

Attrition in the sample reporting SDQ questionnaires between ages 4 and 16 was less than 17 %. To account for attrition in the sample over time, we used the Inverse Propensity Weighting (IPW) method, both for latent models and mean associations (Wooldridge, 2007). After estimating the regression parameters, partial effects were calculated at the means of the covariates. Standard errors were estimated using the bootstrap method, with 1000 repetitions.

We estimated quantile partial effects across the distribution of internalizing symptoms for PoAm at birth, age 11 and age 16, using the method proposed by Machado and Silva (2005) to address count data (such as discrete scales). Each discrete point in the distribution is jittered randomly to obtain a continuous cumulative distribution. In each regression, we set the number of jittered samples to 1000 repetitions, and then estimate the partial effects at each given quantile. Similarly, we explored the heterogeneity over time by modelling class membership for each latent trajectory of internalizing behaviors as a function of PoAm at birth, age 7 and age 15, using a multinomial Logit model, after correcting for potential class misclassification (Asparouhov and Muthén, 2014).

A detailed description of the all methods and formulas used in our analyses is available in Supplemental Appendix A, Section D. All statistical analyses were conducted on Stata, version 16 and R v4.1.1. Code scripts are available upon reasonable request.

3. Results

3.1. Latent trajectories on internalizing behaviors

Table 1 summarizes the distribution of internalizing behaviors at every period measured. Mean values and dispersion decrease as children reach adolescence. Fig. 1 shows the results from the estimated trajectories based on longitudinal latent class analysis. The model with four classes was considered the best fit based on adjusted BIC criteria and likelihood ratio tests (fit indices are reported in Supplemental Appendix A, Section D). Over a quarter of all children showed minimal internalizing behaviors over time (low-risk). A similar proportion of children showed increasing internalizing behaviors during childhood and adolescence (late onset). Similarly, one of every four children showed higher values of internalizing symptoms at age 4, but these behaviors decreased sharply in early childhood (limited). Finally, almost 20 % of participants exhibited large and stable values of internalizing behaviors, only decreasing slightly in late adolescence (early onset).

3.2. Epigenetic age

PoAm does not predict biological age in years, but rather provides a continuous measure of acceleration, relative to the overall population of children observed at the same time point. It is important to note, however, that PoAm in each period reflects the population heterogeneity in accumulated DNAm up to the measurement point. A value of one in the PoAm indicates neither age acceleration nor deceleration relative to the same-age peers, with values lower than unity indicating deceleration, and values greater reflecting acceleration (see Table 1). In our subset of the ALSPAC cohort, PoAm was normally distributed for each timepoint, albeit the variance decreased significantly after birth (see Fig. 2). Preliminary analyses showed differential associations between PoAm and internalizing behaviors, across time points, with higher significance, relative to other DNAm clocks (Knight, Lee, Horvath, Hannum and Levine). However, we note that Levine and Hannum clocks provide

Table 1
Descriptive statistics for the estimation sample.

Internalizing behaviors (score 0–20)					
Age	Mean	SD	p10	p90	N
4	2.66	2.95	0	6	913
6	2.23	2.15	0	5	902
8	2.60	2.46	0	6	874
9	2.32	2.40	0	6	909
11	2.32	2.61	0	6	875
13	2.27	2.20	0	5	843
16	2.12	2.23	0	5	753

Dunedin Pace of Aging					
Age	**Mean**	**SD**	**Min**	**Max**	**N**
birth	0.97	0.09	0.52	1.27	906
7	0.99	0.06	0.72	1.23	972
15	0.99	0.07	0.67	1.29	970

Notes: Statistics include all children with observations in each period. SD stands for standard deviation, p10 and p90 indicate percentiles 10 and 90, respectively.

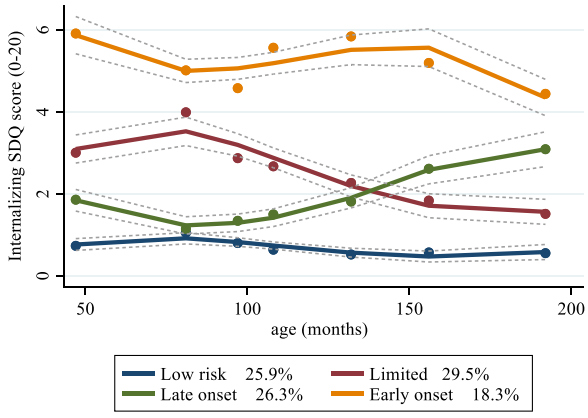

Fig. 1. Trajectories of internalized behaviors based on longitudinal latent class analysis, Notes: Estimated means (dots) and latent classes with longitudinal latent class analysis (with 95% confidence intervals in dashed lines).

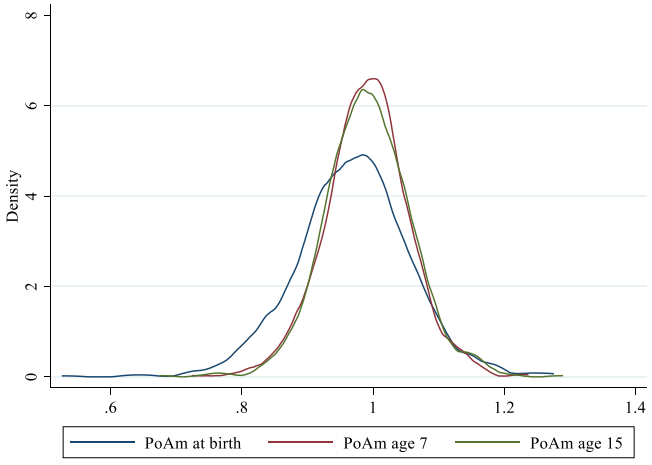

Fig. 2. Estimated kernel densities for PoAm at different ages, Notes: Kernel density estimated for each age at measurement using Epanechnikov method, with bandwidth 0.02.

similar information regarding internalizing behaviors at age 4. Supplemental Appendix A, Section E contains a summary of the comparison across different epigenetic clocks.

3.3. Mean associations between PoAm and internalizing behaviors

We conducted extensive model selection based on the environmental factors that could potentially affect the association between DNAm and internalizing behaviors (see Supplemental Appendix A, Sections C and D). After excluding variables that did not improve model fit, our preferred specification included parity, parental education, gestational age, breastfeeding, home stimulation index at age 3, financial difficulties, income and maternal depression. For more details on the variables used in each regression and the full estimates see Supplemental Appendix B.

Fig. 3 shows the estimated mean partial effects between PoAm and internalizing symptoms, based on the results from the Poisson regressions, with one panel for each measure of PoAm (values reported in Table 2). Since PoAm is standardized, our results show the potential impact of accelerating one SD above the mean on the outcome. Accelerated aging at birth reduced the frequency of internalizing behaviors at age 4, on average by 0.31 units (95 % CI −0.55, −0.12; Table 2). PoAm at birth correlated positively with internalizing behavior scores at age 11 (0.19, 95 % CI −0.02, 0.40; Table 2). At age 7, there was no association

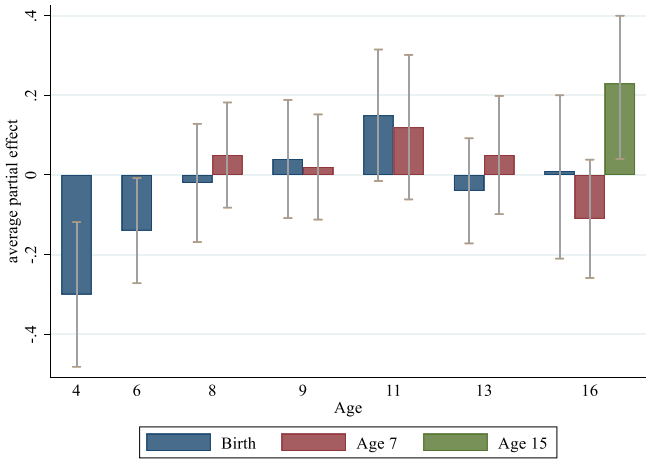

Fig. 3. Estimated partial effects of PoAm on internalizing behaviors, Notes: Estimated partial effects based on Poisson models adjusted by parental education, gestational age, mother's age at birth, parity, gender, diet score during pregnancy, breastfeeding, tobacco exposure at birth, home stimulation index at age 3, income, financial difficulties, and maternal depression. 90 % bootstrap confidence intervals with 1000 repetitions.

between PoAm and internalizing behaviors. Finally, accelerated aging up to age 15 positively correlated with internalizing behavior scores at age 16 (0.22, 95 % CI 0.04, 0.40; Table 2). We conducted extensive sensitivity analysis to validate the robustness of our results (details available in Supplemental Appendix A, Section G). Our estimates remained robust to model selection over a wide range of covariates, and the inclusion of polygenic risks scores as controls.

3.4. Heterogeneity analysis

Fig. 4 shows heterogeneity based on the severity of internalizing behaviors, by estimating the quantile partial effects for PoAm at different deciles of the cumulative distribution function. A low quantile indicates smaller scores of internalizing behaviors, while a high quantile refers to higher scores. In all cases (ages 4, 11 or 16), effect sizes are small and non-significant for children with few or no internalizing behaviors. For those individuals at the top half of the distribution (thus higher values of internalizing symptoms), effects sizes can be almost double, compared to the average partial effects. Additional analyses using logistic regression showed that PoAm did not predict the likelihood of having zero versus any degree of internalizing behaviors (incidence), but rather symptom intensity, which is consistent with the quantile analysis.

Table 3 shows the predicted change in the probability (percent points) to belong to each latent trajectory of internalizing behaviors due to an increase of PoAm of one SD, as well as the relative risk ratios, using the low-risk group as reference. At birth, higher PoAm increases the probability to be classified as low-risk (0.03, 95 % CI 0.00, 0.06), while decreasing the probability to be in the limited group (−0.05, 95 % CI −0.08, −0.02). Higher PoAm, however, does not change the odds to be assigned to the early onset class, relative to the low-risk group. Using the PoAm at age 7, there was no correlation between AA and class membership. At age 15, age accelerated children were more likely to be in the early onset group (0.02, 95 % CI 0.00, 0.05), but less likely to belong to the limited group (−0.03, 95 % CI −0.06, −0.01). Results of the multinomial logistic regressions are included in Supplemental Appendix A, Section H.

4. Discussion

Our results, based on prospective analysis of the ALSPAC cohort are consistent with a dynamic and complex longitudinal relationship

Table 2
Average partial effects of PoAm on internalizing behaviors.

	PoAm at birth			PoAm age 7			PoAm age 15		
Age	Partial effect	95 % CI	p-value	Partial effect	95 % CI	p-value	Partial effect	95 % CI	p-value
4	-0.31	[−0.55 −0.12]	*0.02*						
6	-0.16	[−0.35 0.04]	*0.11*						
8	-0.07	[−0.26 0.12]	*0.48*	0.05	[−0.11 0.21]	*0.54*			
9	0.01	[−0.20 0.21]	*0.95*	0.02	[−0.17 0.21]	*0.81*			
11	0.19	[−0.02 0.40]	*0.07*	0.12	[−0.11 0.34]	*0.33*			
13	-0.03	[−0.19 0.14]	*0.76*	0.05	[−0.14 0.24]	*0.59*			
16	0.01	[−0.20 0.21]	*0.95*	0.11	[−0.30 0.07]	*0.24*	0.22	[0.04 0.40]	*0.02*

Notes: Poisson models adjusted by parental education, gestational age, mother's age at birth, parity, gender, diet score during pregnancy, breastfeeding, tobacco exposure at birth, home stimulation index at age 3, income, financial difficulties, and maternal depression. Bootstrap confidence intervals with 1000 repetitions. P-values corrected for multiple hypothesis testing with the Sidak method.

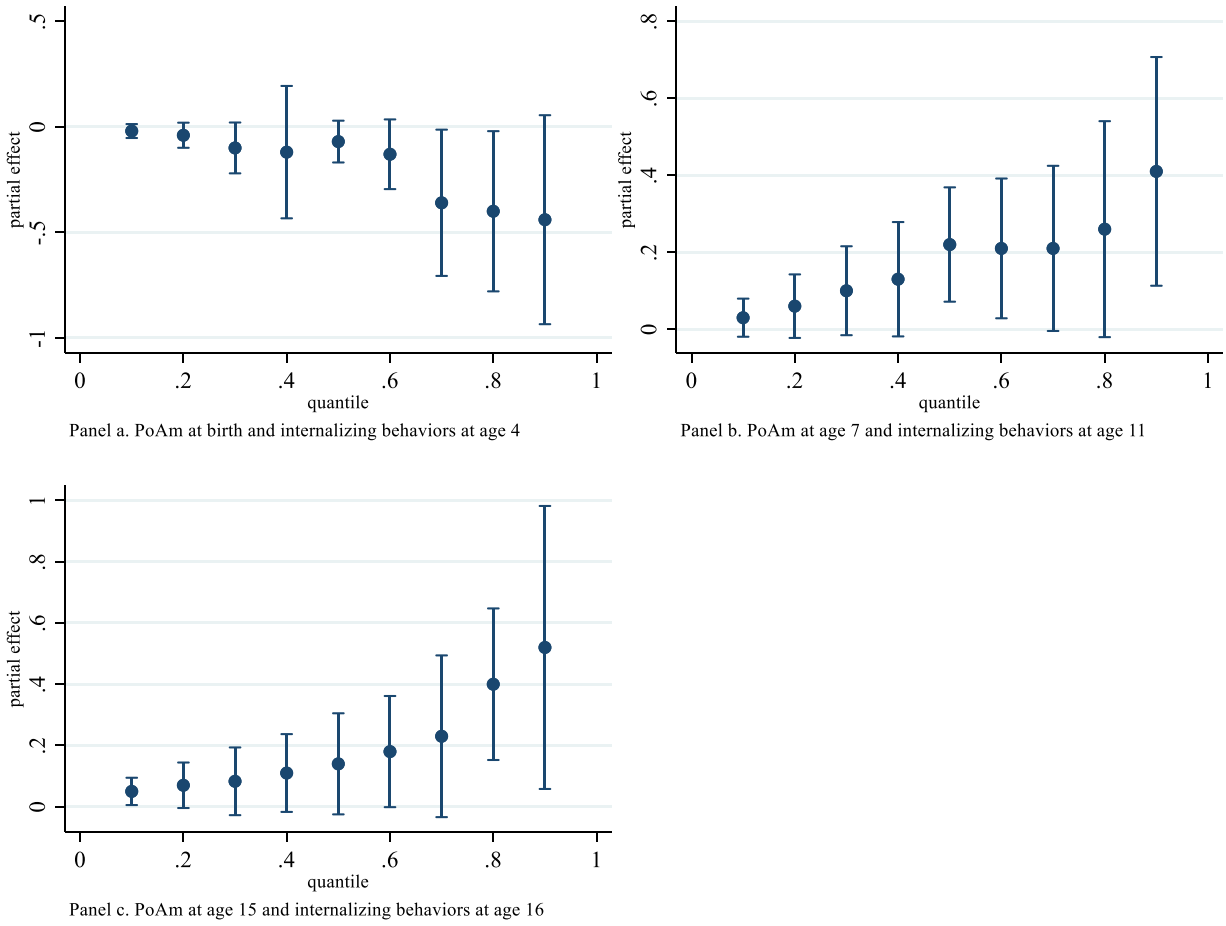

Fig. 4. Quantile partial effects, Notes: Estimated partial effects at each decile calculated with the method proposed by Machando and Santos Silva (2005), using same covariates as in Table 2. PoAm at birth and internalizing behaviors at age 4 (panel a), PoAm at age 7 and internalizing behaviors at age 11 (panel b), and PoAm at age 15 and internalizing behaviors at age 16 (panel c). 90 % bootstrap confidence intervals with 1000 repetitions.

between the biologically-based PoAm marker of epigenetic age acceleration and the development of internalizing behaviors. We found that AA at birth is associated with lower internalizing symptoms in early years for some children, while potentially higher internalizing behaviors at the beginning of adolescence, for others. AA at the end of adolescence was significantly correlated with internalizing symptoms at late adolescence (age 15–17).

In line with previous work (Barker and Maughan, 2009), we found different developmental trajectories of internalizing behaviors across individuals. A large proportion of children showed either persistent absence or presence of internalizing symptoms, which were associated with AA both at birth and during late adolescence. There was also a sizeable proportion of children who showed either decreasing or increasing internalizing symptoms between early childhood and late adolescence.

Our estimates of the associations with PoAm at birth most likely reflect heterogeneity on environmental (adverse or favorable) factors during fetal development and birth. Therefore, while PoAm was not trained on birth data, it still indexes a measure of epigenetic gestational age (GA) acceleration at birth. Similar epigenetic GA clocks have shown to be highly correlated with other markers of biological (e.g., ultrasound imaging) and chronological gestational age (Knight et al., 2016; Suarez et al., 2018). In the ALSPAC sample, the correlation between PoAm at birth and estimated gestational period is 0.13 (comparative results of

Table 3
Estimated partial effects and relative risk ratios of PoAm on class membership (relative to low-risk group).

	PoAm at birth			PoAm age 7			PoAm age 15		
Class	Partial effect	95 % CI	p-value	Partial effect	95 % CI	p-value	Partial effect	95 % CI	p-value
Low risk	0.03	[0.00 0.06]	*0.05*	0.00	[−0.03 0.03]	*0.88*	-0.02	[−0.05 0.01]	*0.26*
Late onset	-0.05	[−0.08 − 0.02]	*0.01*	0.01	[−0.02 0.04]	*0.60*	-0.03	[−0.06 − 0.01]	*0.03*
Limited	-0.01	[−0.03 0.02]	*0.77*	-0.01	[−0.04 0.02]	*0.46*	0.03	[0.01 0.06]	*0.02*
Early onset	0.02	[−0.01 0.05]	*0.20*	0.00	[−0.01 0.04]	*0.99*	0.02	[−0.01 0.04]	*0.16*
	PoAm at birth			PoAm age 7			PoAm age 15		
Class	RRR	95 % CI	p-value	RRR	95 % CI	p-value	RRR	95 % CI	p-value
Late onset	0.75	[0.61 0.91]	*0.01*	1.02	[0.87 1.24]	*0.83*	0.95	[0.78 1.15]	*0.60*
Limited	0.87	[0.71 1.05]	*0.16*	0.95	[0.91 1.33]	*0.59*	1.24	[1.02 1.49]	*0.03*
Early onset	0.98	[0.76 1.25]	*0.88*	0.99	[0.79 1.23]	*0.95*	1.18	[0.96 1.46]	*0.12*

Notes: Multinomial models adjusted by parental education, gestational age, mother's age at birth, parity, gender, diet score during pregnancy, breastfeeding, tobacco exposure at birth, home stimulation index at age 3, income, financial difficulties, and maternal depression. Bootstrap confidence intervals with 1000 repetitions. P-values corrected for multiple hypothesis testing with the Sidak method.

PoAm and other GA epigenetic clocks available in Supplemental Appendix A, Section E). Results suggest that for some children with high AA at birth, the likelihood to develop internalizing symptoms during early childhood is significantly lower. This potential early advantage at birth seems to become less important as children grow up, potentially due to the accumulation of diverse experiences during childhood and adolescence. Moreover, PoAm at birth is also associated with lower probability to have a trajectory of decreasing symptoms as children grow up. In other words, for some children, low AA at birth increases the chances to mitigate initial risk of internalizing behaviors during later development. In addition, we note that accelerated GA potentially plays a larger role in preventing internalizing behaviors in children with higher intensity of symptoms, consistent with previous literature connecting mental health risk factors and DNA methylation in children (Starnawska et al., 2017; Szyf and Bick, 2013).

Epigenetic aging during early childhood (up to age 7 in the ALSPAC cohort) might not track long-term environmental incidents as they relate to internalizing symptoms beyond puberty. Recent evidence suggests that during the first five years of life, over 100,000 CpG sites show within-individual variation or re-modelling, while less than 460 CpG sites show changes in methylation between ages 5 and 10 years old (Pérez et al., 2019). Only 220 CpG sites exhibit changes in DNA methylation in the 5–10 years period but no changes between birth and age 5. During this first period of rapid growth, it is possible that changes in PoAm during early childhood might only weakly reflect potential mediation between environmental factors in early years and mental health during middle childhood and adolescence.

Finally, we found that the accumulated DNA methylation changes tracked by the PoAm algorithm up to late adolescence are strongly associated with the trajectories of increasing internalizing symptoms up to age 16, particularly for individuals developing symptoms during adolescence. Our results are consistent with recent evidence on the association between epigenetic aging and brain structures in adolescents from low-income families, suggesting a connection between AA, neurocognitive impairment and the emergence of internalizing symptoms (Blanken et al., 2017; Hoare et al., 2020). Moreover, several studies have identified links from stress and adversity in early years to DNA methylation and mental health in adolescence (Barker, Cecil et al., 2018; Cam et al., 2017; Essex et al., 2013; Suarez et al., 2018; Sumner et al., 2019). Given the observational nature of our study and data availability, we cannot determine whether these associations reflect contextual or cumulative effects of internalizing symptoms on DNA methylation, or concurrent changes in DNA methylation due to environmental factors.

Taken together, our results suggest that epigenetic age acceleration at birth correlates with trajectories of low (but not decreasing) internalizing behaviors during early life. In turn, trajectories of increasing internalizing symptoms were associated with increased AA at adolescence. In contrast, children with decreasing internalizing behavior profiles were more likely to show epigenetic age deceleration at ages 15–17. Unfortunately, while evidence suggests the existence of two critical periods for concurrent changes in emotional development and DNAm aging, it is not possible to test potential causality or bidirectionality in these associations without exogenous changes in adverse events that can uniquely lead to higher internalizing behaviors alone (Barker, Walton et al., 2018).

4.1. Strengths and limitations

To our knowledge, this is the first study to unveil heterogeneous longitudinal and cross-sectional associations between biological AA and internalizing behaviors, from birth to adolescence. Moreover, unlike previous studies, we rely on multiple measures of both internalizing behaviors and DNA methylation, thus being able to construct dynamic profiles over childhood and adolescence. We conducted multiple sensitivity analyses that provide confidence in the robustness of the estimated associations. Still, our work has several limitations, particularly due to the small sample size. The SDQ sub-scale of internalizing behaviors is ordinal by design, which violates the basic assumptions of traditional statistical methods. In addition, attrition is a serious threat to internal validity. To overcome these challenges, we used statistical approaches that allowed us to maintain the data on its natural scale, avoiding additional assumptions. To address attrition and the small sample size, we balanced the sample using propensity score weights and bootstrapped standard errors whenever possible. We acknowledge that we are not able to replicate the results in this analysis in an independent sample at this time, due to the unique characteristics of the ALSPAC cohort. Moreover, the ALSPAC cohort is representative of a predominantly White and high-income population, thus findings should be extrapolated in context.

5. Conclusions

We present a novel analysis of heterogeneity on the longitudinal associations between biological accelerated aging and internalizing behaviors from birth to adolescence, relying on multiple measures of DNA methylation over time. Our results suggest that accumulated DNA methylation due to adverse experiences in utero and during adolescence have divergent effects on the development of internalizing behaviors, in a representative high-income population. Accelerated aging during adolescence significantly correlates with higher internalizing symptoms in the same period, suggesting common pathways between environmental factors, biological aging and mental health at this age.

From a policy perspective, while DNA methylation markers at birth are useful to determine gestational age, they could also help to identify children that hold the potential to mitigate early developmental risks during adolescence. In a similar vein, prevention strategies to mitigate

the risk of chronic internalizing behaviors during late childhood and adolescence could lead to a slower pace of biological aging in adulthood (Brody et al., 2016; Miller et al., 2015; Richmond-Rakerd et al., 2021).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationshipsthat could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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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.biopsycho.2022.108463.

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