ANALYSIS OF CHILDHOOD TRAUMA AND EPIGENETIC AGE ACCELERATION SHOWED NO APPARENT LINK OR SEX SPECIFIC EFFECTS IN ADOLESCENTS

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Research indicates that adverse childhood events are associated with accelerated biological aging, which is linked to negative effects on mortality and an increased susceptibility to psychiatric diseases. Additionally, differences in pace of biological aging between men and women have been reported. A widely used measure for biological age is epigenetic age, for which so-called epigenetic clocks are developed. We analyzed a dataset of 500 adolescents and assessed childhood trauma using the Childhood Trauma Questionnaire. Epigenetic age acceleration was calculated using five different commonly used epigenetic clocks. Our results revealed no significant associations between childhood trauma and epigenetic age acceleration, and no sex specific effect. Moreover, we examined the role of genetic risk for childhood trauma on epigenetic age acceleration. Similarly, this analysis yielded no significant results or detect any notable sex specific effects. These findings suggest that there may not be a significant correlation between childhood trauma and epigenetic age acceleration in adolescents that experienced moderate childhood trauma exposure. This paper also highlights the need to incorporate larger sample sizes and consider potential sex-specific effects to comprehend these interactions and their implications for overall health and mortality.

I. INTRODUCTION

Childhood trauma, characterized by experiences of neglect, abuse or maltreatment during young age and adolescence, is associated with poorer health outcomes in later life. Several studies showed that adverse childhood events increase the risk for psychiatric and late onset diseases[1–3]. Moreover, the effects of such trauma extend to the molecular level, where we find a relation with epigenetic age – a metric based on DNA methylation patterns[4,5].

DNA methylation plays an important role in the regulation of gene expression, impacting cellular processes that underpin the development of various health conditions. This epigenetic alteration involves the addition or removal of a methyl group to the cytosine base in DNA, often occurring at specific sites known as CpG sites. The methylation state of CpG sites is influenced by factors such as genetics, biological sex and age, and environmental variables, including childhood experiences[6–10]. These epigenetic signatures lay the foundation for a fascinating biomarker in the field of aging: epigenetic clocks.

Epigenetic clocks are algorithms that estimate an individual's biological age based on DNA methylation patterns[11,12]. As these patterns change over time, researchers have leveraged them to train algorithms that predict an individual's age. This calculated epigenetic age can sometimes deviate significantly from the person's chronological age, indicating potential disparities between the pace of biological aging and the passage of time. The concept of epigenetic clocks is proposed as potential marker of overall health and aging[13–16].

Childhood trauma, therefore, has far-reaching consequences, affecting not only psychological well-being but also leaving a lasting impact on the molecular mechanisms that underlie the aging process. A well validated and widely used method to quantify childhood trauma is the Childhood Trauma Questionnaire (CTQ), a 70-item self-administered inventory on a 5-point Likert scale from 1 (never true) to 5 (very often true)[17]. To reduce burden on the participants, the developers created a shortened version with 28 items from the questionnaire, which allows for a more rapid screening of no more than 5 minutes[18]. This shortened CTQ provides an accurate measure for overall childhood trauma and subtypes (emotional abuse, emotional neglect, physical abuse, physical neglect, and sexual abuse)[19,20]. Relatedly, childhood maltreatment has been found to be modestly heritable, exhibiting an explained phenotypic variation of approximately 8%, which aligns with the SNP heritability levels seen in depression[21,22]. Employing genetic risk for childhood trauma as a determinant enables us to assess the role of an individual's genetic predisposition in their risk for childhood trauma on epigenetic age. By examining the relation between genetic susceptibility and epigenetic changes, we can gain a better understanding of how genetic factors may contribute to the acceleration or deceleration of the aging process in in the context of early life experiences. Additionally, recent studies show that the links between trauma and epigenetic age might be moderated by sex, underscoring the need to include biological sex into the analyses[23,24].

Understanding the complexities of these interactions is crucial for gaining knowledge on the adverse effects of childhood trauma, develop interventions to minimize the risk of childhood trauma, and the possible impact of epigenetic aging for psychiatric care. In this paper, we investigate the interplay between childhood trauma, biological sex, and genetic influences on epigenetic age.

II. METHODS

Participants

The participants were all from the longitudinal Research on Adolescent Development And Relationship (RADAR) study as previously described[25]. The dataset comprised 497 participants from the RADAR Young cohort and 244 participants from the RADAR Old cohort. In both cohorts, participants are 12 years old at the start

of the study, with the first wave being in 2001 and 2005 for RADAR Old and RADAR Young respectively. Self-report assessments of participants are included in the study. The study involved two moments of saliva sampling for genotyping: the first one in 2009 (T1), exclusively involving participants from the Young cohort; and the subsequent batch in 2019 (T2), where participants from both the Young and Old cohorts were sampled. Participants in both cohorts were recruited in the Netherlands. For more details on the participants and the timeline of the study, see Figure 1.

Childhood trauma

Childhood trauma was assessed using the Childhood Trauma Questionnaire (CTQ)[17] of which validity of the clinical items (including Dutch translation) has been demonstrated[18,20]. This Questionnaire is a self-report scale with 28 items including emotional neglect, physical neglect, emotional abuse, physical abuse, and sexual abuse. Responses from participants range from 1 (never true) to 5 (true very often) on each item. We used the mean of the total score to assess overall trauma exposure.

DNA Methylation and Epigenetic Age Estimation

DNA was derived from saliva samples using the Oragene DNA kit (DNA Genotek, Ottawa, ON, Canada) followed by bisulfite conversion using ZIMO kits. DNA methylation was measured at two time points, specifically in 2012 (T1) and 2020 (T2), utilizing the Illumina 450K and Illumina EPIC bead chips (Illumina, San Diego, CA) respectively. Using the *minfi*[26] and *meffil*[27] packages in R version 4.2[28] quality control was performed on the DNA methylation data. Initial quality control steps for samples included filtering out samples with mean detection p value > 0.01 and instances of discordance between reported and calculated sex. As suggested by Calen P. Ryan[12], no further quality control was done on the dataset before calculating epigenetic age. The following quality control steps were applied for future downstream analyses of the dataset. Quantile normalization was done and beads failing one or more quality control criteria were removed. These criteria included a detection p value > 0.01, located on the X or Y chromosome, known genetic variation, and susceptibility to cross-reactivity on both the 450K bead chips[29] and EPIC array chips[30]. Additionally, cell-type composition values for saliva were estimated using saliva gse48472 reference from the *meffil* package. Surrogate variable analysis by *meffil* calculated factors contributing to variation. These factors were compared to possible covariates in order to identify possible confounders. To calculate epigenetic age of the participants, we employed principal component (PC) adjusted algorithms, which have been demonstrated to improve their performance[31]. The analysis involved the examination of five different PC-corrected epigenetic clocks from the Morgan Levine Lab (https://github.com/MorganLevineLab/PC-Clocks), namely Horvath1, Horvath2, Hannum, PhenoAge and GrimAge. Each of these clocks relies on different CpG sites – and therefore representing a distinct measure of epigenetic aging. A commonly used measure for evaluating epigenetic age is epigenetic age acceleration (EAA), which was obtained from the residuals of regressing epigenetic age against chronological age. While calculating EAA, we adjusted for cell type proportions (Bcell, Buccal, CD4T, CD8T, Gran, Mono).

Genotype

Additionally, we assessed the role of genetic risk for childhood trauma on epigenetic age acceleration. We used genetic data of the RADAR participants, from the datasets on different timepoints of saliva sampling, using the Affymetrix SNP Array and Infinium SNP Array for

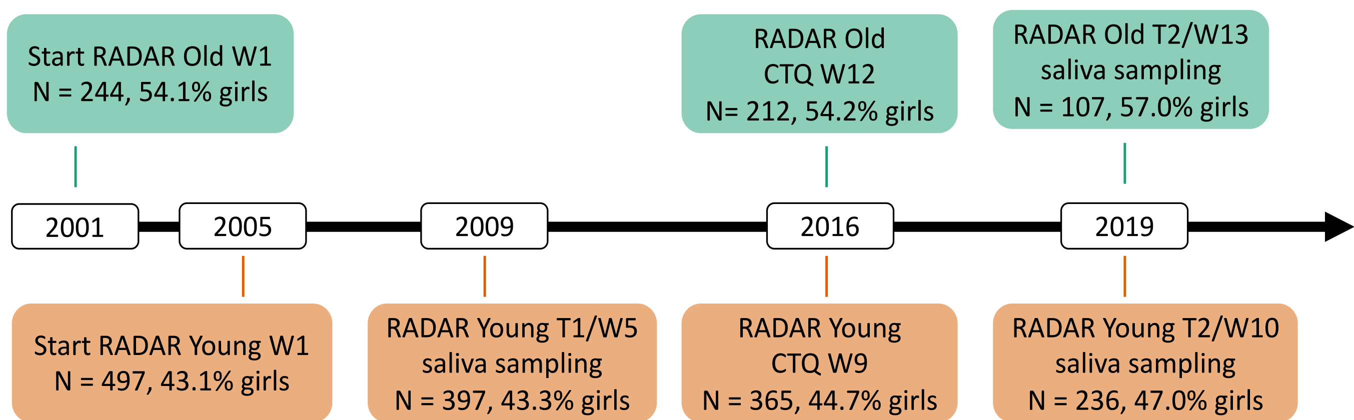

Figure 1: ***Timeline of the Research on Adolescent Development And Relationship (RADAR) study.*** *The figure depicts the study's timeline, including the initiation of the study for both RADAR Old and RADAR Young cohorts, the moments of saliva sampling for genotyping (T1 and T2), and the administration of the Childhood Trauma Questionnaire (CTQ)*

T1 and T2 respectively. Genetic data from T1 and T2 was cleaned separately in several quality control (QC) steps using the PLINK software version 1.9[32,33]. An initial QC removed low quality variants, setting thresholds at a call rate of over 95%, a minor allele frequency exceeding 99%, and a Hardy-Weinberg equilibrium p-value greater than 1e-5. Initial sample QC included assessment of comparing reported and estimated sex, maintaining a call rate above 95%, keeping samples within a range of ±3 standard deviations from the mean heterozygosity, removing related samples through identity by descent (IBD) with a threshold of 0.1, and identifying and excluding ethnic outliers based on visual inspection. Additionally, related samples based on IBD between T1 and T2 were removed. Genotype data was imputed through the TOPMed Imputation Server[34] using the TOPMed r2 reference panel (1.0.0). Principal components were computed using the *plink* software to assess batch effects. Lastly, polygenic risk scores (PRS) were computed by *PRSice* version 2.3.5[35] based on summary statistics from a genome-wide association study (N=185 414) on childhood maltreatment by Warrier et al[22].

Statistical analysis

Our primary aim was to explore how childhood trauma, biological sex, and genetic risk for childhood trauma impact epigenetic age. To achieve this, we utilized multiple linear regression models. Model 1 considered the influence of CTQ value and sex with EAA as outcome (EAA ~ CTQ + Sex). Model 2 assessed the relationship between EAA and PRS for childhood trauma, while also accounting for sex (EAA ~ PRS_CT + Sex). Statistical analyses were performed using R version 4.2[28].

III. RESULTS

Preliminary data analysis

A total of 788 samples passed DNA methylation quality control. Chronological age is crucial for calculating epigenetic age acceleration, so we excluded samples without reported age which resulted in 722 remaining samples. As our study design is cross-sectional, participants with repeated measures were only included once. Many participants from the Young cohort were younger than 18 at timepoint 1, and since the CTQ refers to experiences up to this age, we prioritized data from timepoint 2 when available. The final dataset contains 511 samples from both the Young (N=408) and Old (N=103) cohort. Values from the childhood trauma questionnaire (CTQ) range between 1 and 2.76 (Table 1). With a standard deviation of 0.03, our cohort has a moderate spread of polygenic risk for childhood trauma (CT). Our dataset is almost evenly divided by biological sex (46.8% girls, 53.2% boys).

Table 1: ***Dataset summary:*** *This table provides a description of the final dataset. It presents statistics for CT Questionnaire, polygenic risk scores (PRS) related to childhood trauma, and the sex distribution within the cohort.*

Variables	Total (N=511)				
	N	Mean	SD	Min	Max
CT Questionnaire	449	1.33	0.31	1.00	2.76
PRS CT	446	0.13	0.03	0.01	0.19
Sex					
Girls	239	46.77%			
Boys	272	53.22%			

CT Childhood Trauma, *PRS* Polygenic Risk Score

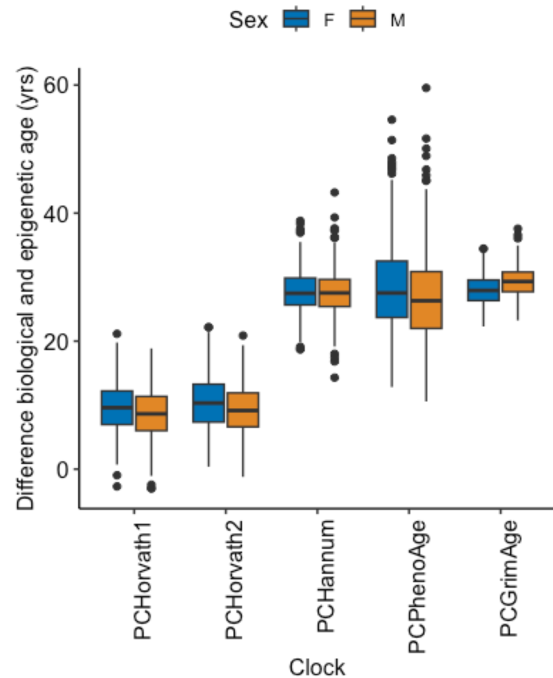

Figure 2: ***Age discrepancy between biological and epigenetic age per clock****. Almost all epigenetic ages are overestimated, indicated by a positive difference. PCHorvath1 and PCHorvath2 show the least discrepancies.*

Epigenetic age analysis

We investigated epigenetic age using the principal component (PC) corrected clocks for Horvath1, Horvath2, Hannum, PhenoAge and GrimAge. Within our study, age discrepancies varied considerably across different clocks (Figure 2). Epigenetic ages seem to be overestimated in all clocks. However, it is worth highlighting that both Horvath epigenetic clocks stand out with the least deviation, and therefore providing the most accuracy in predicting chronological age. These variations in age estimation directly impact the calculation of epigenetic age acceleration (EAA). Discrepancies, whether positive or negative, result in

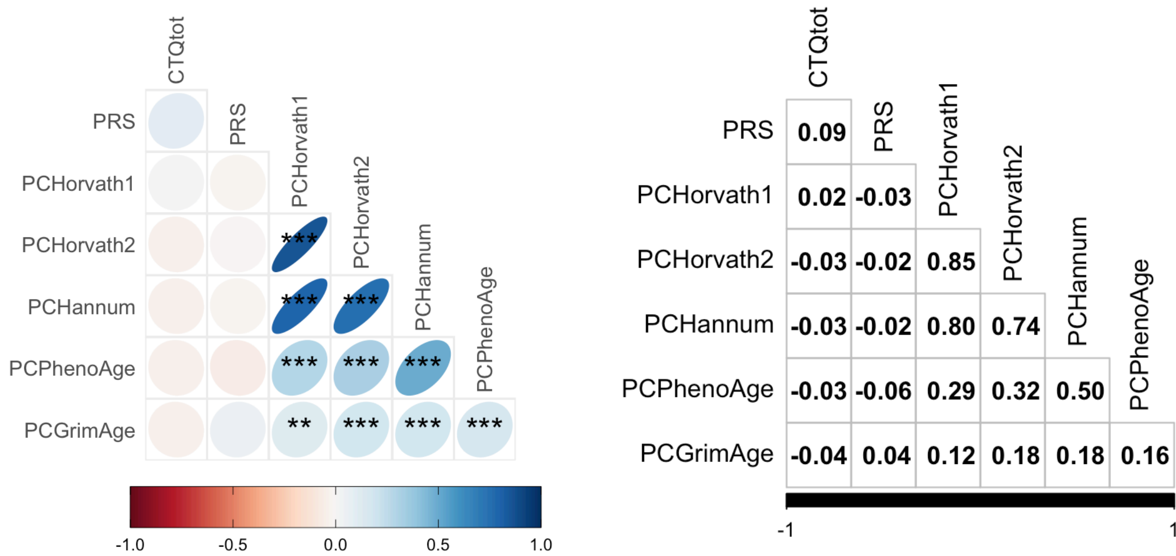

Figure 3: ***Pearson correlation between CTQ value, PRS for childhood trauma and EAA for the different clocks.*** *Left: Correlation indicated by color. Significant correlations are indicated with one or more asterisks where ***: p < 0.001, **: p < 0.01, *: p < 0.05. Right: Correlation values range between -1 and 1.*

increased or decreased EAA values. To compute EAA, we regressed EA on chronological age and corrected for cell-type composition, which included Bcell, Buccal, CD4T, CD8T, Gran, and Mono. The residuals from these linear models were used to quantify EAA for each clock (Table 2).

Among the clocks, EAA values were consistently centered around 0, with standard deviations ranging from 1.69 to 2.84. The most variation is seen in PhenoAge while GrimAge showed the least deviation. As shown in Figure 3, EAA for both Horvath clocks are positively correlated with CTQ and PRS for CT, while the other 3 clocks (PCHannum, PCPhenoAge and PCGrimAge) correlate negatively with these variables.

Table 2: ***Epigenetic Age Acceleration Summary.*** *This table provides a comprehensive summary of age acceleration metrics, including mean values, standard deviations, minimum and maximum values, for the PC-corrected epigenetic clocks. Epigenetic age acceleration is adjusted for cell type compositions (EA ~ Chronological age + Bcell + Buccal + CD4T + CD8T + Gran +Mono)*

Variables	Total (N=511)				
	N	Mean	SD	Min	Max
PCHorvath1	511	0.00	2.81	-7.24	9.13
PCHorvath2	511	0.00	2.84	-10.04	11.47
PCHannum	511	0.00	2.22	-7.28	7.92
PCGrimage	511	0.00	1.69	-4.71	6.22
PCPhenoAge	511	0.00	2.59	-8.52	9.84

Association Between Traumatic Childhood Experiences and Epigenetic Age Acceleration

By using multiple linear regression analysis, we examined the associations between childhood trauma, as assessed by the Childhood Trauma Questionnaire (CTQ), and epigenetic age acceleration (EAA) across various epigenetic clocks (Table 3). For examining the validity of the model, we examined the residuals plot where we confirmed linearity and variance of errors (Supplementary Figure 4). Additionally, the QQ plots showed normality of the errors by following the expected diagonal line.

Although not statistically significant, the results revealed distinct associations between CTQ value and EAA. The analysis showed a positive association of EAA calculated by the PCHorvath1 clock and CTQ (Beta = 0.196 ± 0.443, P = 0.647), indicating that higher CTQ scores were linked to increased EAA. Conversely, PCHorvath2 (Beta = -0.289 ± 0.421, P = 0.421), PCHannum (Beta = -0.242 ± 0.337, P= 0.337), PCPhenoAge (Beta = -0.255 ± 0.366, P = 0.366) and PCGrimAge (Beta = -0.216 ± 0.235, P = 0.235) all displayed negative associations with CTQ, indicating that higher CTQ scores were linked with decreased EAA. Additionally, sex showed to be significantly associated with EAA for PCHorvath2, PCPhenoAge and PCGrimAge.

Polygenic Risk Score for Childhood Trauma (PRS CT) as predictor of Epigenetic Age Acceleration

Given the genetic basis of childhood trauma, we

Table 3: Associations between Childhood Trauma (CT) and Epigenetic Age Acceleration (EAA). Results of multiple linear regression analysis across various epigenetic clocks.

Model: EAA ~ CTQ + Sex

EAA	Beta ± SD (P)	Beta ± SD (P)	R adjusted
	CTQ	SexM	Model
PCHorvath1	0.196 ± 0.443 (0.647)	-0.326 ± 0.265 (0.22)	-0.001
PCHorvath2	-0.289 ± 0.421 (0.493)	-0.745 ± 0.262 (0.005)	0.015
PCHannum	-0.242 ± 0.337 (0.472)	-0.368 ± 0.209 (0.08)	0.004
PCPhenoAge	-0.255 ± 0.366 (0.487)	-1.674 ± 0.228 (9.72e-13)	0.105
PCGrimAge	-0.216 ± 0.235 (0.359)	1.211 ± 0.146 (1.407e-15)	0.131

EEA Epigenetic Age Acceleration*, CTQ* Childhood Trauma Questionnaire, *SD* Standard Deviation

Table 4: Associations between Polygenic Risk Score (PRS) for Childhood Trauma (CT) and Epigenetic Age Acceleration (EAA). Results of multiple linear regression analysis across various epigenetic clocks.

Model: EAA ~ PRS_CT + Sex

EAA	Beta ± SD (P)	Beta ± SD (P)	R adjusted
	PRS_CT	SexM	Model
PCHorvath1	-2.258 ± 4.195 (0.591)	-0.064 ± 0.26 (0.806)	-0.004
PCHorvath2	-0.863 ± 4.189 (0.837)	-0.441 ± 0.26 (0.091)	0.002
PCHannum	-1.182 ± 3.322 (0.722)	-0.277 ± 0.206 (0.181)	0.000
PCPhenoAge	-2.77 ± 3.626 (0.445)	-1.728 ± 0.225 (1.073e-13)	0.116
PCGrimAge	1.119 ± 2.486 (0.653)	1.085 ± 0.154 (7.927e-12)	0.098

EEA Epigenetic Age Acceleration*, PRS* Polygenic Risk Score, *SD* Standard Deviation

assessed the predictive capacity of the polygenic risk scores (PRS) for childhood trauma (CT) on epigenetic age acceleration (EAA). To obtain the PRS for CT, we used the best-fit PRS as calculated by *PRSice*. This best-fit PRS contained the top 7 Single Nucleotide Polymorphisms (SNPs) from the CT study, which included the genome wide significant SNPs (P = 5e-8). The model we used regressed EAA on the CT PRS across the different epigenetic clocks while taking biological sex into account (Table 4). These regression models also showed variance, linearity, and normality of errors, as evidenced by the regression and QQ plots (Supplementary Figure 5).

The results revealed both positive and negative associations, albeit not statistically significant. Most clocks show a negative beta coefficient suggesting a potential role of genetic predisposition to childhood trauma in reducing EAA while the positive beta value for PCGrimAge (Beta = 1.119 ± 2.486, P = 0.653) value suggests an increased EAA in this relation. Similar to the model on CTQ, we find significant associations between EAA and sex for PCPhenoAge and PCGrimAge.

Sex differences

We performed a sex-specific analysis of the relationship between epigenetic age acceleration (EAA) and both childhood trauma (CTQ) and polygenic risk scores for childhood trauma (PRS CT) across different epigenetic clocks (Table 5). Figure 4 shows scatterplots for CTQ and EAA in the top row and PRS and EAA in the bottom row, with separate regression lines for boys and girls. Although not significant, the results demonstrated varying associations between EAA and CTQ or PRS CT for different clocks, with some distinctions. In the context of CTQ, the clocks PCHorvath1 and PCHannum showed inconsistent results for boys and girls, with differing beta coefficients suggesting potential sex-specific effects. Conversely, the clocks PCHorvath2, PCPhenoAge, and PCGrimAge displayed consistent patterns for both sexes in relation to CTQ. These inconsistencies were also visible regarding PRS CT. We saw variability in the beta coefficients for boys and girls in the PCHorvath1, PCHorvath2, PCHannum and PCGrimage clocks while PCPhenoAge demonstrated consistent associations for both sexes. Additionally, we tested sex as interaction term. None of the interactions between sex and CTQ or PRS CT significantly influenced the associations.

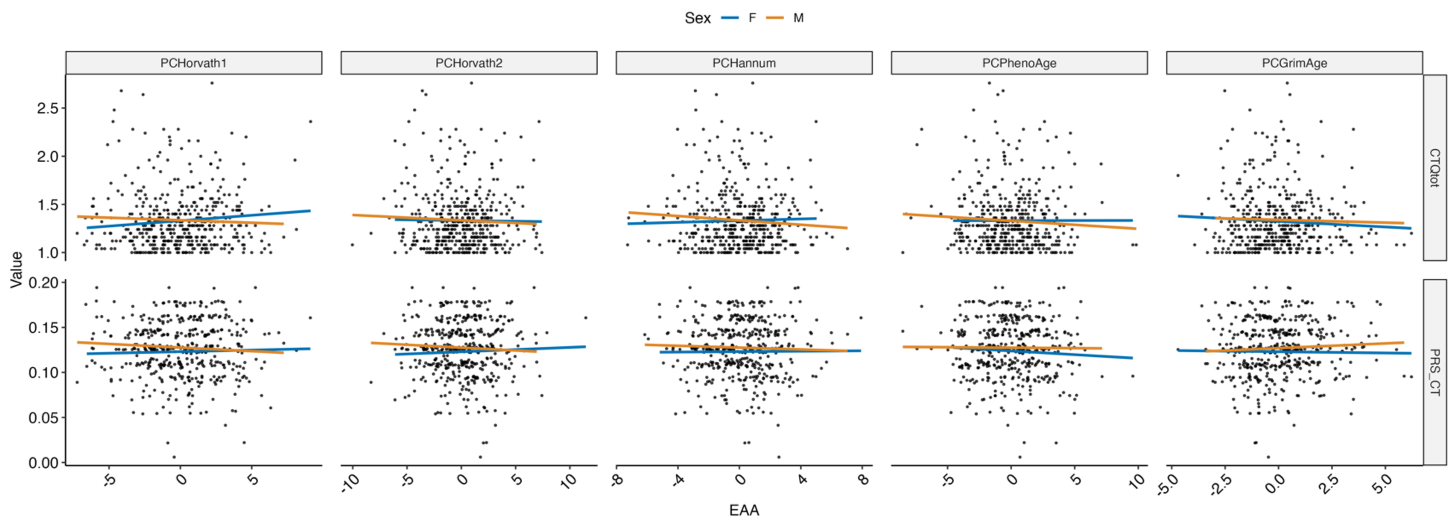

Figure 4: ***Scatterplots illustrating the associations between Childhood Trauma Questionnaire (CTQ) and epigenetic age acceleration (EAA) in the top row and between Polygenic Risk Score for Childhood Trauma (PRS CT) and EAA in the bottom row****. Each grid includes regression lines for boys and girls, showing the variations in these relationships across different epigenetic clocks.*

Table 5: Analysis of EAA for CTQ and PRS CT stratified by sex.

Model: EAA ~ CTQ / EAA ~ PRS_CT			
EAA	Beta ± SD (P)	Beta ± SD (P)	Beta ± SD (P)
	Boys	Girls	Sex interaction (boys)
PCHorvath1			
CTQ	-0.49 ± 0.63 (0.44)	0.80 ± 0.57 (0.17)	-1.29 ± 0.85 (0.13)
PRS CT	-7.14 ± 6.17 (0.25)	2.37 ± 5.68 (0.68)	-9.52 ± 8.39 (0.26)
PCHorvath2			
CTQ	-0.50 ± 0.63 (0.42)	-0.10 ± 0.57 (0.86)	-0.40 ± 0.84 (0.64)
PRS CT	-5.41 ± 6.04 (0.37)	3.45 ± 5.81 (0.55)	-8.86 ± 8.38 (0.29)
PCHannum			
CTQ	-0.71 ± 0.52 (0.17)	0.17 ± 0.43 (0.69)	-0.89 ± 0.68 (0.19)
PRS CT	-2.94 ± 4.95 (0.55)	0.49 ± 4.43 (0.91)	-3.43 ± 6.65 (0.61)
PCPhenoAge			
CTQ	-0.55 ± 0.54 (0.31)	0.01 ± 0.50 (0.99)	-0.56 ± 0.73 (0.45)
PRS CT	-0.60 ± 4.99 (0.9)	-4.82 ± 5.29 (0.36)	4.22 ± 7.26 (0.56)
PCGrimAge			
CTQ	-0.15 ± 0.34 (0.65)	-0.27 ± 0.33 (0.41)	0.12 ± 0.47 (0.80)
PRS CT	3.05 ± 3.53 (0.39)	-0.71 ± 3.51 (0.84)	3.76 ± 4.98 (0.45)

EEA Epigenetic Age Acceleration, *PRS* Polygenic Risk Score, *CT* Childhood Trauma, *SD* Standard Deviation

IV. DISCUSSION

This study investigated the interplay between childhood trauma, epigenetic age acceleration (EAA), and biological sex. We used five distinct principal component (PC) corrected epigenetic clocks, namely Horvath1, Horvath2, Hannum, PhenoAge, and GrimAge, each reliant on different CpG sites, thus representing varied measures of epigenetic aging. Although suggestive patterns were revealed, it is important to note the lack of statistically significant findings across the epigenetic clocks. Moreover, the analysis of polygenic risk scores (PRS) for childhood trauma found no significant associations for all clocks. A sex-specific analysis of the relation between EAA and childhood trauma or genetic risk for childhood trauma lacked the support of statistically significant results for sex disparities. While previous studies have repeatedly reported an association of childhood trauma and accelerated epigenetic aging[23,36,37], our results have not replicated this pattern. One potential explanation for this could be the relatively low proportion of participants who reported high levels childhood trauma in combination with our moderate sample size.

As the different epigenetic clocks focus on different underlying processes, they may present distinct effects in relation to childhood trauma. Previous research has established GrimAge as most reliable epigenetic predictor of mortality risk[38], yet it has showed inconsistent associations with stress. Recent studies indicate an association between accelerated aging in GrimAge due to lifetime psychosocial stress, while others find no significant correlation[39]. Another study on early life adversity and age acceleration using GrimAge showed no significant association with physical or sexual childhood abuse but found accelerated aging of 2.04 years in relation to childhood poverty[40]. Their mediation analysis estimated that smoking - one of the factors associated with GrimAge DNA methylation sites - explained over 50% of the accelerated epigenetic aging for participants who experienced childhood poverty. Moreover, a study by Hamlet EJ. et al linked accelerated GrimAge (but not Horvath, Hannum or PhenoAge) to early life adversity. In line with this, another study by Joshi D. et al showed positive EAA for GrimAge with adverse childhood experiences, while this link was not found for PhenoAge EAA[5]. The specific association of GrimAge with accelerated epigenetic aging related to childhood adversity aligns the trend in our findings from the PRS models. This indicates genetic risk for childhood trauma can lead to accelerated biological aging in relation to GrimAge, which assesses the risk of mortality.

Several studies with epigenetic clocks have indicated diverging aging between men and women[41–43]. The observed distinctions are thought to be influenced by environmental factors and life-style choices. In a study on sex differences in lifespan, the male participants tended to be biologically older, but they showed unhealthier life habits as well[41]. Furthermore, it is well-documented that responses to stress can differ between individuals of different sexes[44,45]. However, the current research lacks studies on sex specific epigenetic aging in relation to childhood trauma. Our study, although lacking significant sex-specific effects, contribute to this field and emphasize the need for further investigations into the interplay between sex-specific responses to childhood experiences and the epigenetic aging process.

Limitations of the study include the need to account for potential confounders like smoking, BMI, and other health-related variables. Age acceleration has been shown to be associated with sex[15,46], smoking status[15,47], BMI[11,15] and cell type proportions[48]. Including these factors in the analysis can lead to different results and highlights the importance of carefully selecting the covariates of your model. This was also concluded in a paper by Protsenko et al., where they encouraged researchers to correct for tobacco, alcohol use, physical activity, race, sex, adult socioeconomic status, medical comorbidity, and blood cell composition[39]. Moreover, while this study focusses on childhood trauma, the trauma we measured focused on diverse forms, including items focusing on emotional neglect, physical neglect, emotional abuse, physical abuse, and sexual abuse, which may impact epigenetic aging differently. The SNP heritability for childhood trauma showed to be rather modest, with an explained phenotypic variance of around 8%, which means non-genetic factors cannot be ignored[22]. Additionally, we used saliva samples for DNA methylation extraction, which can lead to different results in comparison to other tissues, such as blood samples. Further studies may also benefit from the use of a new third-generation clock in the analysis, namely the DundinPace clock, which has also been associated with early life stress[40].

Our study also provided some strengths, with the first one being using PC corrected clocks, which have been shown to result in more accurate and reliable epigenetic age predictions. Additionally, we used polygenic risk for childhood maltreatment as a distinct measure for epigenetic aging and delved into the sex specific effects of epigenetic aging in relation to childhood trauma.

V. CONCLUSIONS

This study aimed to gain knowledge on the overall and sex specific effects of childhood trauma and genetic predisposition of childhood trauma on epigenetic aging. Employing 5 different principal component corrected

epigenetic clocks (Horvath1, Horvath2, Hannum, PhenoAge and GrimAge) resulted in no significant associations. Additionally, we found no significant results in the sex-specific analyses. Further studies are required to identify these interactions and their potential implications for overall health and mortality.

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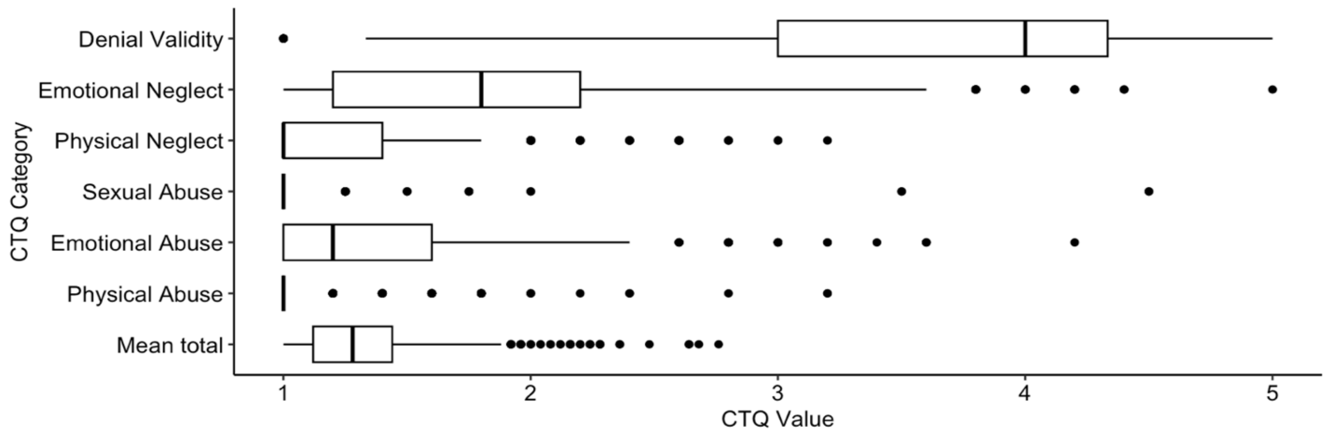
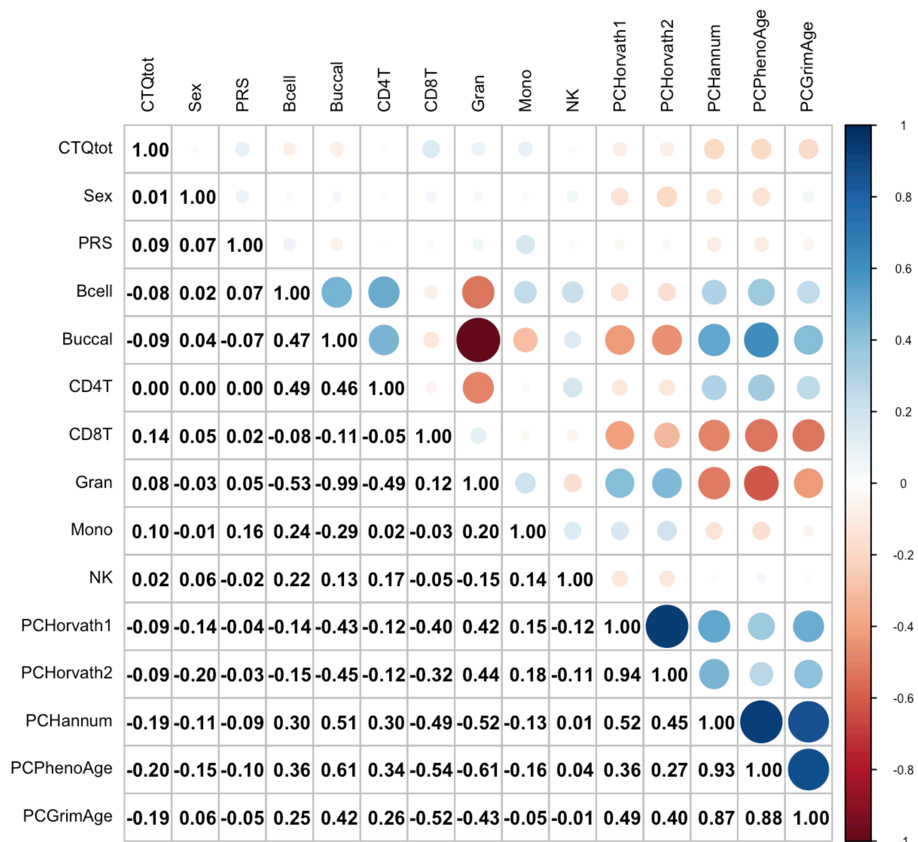
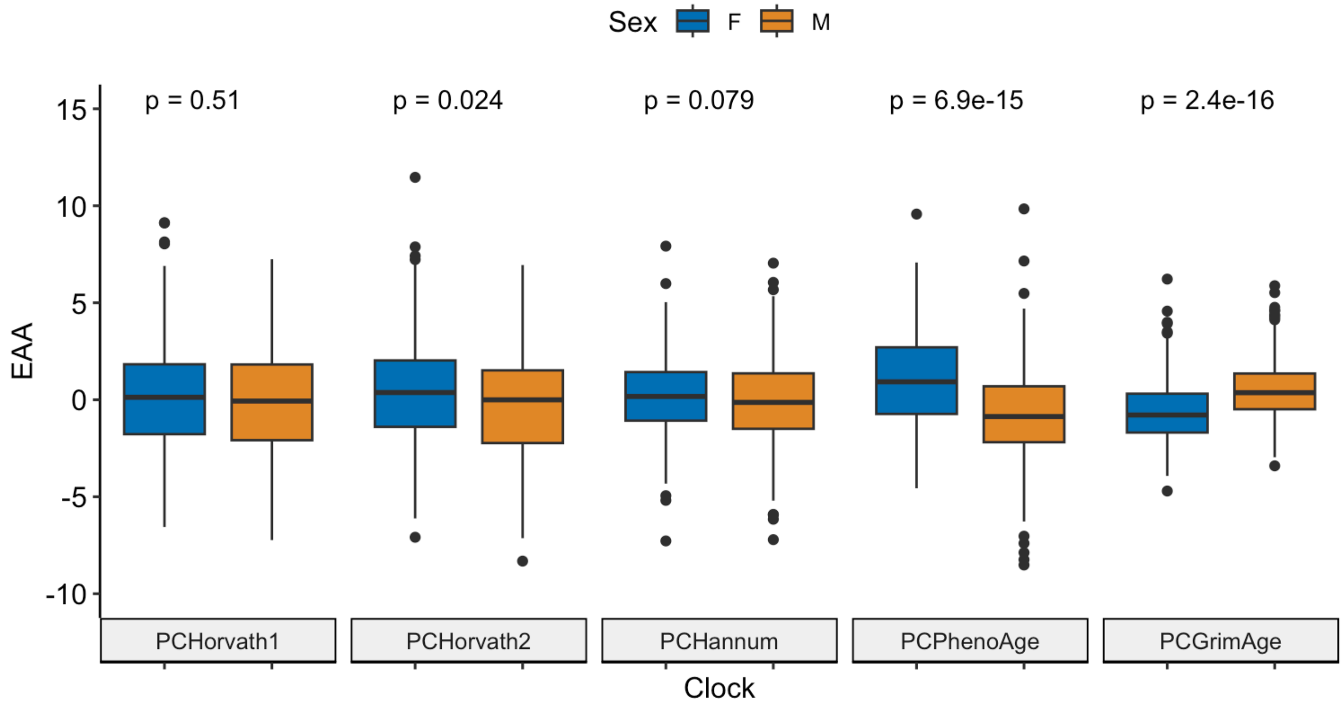
VII. SUPPLEMENTARY DATA



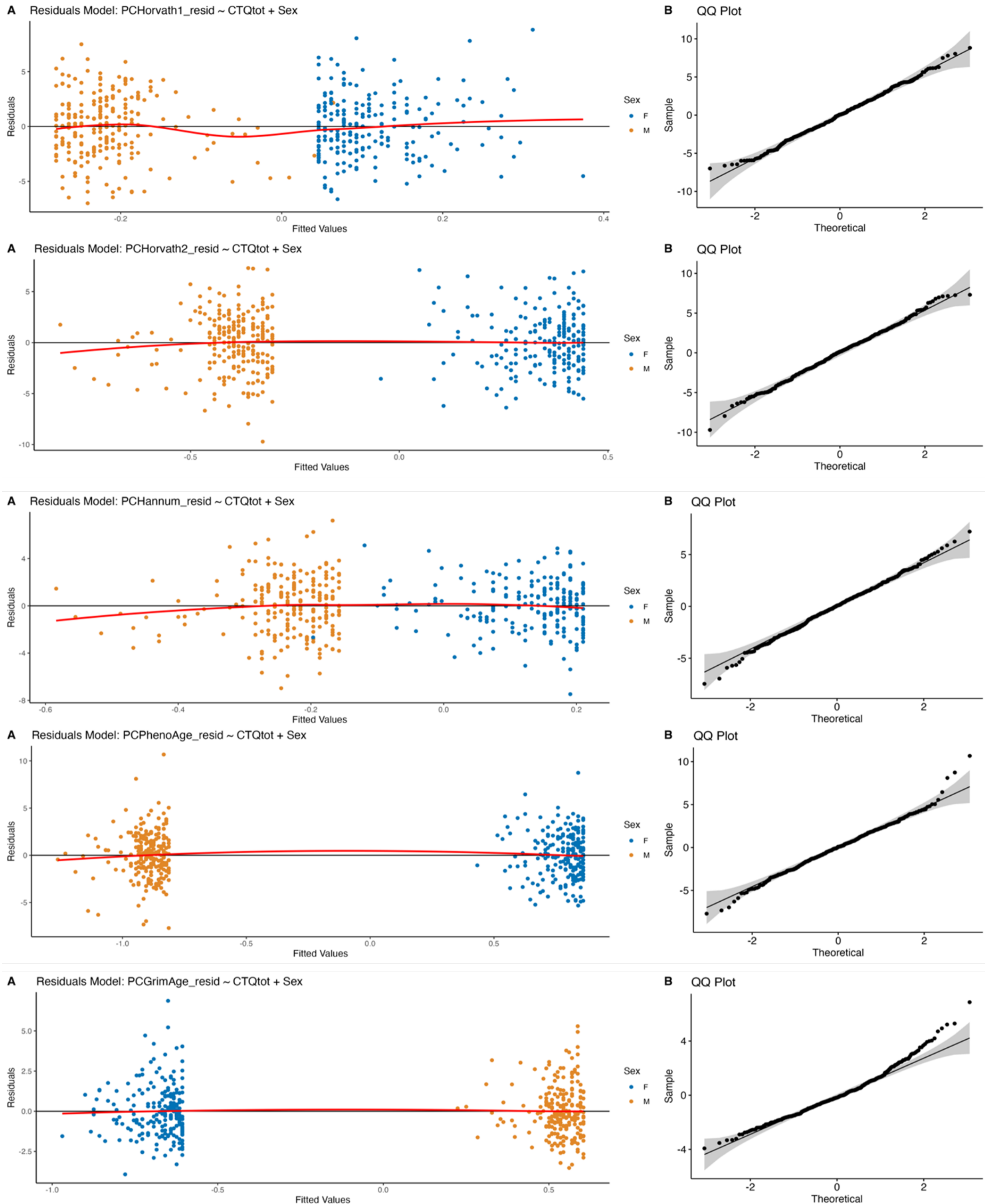
Supplementary Figure 1: ***Boxplot Childhood Trauma Questionnaire (CTQ) values total and per category of the 450 participants included in the analysis who filled in the questionnaire.*** *The plot shows low values for sexual and physical abuse, while a lot of participants had a high score for denial validity.*



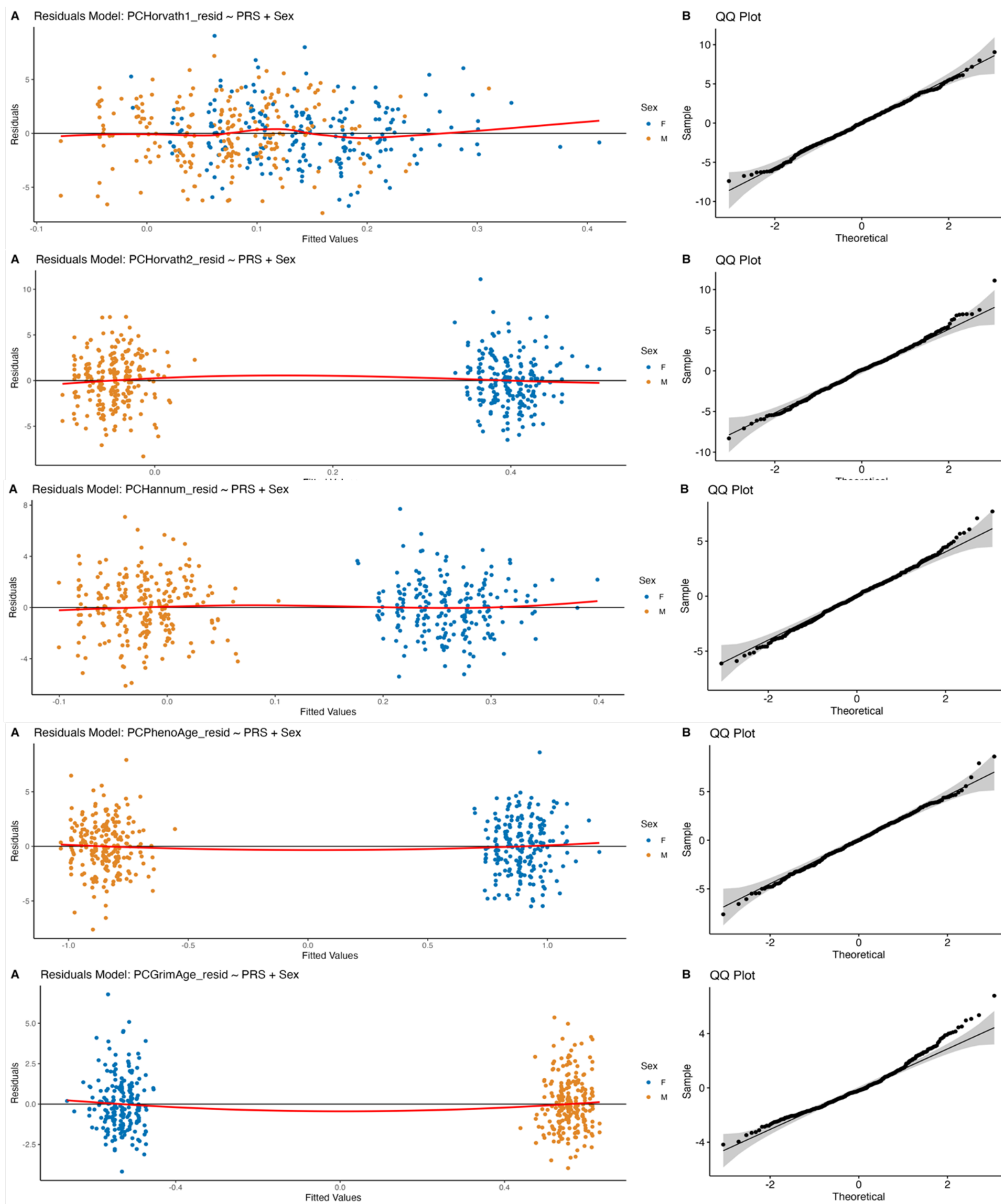
Supplementary Figure 2: ***Pearson correlation for Epigenetic Age (EA) for the different clocks (PCHorvath1, PCHorvath2, PCHannum, PCPhenoAge and PCGrimAge) with Childhood trauma questionnaire (CTQ) values, Polygenic Risk Score (PRS) for childhood trauma, sex and cell type compositions.*** *The plot shows high correlation values between the EA from different clocks and EA values with cell type composition values.*



Supplementary Figure 3: ***Boxplot of Epigenetic Age Acceleration (EAA) per epigenetic clock.*** *The plot is grouped by sex and means are compared using Wilcoxon test. EAA differs significantly between boys and girls for the PCHorvath2, PCPhenoAge and PCGrimAge clocks when maintaining a p value significance level of 0.05. Notably, PCPhenoAge shows a higher EAA for girls while PCGrimAge indicates a lower EAA for girls.*



Supplementary Figure 4: ***Validation of the linear regression model of Epigenetic Age Acceleration (EAA) with Childhood Trauma Questionnaire (CTQ) values and Sex as predictors.*** *The five rows represent the five different clocks (PCHorvath1, PCHorvath2, PCHannum, PCPhenoAge, and PCGrimAge) indicating EAA values. (A) Scatterplot with residuals versus fitted values. Residuals are evenly scattered around 0, signifying the model's satisfactory variance of errors and linearity. The distinct groupings in the fitted values corresponding to different sexes suggest the substantial role of sex in predicting EAA. (B) QQ plot illustrating the normality of errors, as indicated by the expected vertical line for all five clocks.*



Supplementary Figure 5: ***Validation of the linear regression model of Epigenetic Age Acceleration (EAA) with Polygenic Risk Scores (PRS) for childhood trauma and Sex as predictors.*** *The five rows represent the five different clocks (PCHorvath1, PCHorvath2, PCHannum, PCPhenoAge, and PCGrimAge) indicating EAA values. (A) Scatterplot with residuals versus fitted values. Residuals are evenly scattered around 0, signifying the model's satisfactory variance of errors and linearity. The distinct groupings of the fitted values in all clocks except for PCHorvath1 corresponding to different sexes suggest the substantial role of sex in predicting EAA. (B) QQ plot illustrating the normality of errors, as indicated by the expected vertical line for all five clocks.*