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Research Article

Interaction of mercury exposure and DNA methylation with sustained attention in children in a novel analysis of epigenetic susceptibility

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Abstract

The etiology of attention-deficit/hyperactivity disorder (ADHD) remains poorly understood, despite it being one of the most common neurodevelopmental disorders worldwide. Past research suggests methylmercury exposure and DNA methylation (DNAm) levels are each associated with ADHD in children, yet whether they interact to affect ADHD is unknown. Leveraging data from a longitudinal cohort of children in Mexico, this novel epigenetic–environment interaction study identified significant interactions between childhood mercury exposure (measured at 6–12 years of age) and adolescent blood leukocyte DNAm in their association with sustained attention [quantified via the Conners continuous performance test, 3rd edition (CPT3)] measured on average 5.6 ± 0.99 years later. Using adjusted linear regression, we assessed associations between hair and urine mercury concentrations and CPT3 scores reflecting inattention, impulsivity, vigilance, and sustained attention ($N = 399$). We then tested the interaction between mercury and DNAm at loci previously associated with the CPT3 outcomes ($N = 374$). Significant associations between mercury and CPT3 differed in magnitude and direction depending on the mercury biomarker and CPT3 variable. These associations often differed by gender. For example, urine mercury was positively associated with vigilance scores in males [$\beta = 1.31$(SE = 0.65), $P = .045$] but not in females [$\beta = -0.20$ (SE = 0.81), $P = .80$). In all children, three significant mercury–DNAm interactions were identified for either inattention or vigilance outcomes. Among females, 155 significant interaction terms were identified for the inattention models. In males, three significant interactions were identified for the impulsivity model. Overall, results suggest in some cases DNAm can influence the association between mercury exposure and ADHD-like symptoms.

Keywords: mercury; epigenetics; gene-environment; children's health

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a highly prevalent and complex neurodevelopmental disorder characterized by a dysregulation of normal executive function. In USA, 10.5% of children and 4.4% of adults are affected by this disorder [1, 2]. The prevalence varies from country to country by influences such as socio-economic (SES) factors, environmental exposures, and access to healthcare systems [1–3]. ADHD along with autism are considered to be the two most common neurodevelopmental disorders worldwide [3]. Despite this, the aetiology of ADHD is poorly understood.

Several studies have linked mercury (Hg) exposure to ADHD and ADHD-like behaviours in children and adolescents [4–8]. Mercury exists environmentally in multiple forms, including organic, inorganic, and elemental, and can cycle between these forms over time. The most prevalent form of organic Hg is methylmercury (meHg), which humans are predominantly exposed to through consumption of contaminated seafood [9]. As testament to its broad prevalence, nearly all people in the world have at least some level of meHg in their bodies according to the US Environmental Protection Agency [10]. This is concerning because it is known to be neurotoxic in high doses, with less certainty on the effects

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of chronic low-dose exposures. Notably, the majority of studies relating meHg to adverse neurodevelopment focus on prenatal Hg exposures [4–6]. While this represents a neurodevelopmental period that is highly vulnerable to toxic insults, childhood and adolescence are also important timeframes for brain development, specifically for the frontal lobe [11–14]. Exposure to Hg during this time may adversely impact the still developing frontal lobe of the brain, dysregulate normal executive functioning, and contribute to the aetiology of ADHD [11–14]. Though less frequent than prenatal meHg exposure studies, associations between childhood and adolescent Hg levels and adverse neurobehavioural and ADHD-related symptoms have been reported in different cohorts [7, 15]. Relevant to the present study, not all researchers have reported adverse relationships between Hg exposure and neurocognitive and behavioural outcomes; some examples even imply a beneficial relationship [16–18]. This is counterintuitive to the current consensus that meHg is a potent neurotoxicant, implying that there may be important confounders missing or differences in susceptibility across populations. Selenium and polyunsaturated fatty acids (PUFA), e.g., are neuroprotective nutrients found in seafood and are often consumed with meHg through contaminated fishes. Including these in statistical analyses has been shown to improve the ability to detect associations between meHg exposure and neurological effects [17, 19, 20]. Another important consideration for these studies is the type of biomarker used, as Hg from different biospecimens tends to reflect different sources. Urine Hg, e.g., generally indicates inorganic Hg exposure, whereas Hg in blood or hair is more indicative of exposure to meHg [21–23]. People can be exposed to inorganic or elemental Hg through several common everyday sources, with dental amalgams one common source. While meHg is thought to be more neurotoxic, inorganic Hg exposure is also known to be neurotoxic and exhibits greater nephrotoxicity compared to meHg [24, 25].

In addition to chemical exposures, epigenetic influences are also hypothesized to contribute to risk of ADHD, including aberrant DNA methylation (DNAm) [26, 27]. DNAm is a mitotically heritable epigenetic modification that is stable, but reversible. This modification helps to regulate gene expression, generally acting to suppress it. Dysregulation of DNAm can contribute to adverse health outcomes, and researchers have recently started to explore it as a possible mechanism underlying environmental toxicant-associated neurodevelopmental disorders [26–29].

Despite the fact that both DNAm and Hg exposure may influence ADHD aetiology, only a handful of publications have explored the effects of both these variables on neurobehavioural outcomes within the same study, and of those examples, the analyses focus on the potential mediating role of DNAm in Hg-related neurobehavioural impairments [27, 29]. We previously reported associations between DNAm and attention-related symptoms in adolescent Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) participants; symptoms were quantified by the Conners Continuous Performance Test, 3rd edition (CPT3). This study was conducted in a population of Hispanic individuals between the ages of 9 and 18 years, and the current research builds upon this finding in the same study population [30]. The present study aims to examine the combined effects of childhood Hg exposure and adolescent DNAm on adolescent ADHD-like symptoms not through mediation, but rather through a novel assessment that employs similar concepts that underlie a standard gene–environment interaction (G × E) study. The basic principle behind a G × E study states that the effect of an environmental exposure on a person's health risk may vary depending on their genotype, meaning a person with a distinct genetic polymorphism in a gene may be predisposed to toxicant-related disease, whereas people with another variant of that gene are less likely to develop the disease. Genetic predisposition or susceptibility to mercury-induced neurotoxicity has been proposed in past studies in human cohorts [7, 8, 31, 32]. We propose a similar concept—epigenetic susceptibility. We hypothesize that the effect of Hg exposure on a person's risk of developing ADHD-like symptoms will vary depending on the methylation state of DNA at a given gene. We test this hypothesis using mercury biomarker, blood leukocyte DNAm, and CPT3 performance data from child participants of the ELEMENT cohort. The relationship between DNAm and CPT3 scores was previously identified in our prior publication [30]. The current study aims to identify associations between child hair and urine Hg exposure and adolescent CPT3 performance in the same study population using adjusted linear regression models. Finally, in a novel approach to model Epigenetic–Environment (E × E) interaction, we test the interaction between Hg and DNAm. This unique approach could serve as a model for future research examining the concept of epigenetic susceptibility to toxicant-associated health effects.

Materials and methods

Study population

Recruitment for the ELEMENT cohort took place between 1994 and 2004. During this period, 1012 mother/child dyads were recruited during pregnancy or at birth from hospitals and prenatal clinics from the Mexican Social Security Institute, serving low- to middle-income populations in Mexico City. Data were periodically collected from these women and their children at various timepoints from their recruitment through present day, and data from two follow-up visits were used in this analysis [33]. A schematic representation of the timing of key predictors and outcomes in ELEMENT that are the focus of the present study are outlined in Fig. 1. During the child visit (2008–2012), biological samples were collected from the children (hair, blood, and urine) and survey data were obtained on health, diet, and demographic characteristics. Hair and urine Hg levels were measured. In a subsequent follow-up visit starting in 2015, referred to as the adolescent timepoint, biological samples, e.g. blood, and survey data were collected. Epigenome-wide DNAm was quantified in blood leukocytes for 526 participants. Participants were also administered the CPT3 to quantify ADHD-like tendencies [33]. In a previous publication from our lab group, associations between aberrant DNAm and CPT3 scores from these same participants were identified at the adolescence timepoint [30]. The present study builds upon this work by considering the potential interaction between DNAm and childhood exposure to Hg. It focusses on a subset of 399 participants for whom childhood Hg and adolescent CPT3 data were collected. In the interaction models with DNAm, 374 participants that also have adolescent DNAm are included. The institutional review boards of the Mexico National Institute of Public Health and the University of Michigan approved all research protocols related to data and research relevant to both timepoints; both parental informed consent and child assent was obtained for all participants in this study.

Mercury biomarkers

Hair and urine were collected from participants at the childhood study visit and total Hg was quantified in both biospecimens using a Direct Mercury Analyzer 80 (DMA-80, Milestone Inc., CT, USA) as described in previous studies [34, 35]. In short, hair was cut from the proximal end in 2 cm segments, washed in acetone, rinsed

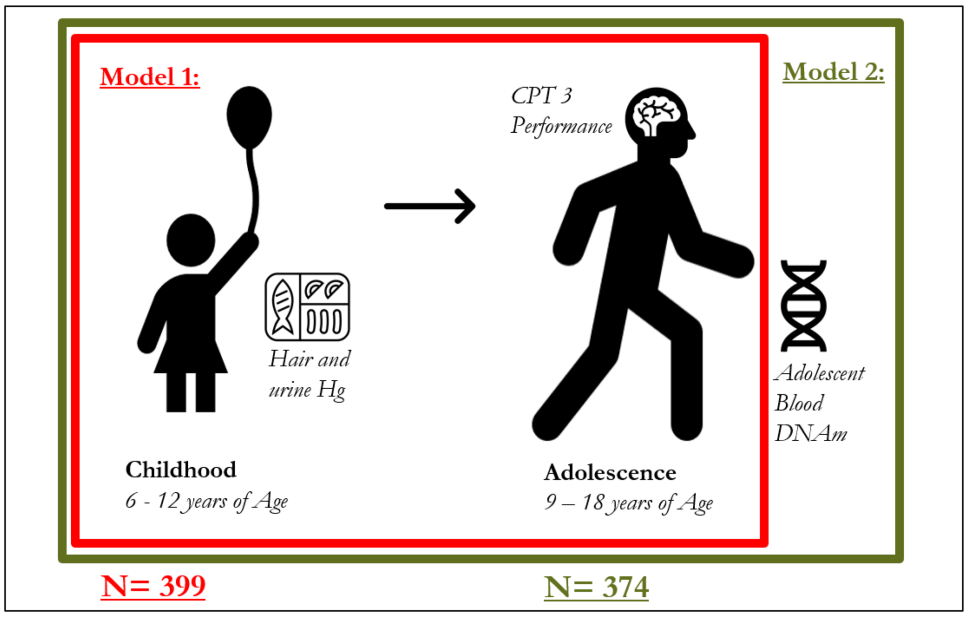

Figure 1. Schematic of the timing of key predictors and outcomes in ELEMENT that are the focus of this research. The inner box outlines the timepoints, variables, and sample size relevant to model 1 and the outer box represents those pertinent to model 2. Note that blood DNAm and CPT3 performance were measured at the same time point while Hg was measured in samples collected several years prior.

three times with sterile water, dried overnight, and ~5 mg of sample was placed into a nickel sampling boat. While the DMA-80 is unable to distinguish between the different types of Hg, it is known that hair Hg predominantly reflects exposure to organic Hg [21, 22]. Prior to Hg analysis in urine, which is generally a biomarker for its inorganic form, the sample was vortexed, a portion was transferred to a quartz sampling boat and specific gravity was measured via a refractometre (PAL-10S, Atago) [21, 23]. The instrument was calibrated daily and procedural blanks and replicates were also utilized for quality control. CRM #13 for hair (National Institute for Environmental Studies, Japan) and QMEQAS for urine (Institut National de Santé Publique du Québec) were used as certified reference materials. Reference material recoveries were between 80% and 110% and the analytical limit of detection was <1 ng Hg per measure. Urine Hg levels were adjusted for specific gravity.

Epigenetic analysis

As outlined in a publication by Ehlinger *et al*, an epigenome-wide DNAm analysis was conducted using an EPIC array (Illumina Infinium Methylation EPIC BeadChip; CA, USA) for all ELEMENT participants who provided an adequate blood sample at the adolescent study visit [30]. The Bead Chip array is a probe-based assay quantifying methylation at >850 000 CpG sites across the human genome [36, 37]. Prior to analysis on the Bead Chip array, we extracted DNA from blood leukocytes of participants via Qiagen Flexigene kits (Hilden, Germany) and performed bisulphite conversion on the DNA samples. Subsequently, bisulphite-converted samples were submitted to the University of Michigan Advanced Genomics Core where they were randomized across chips and positions and hybridized to BeadChips, and signals were read, subjected to initial processing, and underwent thorough quality control measures [38].

For data processing, the raw image data files were read into R using the minfi package and RELIC was employed to correct for background noise and dye bias [39, 40]. On each chip, unwanted technical variability, estimated from control probes, was accounted for via quantile normalization [40, 41]. Probes with probable cross-reactivity, poor detection above background (in <5% of samples), and/or polymorphisms in the CpG or single base extension sites were excluded from analysis [42]. As added quality control measures, documented gender was compared to that which was estimated from the X/Y chromosome DNAm profiles, and we checked for low intensity across all probes and/or >5% probes failing. All 526 adolescent samples submitted for analysis passed the initial quality control check.

To account for heterogeneity, cell-type proportions were estimated using tissue-specific differentially methylated region (DMR) data included on the chips [43]. Intensity values from the non-negative control probes were utilized to generate variables representative of technical variation influencing the DNAm data via surrogate variable analysis [44]. β-values reflecting the proportion of total methylated cytosine at each site were used in all downstream statistical analyses outlined in this research. These data were also used in a previously published EWAS (epigenome-wide association study) which identified relationships between DNAm and ADHD-like behaviours [30]. From this foundational research, CpG sites having the 1000 smallest raw *P*-values for their association with each CPT3 outcome were selected for the present study to test interactions between ADHD-associated CpG sites and Hg biomarkers. These CpG sites are enriched in gene pathways related to ferroptosis, neurotransmission, inflammation, and immune response as previously described [30].

Cognitive performance test

The CPT3 is a computerized test designed to assess four distinct aspects of attention in children: inattentiveness, impulsivity/hyperactivity, sustained attention, and vigilance. During the examination, children are presented with a series of stimuli to which they must either respond or not by hitting a button. Continuous variables are generated from the response data to reflect the aforementioned aspects of attention [45, 46]. The CPT3 was administered by trained research staff in Mexico during the adolescent study visit. While inter-examiner variability is not a major

concern due to the computerized nature of the exam, the examiners took measures to ensure all participants understood the directions and were provided a distraction-free environment within the research centre to take the exam. For the present study, Omissions Errors, Perseverations, Interstimulus Interval (ISI) Change, and Hit Reaction Time (HRT) Block Change variables were selected as outcomes based on ease of interpretation and prevalence of use in the literature, including use in Ehlinger *et al*. [30]. They respectively reflect inattentiveness, impulsivity/hyperactivity, vigilance, and sustained attention. Response style is another relevant variable generated by the CPT3 test that alone does not accurately represent any aspect of attention but rather accounts for individualistic style of responding and may act as a confounding factor [45–47].

Covariates

Covariates were carefully selected on basis of biological relevance to the present research question or correlation with the outcomes of interest in this study population. A more complete assessment of covariate selection is described by Ehlinger *et al* [30]. Briefly, child age, gender, SES, and PUFA levels estimated from food frequency questionnaires (FFQs) were documented at both the childhood and adolescent timepoint. The CPT3 Response Style, cell-type proportions in whole blood (CD8+ T cells, B cells, and granulocytes), and batch effects from the EPIC data were generated at the adolescence study visit only and were also considered as confounders or precision covariates in the analyses that included DNAm data. The Mexican Association of Marketing Research and Public Opinion Agencies (AMAI) scaling system was specifically designed for Mexican populations and was selected for use in this study as a metric to assess SES; the variable ranks individuals in groups numerically from lowest SES (1) to highest SES (7) [48, 49]. As previously described, numerous other variables including selenium exposure (derived from FFQ), folate levels (derived from FFQ), body mass index, Tanner staging (an estimation of pubertal status), and additional cell-type proportion (monocytes, natural killer T cells, and CD4+ T cells) were considered. They were ultimately excluded from the final models due to the lack of association with the outcome variables or limited biological relevance to the research questions as described in Ehlinger *et al* [30].

Statistical methods

All statistical methods were conducted using R (version 4.0.3). Mercury biomarkers, CPT3 Omissions errors, and perseverations were natural log-transformed. After performing descriptive statistics examining distribution, sample size, mean, and standard deviation of variables, we answered the two research questions using linear regression. The study schematic is depicted in Fig. 1. The first model aims to identify the associations between Hg exposure and CPT3 performance without consideration of DNAm. The second model addresses the research question of whether DNAm at CPT3-associated loci modifies the relationship between Hg and outcomes, modelled through an interaction term between the two predictors. The models can be described by the equations below.

Equations

Model 1: $\text{CPT3 score} = \boldsymbol{\beta_{Hg}X_{Hg}} + \beta_i X_i \ldots + \beta_0 + \varepsilon$

Model 2: $\text{CPT3 score} = \boldsymbol{\beta_{int}X_{Hg}{}^{*}X_{DNAm}} + \beta_{Hg}X_{Hg} + \beta_{DNAm}X_{DNAm} + \beta_i X_i \ldots + \beta_0 + \varepsilon$

- **Annotations**: CPT3 score = one of four outcomes from the CPT3; β_{DNAm} = slope of the DNAm coefficient for 1–1000 distinct CpG sites in the genome; β_{Hg}= slope of the Hg variable (both log hair Hg or log specific gravity-adjusted urine mercury was used in otherwise identical models); β_{int} = slope of the interaction between Hg and DNAm; β = slope of the coefficient; X = value of covariate; i = covariates controlled for in each model; β_0 = intercept; ε = residual error

Associations of Hg exposure with CPT3 performance (Model 1)

Multiple linear regression models controlling for age, gender, SES, and CPT3 response style were employed to examine the relationships between hair and urine Hg levels and four CPT3 outcomes representative of inattention (Omissions Errors), impulsivity (Perseverations), vigilance (HRT ISI Change), and sustained attention (HRT Block Change). This was conducted in total study population models (N = 399) and gender stratified models (N = 203 females; N = 196 males). PUFA levels (calculated from FFQs administered at childhood) were also included as an additional covariate in a sensitivity analysis (N = 262 based on the availability of childhood PUFA data) to account for potential protective effects of beneficial seafood nutrients co-exposed with Hg during childhood.

Interaction of Hg exposure and DNAm with CPT3 performance (Model 2)

We designed a model that would consider how one predictor might modify the relationship of the other with the outcome, by adding an interaction term between DNAm and Hg exposure to linear regression models assessing the relationships between the CPT3 outcome and the exposures. The analysis was run using each of the four CPT3 variables previously described, with all models controlling for age, gender, SES, CPT3 response style, and adolescent PUFA levels. For each outcome, only the CpG sites corresponding to the 1000 lowest raw *P*-values identified in our previous publication as associated with CPT3 outcomes were used [30]. Benjamini–Hochberg test was used to adjust for multiple comparisons. Gender-stratified models were also conducted.

Since epigenetic data are traditionally modelled as a mediator (Fig. 2a) instead of an effect modifier (Fig. 2b), we conducted a mediation analysis for the significant CpG sites in the interaction models ($q_{int} < 0.10$) to infer whether these sites also had evidence for mediation. We tested whether DNAm at these sites were potential mediators in the relationship between Hg exposure and CPT3 performance. For this, the R package 'mediation' was used to infer whether these sites were modifying and not mediating associations. This package employs the quasi-Bayesian Monte Carlo method to estimate the average causal mediation effects (ACME; indirect effect) of DNAm for each model across 1000 simulations [50].

Results

The study population was comprised of a near equivalent percentage of males (49%) and females (51%). Median (interquartile range) hair and specific gravity adjusted urine Hg measured at the childhood visit were, respectively, 0.46 (0.28–0.80) μg/g and 0.54 (0.32–1.06) μg/l (Supplemental Figure S1); for Hg by gender see Supplemental Figures S2 and S3. ELEMENT participants comprised varying levels of SES, with the majority of participants representing low- to middle-income families (Figure S4). Adolescent CPT3 scores, recorded after following up the same participants an average of 5.6 years later, were on average (standard deviation) 50 (10), 51 (11), 51 (10), 51 (9), and 52 (9) for omissions errors, perseverations, ISI change, HRT block change, and response

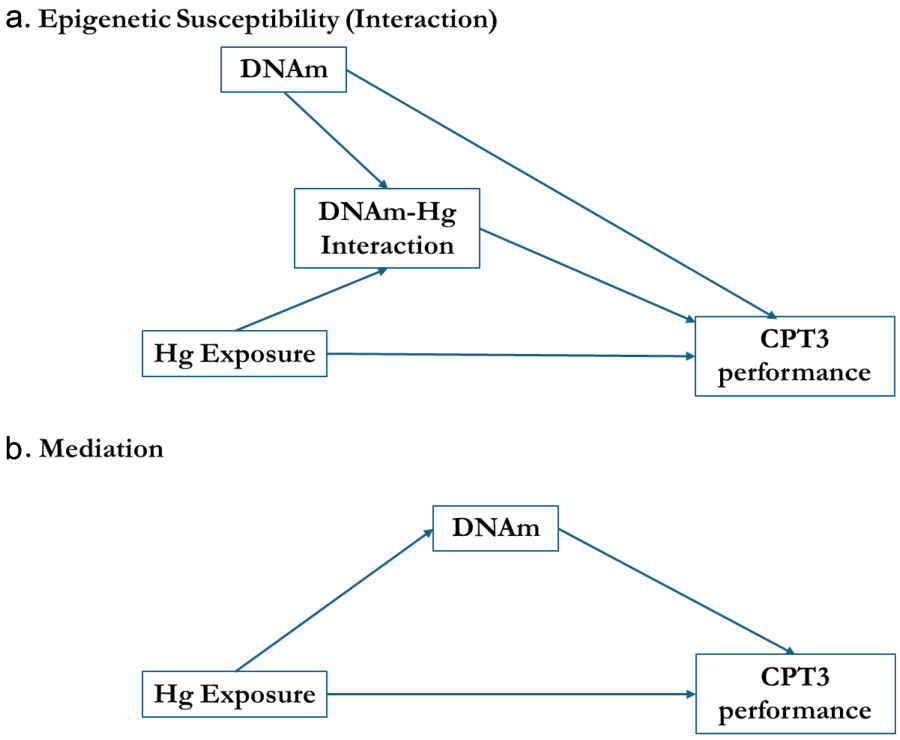

Figure 2. Schematic representing the possible relationships between Hg, DNAm, and CPT3 performance. (a) In the case of epigenetic susceptibility, Hg and DNAm could have independent effects on the CPT3 outcome and the interaction between the two has an additional effect when both are present. (b) In the case of mediation, DNAm is a mediator in the relationship between Hg exposure and CPT3 performance.

style, respectively, exhibiting broad ranges (Table 1; for descriptive statistics stratified by gender see Supplemental Table S1).

Associations of Hg exposure with CPT3 performance

Childhood hair and specific gravity-adjusted urine Hg levels were associated with CPT3 performance at adolescence in the full analytic sample and in gender stratified analyses. The nature of these relationships was dependent upon the Hg biomarker and CPT3 variable used for each analysis. With omissions errors as the outcome, for example, hair Hg was associated with reduced inattentiveness [$\beta = -0.021$ (0.0095), $P = .027$]. However, when urine Hg was used instead, no significant associations were identified, but the direction of effect remained the same [$\beta = -0.0045$ (0.0078), $P = .57$]. In many instances, gender also modified associations between Hg exposure and outcome. For example, specific gravity adjusted urine Hg exposure was significantly related to loss of vigilance in males [$\beta = 1.31$ (0.65), $P = .045$], but not in females [$\beta = -0.20$ (0.81), $P = .80$] (Table 2). Lastly, the significance level and direction and magnitude of effect were similar for most associations with the addition of childhood PUFA in a sensitivity analysis (Table 2 and Supplemental Table S2).

Combined associations of Hg exposure and DNAm with CPT3 performance

In the 'Epigenetic' × Environment interaction analysis, based on a significance threshold of $q < 0.10$, in models including all children, hair Hg significantly interacted with DNAm at one CpG site cg05093254 (annotated to the *ABCA3* gene) ($\beta_{int} = 5.01$, SE = 1.24, $q = 0.067$) in its association with HRT ISI change score (Tables 3 and 4). For the omissions errors models, urine Hg significantly interacted with DNAm at two CpG sites: cg10396484 (annotated to *LBX1*) ($\beta_{int} = 0.048$, SE = 0.012, $q = 0.057$) and cg21442182 (intergenic; $\beta_{int} = -0.013$, SE = 0.0033, $q = 0.075$) (Tables 3 and 5). No significant interactions were identified for the perseverations or HRT block change outcomes in models including all children. For the perseveration outcome when stratified by gender; however, urine Hg in males significantly interacted with 3 CpG sites, cg03760895 (*RSL1D1*), cg17227358 (*DRGX*), and cg19505184 (intergenic; Tables 6 and 7). In females, significant interactions were identified between DNAm at 155 CpG sites and hair Hg for the omissions error outcomes (Tables 6 and 8). Select observations are presented in Table 8; see Supplemental materials for all 155 statistically significant interaction results, with both Hg biomarkers for comparison.

In the mediation analysis, there was no evidence of mediation identified in all children or male-only models (Supplemental Tables S3 and S4). Based on a significance threshold of $P < .05$, only one out of the 155 CpG sites tested exhibited a significant ACME, suggesting possible mediation which could have occurred by chance alone (Supplemental Table S5).

Discussion

Previous publications associate aberrant DNAm and Hg exposure with ADHD and ADHD-like symptoms, but few studies consider their combined effects, as is done in the present study through a novel analysis of epigenetic–environment interaction. In a study population of children followed up through adolescence in Mexico City, significant associations between adolescent DNAm and CPT3 performance were previously identified [30]. In this study, we identify associations between childhood Hg exposure and adolescent CPT3 performance. After first identifying these initial associations, in a subsequent analysis including an interaction term

Table 1. Descriptive statistics for key variables

Variable	N	Mean	Std. Dev.	Minimum	25th Percentile	Median	75th Percentile	Maximum
Urine Hg (specific gravity adjusted, μg/l)	399	1.085	2.855	0.087	0.319	0.54	1.057	49.672
Hair Hg (μg/g)	395	0.625	0.575	0.062	0.277	0.46	0.799	6.217
Omissions errors (T score)	399	50.38	10.46	41	44	47	51.5	90
Perseverations (T score)	399	50.92	11.08	44	45	46	51	90
HRT ISI change (T score)	399	50.68	9.68	27	44	50	56	87
HRT block change (T score)	398	51.01	8.96	13	46	51	56	88
Response style (T score)	399	52.1	9.19	31	46	52	57	90
Age (years) at Childhood visit	378	8.9	1.3	5.7	7.5	9.1	9.9	12
Age (years) at Adolescence visit	399	14.3	2.1	9	12.5	15	16	18
Years between visits	378	5.6	1.0	2.9	4.8	5.8	6.3	7.5
Childhood PUFA (g/day)	262	10.6	5.1	2.8	6.6	10.1	13.3	28.9
Adolescent PUFA (g/day)	399	14	6.9	1.7	8.6	12	18	49
	N	**%**						
Gender	399							
.. Female	203	50.90						
.. Male	196	49.10						
AMAI	399							
.. E	1	0.30						
.. D	99	24.80						
.. D+	90	22.60						
.. C-	103	25.80						
.. C	76	19						
.. C+	27	6.80						
.. A/B	3	0.80						

Note: AMAI is the Mexican Association of Marketing Research and Public Opinion Agencies scaling system; categories are listed sequentially with E reflecting the lowest SES level (*E* = 1) and A/B representing the highest SES level (A/B = 7). Abbreviations: HRT = Hit Reaction Time, ISI = Inter-Stimulus-Interval, μg/L = micrograms of Hg per litre of urine, μg/g = micrograms of Hg per gram of hair, g/day = grams per day, PUFA = polyunsaturated fatty acids.

between Hg and DNAm, significant interaction terms were identified at one CpG site (cg05093254; annotated to *ABCA3*) with hair Hg for the HRT ISI change models and at two CpG sites (cg10396484 and cg21442182; annotated to *LBX1* and an intergenic region, respectively) with urine Hg for the omissions error models. Genes annotated to the significant CpG sites, such as *ABCA3* and *LBX1*, may play potential biological roles in neurological function in general or specifically in neurotoxicity from Hg. *ABCA3* (encoding the protein ATP-binding cassette subfamily A, member 3) is an important lipid transporter involved in phosphatidylcholine and phosphatidylglycerol trafficking that plays a role at the blood-brain barrier [51–53]. *LBX1* (encoding Ladybird Homeobox Protein Homolog 1) is an important transcription factor that helps regulate neuron-specific gene expression patterns that influence cell fate during neurodevelopment, is involved in regulating cell migration, and is essential for GABAergic interneuron development in the dorsal horn of the spinal cord [54]. Without direct mechanistic experiments, conclusions regarding the precise role of each gene in the relationship between mercury and CPT3 outcomes cannot be made. Overall, this study provides an example of epigenetic susceptibility to toxicant-related effects and a framework for investigating similar questions.

Associations between the exposures, DNAm, and outcomes were often different by gender, especially in the interaction models. Gender-stratified assessments revealed 155 significant interactions between DNAm and hair Hg in females for the omissions error outcome and 3 significant interactions between DNAm and urine Hg in males for the perseverations outcome. These specific outcomes may be related to key differences in how ADHD symptoms tend to present in males versus females. The omissions error outcome, for example, is meant to reflect inattentiveness, the predominant symptom profile exhibited by females according to clinical trends [55, 56]. Similarly, the perseverations outcome represents impulsivity, which tends to be more common in males [55, 56]. The present findings suggest that both gender and DNAm may influence the relationship between Hg exposure and CPT3 performance and that females may be more susceptible to effect modification by DNAm than males. Future research examining the precise mechanisms underlying these proposed gender differences is warranted to elucidate implications of these results.

With regard to Hg exposure, we observed both positive and negative associations with CPT3 performance; epidemiological studies previously published on this topic have also observed associations in both directions [7, 16]. In the analyses including all children, higher Hg biomarker concentrations often associate with more severe ADHD-like symptoms (positive relationship), as hypothesized for a neurotoxicant, yet none are statistically significant in either the main model or the sensitivity analysis. There was, however, an example of a significant negative association between hair Hg and omissions error which remained significant in the sensitivity analysis adjusting for PUFAs. PUFAs are beneficial seafood nutrients that promote healthy neurodevelopment and may play a role in Hg detoxification. Past studies have shown that controlling for PUFAs in analyses focused on meHg improves the statistical model, as it is an important confounding factor [17, 19, 20]. In our study, adjusting for PUFA did not substantially change results. However, unlike the Hg variables which were measured in biological materials, PUFA levels were calculated from FFQs. While we use a validated FFQ, innate biological differences between participants in how they process the nutrients and potential recall bias in the responses can impact this measure. Another consideration is the reduction of the sample size from 399 in the main model to 262 in the sensitivity analyses; not all participants had data recorded for the PUFA variable, thus reducing the statistical power.

Table 2. Summary of associations between mercury exposure biomarkers and CPT3 outcomes in adjusted linear regression models in all children and stratified by gender

CPT 3 variable:	Omissions errors β (SE) *P*-value		Perseverations β (SE) *P*-value		HRT ISI change β (SE) *P*-value		HRT block change β (SE) *P*-value	
aspect of attention	Inattention		Impulsivity		Vigilance		sustained attention	
Hair Hg	−0.021 (0.0095) .027*	M = −0.018 (0.014) 0.194 F = −0.026 (0.013) 0.045*	−0.017 (0.012) .162	M = −0.001 (0.017) 0.95 F = −0.034 (0.017) 0.048*	−0.22 (0.64) 0.74	M = 0.59 (0.82) 0.47 F = −1.13 (0.99) 0.26	0.45 (0.58) .44	M = 0.85 (0.83) 0.31 F = 0.104 (0.80) 0.897
Urine Hg	−0.0045 (0.0078) .56	M = 0.0086 (0.011) 0.45 F = −0.018 (0.011) 0.085•	−0.012 (0.0097) .23	M = 0.0074 (0.013) 0.59 F = −0.030 (0.014) 0.027*	0.61 (0.52) 0.25	M = 1.31 (0.65) 0.045* F = −0.20 (0.81) 0.80	−0.095 (0.47) .84	M = 0.28 (0.68) 0.69 F = −0.48 (0.65) 0.46

Note: higher scores for CPT3 variables reflect poorer performance. Omissions errors and perseverations were log transformed. * reflects a *P*-value ≤ .05 and • reflects a *P*-value ≤ .10. M = males only models. F = females only models. Models include all children, if not otherwise specified. All models adjusted for age, gender, SES, and response style. SE = Standard Error.

Significant associations were also identified in the gender-stratified models. Hair Hg levels in females were associated with fewer omissions errors and perseverations. The direction and strength of associations were similar when urine was used as the Hg biomarker instead of hair for female omissions errors scores and perseverations scores. No associations were significant in males for either of these CPT3 outcomes regardless of the Hg biomarker used. For the HRT ISI change outcome, however, urine Hg exposure was significantly associated with poorer CPT3 scores in males, but not in females. In models that utilized hair Hg instead, the direction and magnitude of effect were comparable to the urine Hg models in males and females but were not statistically significant. Hair Hg is better representative of organic Hg (meHg exposure), and urine Hg better reflects inorganic Hg [21–23]. Notably, the two exposure biomarkers are correlated to one another (Spearman rho = 0.39, $P = 2.2 \times 10^{-16}$), and it has been shown that urine Hg may reflect meHg exposure when sources of exposure to inorganic Hg are low [57]. Since the direction of association was similar for both biomarkers, this may be an artefact of that correlation between them.

There is a lack of consensus in the literature linking chronic exposure to low levels of Hg during childhood to adverse neurodevelopment in human cohorts. One study by Lozano *et al*, for example, identified significant adverse relationships between childhood hair Hg levels and neurobehaviour as quantified via the Child Behaviour Checklist test (CBCL), the Conners Parent Rating Scales-Revised: Short Form (CPRS-R:S), and the Attention Network Test (ANT) [7, 58–60]. This study was conducted in a birth cohort based in Spain, using data generated from participants at study visits when participants were 9 (hair Hg, CBCL, and CPRS-R:S) and 11 years of age (CBCL, CPRS-R:S, and ANT). Adjustment for seafood intake had little impact on the relationships observed for any model, similar to the present study's sensitivity analysis. In the Lozano *et al*.study, gender significantly modified associations for both the CBCL externalizing behaviour and total problems scales. Authors noted that the associations were negative for females and positive for males in models for both outcomes, though not statistically significant. This is possibly due to the reduced sample size. The same phenomenon of effect modification by gender was observed for models using the ADHD index scores of the CPRS-R:S test [7]. These findings are particularly interesting when compared to the results of our present study, because associations between Hg exposure and CPT3 scores were often different in males and females in our gender stratified models. Though not statistically significant for all models, we identified positive associations in males and negative associations in females regardless of the Hg biomarker used for the ISI change (reflecting vigilance) outcome (Table 2). Taken together with the Lozano *et al*.paper, results suggest that males may be more susceptible to the neurotoxic effects of meHg than females and/or less responsive to the beneficial effects of PUFAs or other seafood nutrients often co-exposed with meHg. Studies in several other cohorts, such as those based in Canada and the Faroe Islands, have also reported significant adverse relationships between prenatal Hg exposure and neurobehaviour and neurodevelopment in children, and also suggest effect modification by gender [4, 5].

In other publications, however, adverse relationships could not be identified between Hg exposure and neuro-outcomes in children, with several studies implicating a positive impact of Hg on neurodevelopmental and behavioural outcomes [16–18]. These inconsistencies in results are exemplified in a publication by Davidson *et al*. conducted in a seminal cohort, the Seychelles Child Development Study (SCDS) that was specifically designed

Table 3. Number of CpG sites with significant interactions ($q < 0.1$) between DNAm and Hg exposure in all children

	Omissions Errors	Perseverations	HRT ISI Change	HRT Block Change
Hair Hg	0	0	**1**	0
Urine Hg	**2**	0	0	0

Table 4. Assessing interactions between DNAm and Hg exposure in their association with CPT3 ISI change scores in all children

	DNAm at *ABCA3* (cg05093254)		Hg		Hg x DNAm	
Hg biomarker	**β (SE)**	***q*-value**	**β (SE)**	***q*-value**	**β (SE)**	***q*-value**
Urine Hg	−1.12 (0.64)	0.097	−334.15 (132.97)	1.0	3.39 (1.35)	1.0
Hair Hg	−1.60 (0.52)	0.016	−494.99 (122.64)	0.066	5.01 (1.24)	0.067

Note: Results with interaction terms significant at the threshold $q < 0.10$ are shown; models adjust for age, gender, SES, CPT3 response style, and adolescent PUFA levels.

Table 5. Assessing interactions between DNAm and Hg exposure in their association with CPT3 omission error scores in all children

		DNAm		Hg		Hg x DNAm	
Hg Biomarker	***Gene* (Probe ID)**	**β (SE)**	***q*-value**	**β (SE)**	***q*-value**	**β (SE)**	***q*-value**
Urine Hg	*LBX1* (cg10396484)	0.049 (0.010)	9.60×10^{-5}	−0.11 (0.027)	0.040	0.048 (0.012)	0.057
	NA (cg21442182)	−0.020 (0.0036)	2.71×10^{-5}	1.06 (0.28)	0.080	−0.013 (0.0033)	0.075
Hair Hg	*LBX1* (cg10396484)	0.045 (0.015)	0.040	−0.050 (0.032)	0.66	0.013 (0.013)	0.92
	NA (cg21442182)	−0.013 (0.0043)	0.034	0.19 (0.33)	0.86	−0.0025 (0.0039)	0.92

Note: Significance threshold $q < 0.10$. models adjust for age, gender, SES, CPT3 response style, and adolescent PUFA levels.

Table 6. Number of CpG sites with statistically significant interactions between DNAm and Hg exposure in the gender stratified models

	Omissions Errors	Perseverations	HRT ISI Change	HRT Block Change
Hair Hg	M = 0 **F = 155**	M = 0 F = 0	M = 0 F = 0	M = 0 F = 0
Urine Hg	M = 0 F = 0	**M = 3** F = 0	M = 0 F = 0	M = 0 F = 0

Note: Significance threshold $q < 0.10$; F = female only models; M = male only models.

Table 7. Assessing interactions between DNAm and Hg exposure in their associations with CPT3 perseverations scores in males

		DNAm		Hg		Hg x DNAm	
Hg biomarker	***Gene* (Probe ID)**	**β (SE)**	***q*-value**	**β (SE)**	***q*-value**	**β (S.E.)**	***q*-value**
Urine	*RSL1D1* (cg03760895)	4.09×10^{-3} (0.02)	0.86	−6.7 (1.5)	0.02	0.07 (0.02)	0.02
Hair	*RSL1D1* (cg03760895)	−0.03 (0.03)	0.48	−1.92 (2.49)	0.98	0.02 (0.03)	0.98
Urine	*DRGX* (cg17227358)	−0.01 (0.01)	0.13	−2.05 (0.5)	0.03	0.02 (0.01)	0.03
Hair	*DRGX* (cg17227358)	1.67×10^{-3} (0.01)	0.91	−2.04 (1.2)	0.98	0.02 (0.01)	0.98
Urine	NA (cg19505184)	0.02 (0.02)	0.38	−5.71 (1.49)	0.06	0.06 (0.02)	0.06
Hair	NA (cg19505184)	0.02 (0.02)	0.5	−3.7 (2.01)	0.98	0.04 (0.02)	0.98

Table 8. Assessing interactions between DNAm and Hg exposure in their associations with CPT3 Omissions Error Scores in females (select results from 155 total statistically significant observations)

		DNAm		Hg		Hg x DNAm	
Hg biomarker	***Gene* (Probe ID)**	**β (SE**	***q*-value**	**β (SE)**	***q*-value**	**β (SE)**	***q*-value**
Hair	*GABRG3* (cg27553667)	0.02 (0.02)	0.96	0.09 (0.04)	0.15	−0.04 (0.02)	0.09
Urine	*GABRG3* (cg27553667)	0.06 (0.01)	4.57×10^{-4}	0.01 (0.03)	1	−0.01 (0.01)	1
Hair	*POLD4* (cg16509978)	-1.85×10^{-3} (0.01)	0.98	−0.43 (0.17)	0.07	0.01 (4.99×10^{-3})	0.09
Urine	*POLD4* (cg16509978)	−0.01 (4.71×10^{-3})	0.04	−0.04 (0.16)	1	7.40×10^{-4} (4.91×10^{-3})	1
Hair	*DAAM2* (cg06159534)	-3.80×10^{-3} (0.01)	0.96	2.01 (0.75)	0.07	−0.02 (0.01)	0.08
Urine	*DAAM2* (cg06159534)	0.01 (0.01)	0.37	0.65 (0.65)	1	−0.01 (0.01)	1
Hair	*TNFRSF10A* (cg23303108)	-4.88×10^{-3} (0.01)	0.96	−2.56 (0.77)	0.04	0.03 (0.01)	0.05
Urine	*TNFRSF10A* (cg23303108)	−0.02 (0.01)	0.04	−0.25 (0.73) 1	1	2.35×10^{-3} (0.01)	1
Hair	*STAT1* (cg07052015)	4.41×10^{-3} (0.02)	0.98	−0.24 (0.07)	0.04	0.06 (0.02)	0.06
Urine	*STAT1* (cg07052015)	−0.03 (0.01)	0.05	−0.01 (0.07)	1	-1.57×10^{-3} (0.02)	1
						… 300 more rows	

to study the effects of prenatal Hg exposure on neurological outcomes. To date, it is the longest longitudinal cohort of its kind. Davidson *et al* did not find consistent adverse patterns in the associations between prenatal Hg exposure and any of the 27 neurological outcomes tested, but some models suggested neuroprotective effects of Hg [16]. This is consistent with findings of earlier SCDS publications, utilizing data from younger participants [61, 62]. Davidson *et al*.noted that no PUFAs or other measures related to seafood intake were included in these models and that the beneficial relationships observed are likely due to confounding by these variables [16]. Another important factor to consider is the timing of the exposure, which was *in utero* for the Davidson *et al*.study. In a subsequent SCDS study, Thurston *et al*.describe several significant adverse associations between time-weighted *postnatal* hair Hg measures (from childhood to early adulthood) and outcomes related to attention, executive, and achievement domains observed in participants at 9, 17, 22, and 24 years of age. This study also did not control for seafood nutrient intake [15]. In summation, unmeasured confounding variables, innate population differences, variation in study design and sampling techniques, and potential epigenetic susceptibility may all contribute to contradictory findings across studies. Effort was taken in the present study to build upon past work, select the most appropriate combination of potential confounding variables as covariates, and consider the possible influence of epigenetic susceptibility in the relationship between Hg exposure and CPT3 performance.

The epigenetic mechanism DNAm has been shown to be associated with neurodevelopmental outcomes both in this cohort and in other studies. In Ehlinger *et al* 2023, we described statistically significant associations in ELEMENT adolescents between poorer ISI change performance and increased DNAm (hypermethylation) at four probes annotated to three genes, *ZNF814, ELF4*, and *OR6K6*. Two of these probes were annotated to *ZNF814*, encoding Zinc Finger Protein 814, and one of which was also significantly related to the block change outcome. In all examples, hypermethylation was associated with higher CPT3 scores, or poorer test performance. Notably, when models were stratified by gender, DNAm at two additional probes were significant in females for their associations with HRT block change scores. One of the probes, annotated to the *DSCR9* (Down Syndrome Critical Region 9) gene, exhibited greater methylation related to poorer test performance. The second probe (annotated to the *GLI3* gene), exhibited the opposite relationship, with lower DNAm levels related to poorer CPT3 scores. Interestingly, this gene encodes GLI Family Zinc Finger 3, which belongs to the C2H2-type zinc finger protein family similar to Zinc Finger Protein 814.

In addition to our study, previous publications have identified associations between DNAm and ADHD or ADHD-like symptoms in human cohorts. Mooney *et al*. 2020, for example, which was one of the first ADHD EWAS of its kind, identified potential novel epigenetic biomarkers for this disorder. This was accomplished through modelling associations between peripheral saliva DNAm and both ADHD diagnosis and polygenic risk burden in children via adjusted linear regression analyses [63]. In a more recent analysis conducted by Sun *et al*., researchers identified significant relationships between blood leukocyte DNAm and externalizing behaviours, grey matter volumes (reflecting structural brain networks), and behavioural problems during adolescence [64]. The results of this and other studies suggest DMRs may serve a role in the development of the brain and potentially contribute to the etiology of neurodevelopmental disorders like ADHD in children and adolescents [27, 28, 64]. Furthermore, specific EWAS results, especially when partnered with functional pathways analyses, can provide mechanistic implications regarding biological processes that might underlie disease onset and inform future research that takes a more targeted approach, as is necessary to corroborate any mechanistic insights suggested by such EWAS studies. Li *et al*., for example, identified a list of potentially relevant differentially methylated genes based on small EWAS analysis (9 controls and 12 ADHD cases) using whole blood DNA of Taiwanese children. Subsequently, pyrosequencing was used to quantify DNAm levels at candidate genes, for which significant associations were identified between hypermethylation at *LIME1* and hypomethylation at *SPTBN2* and poorer CPT performance [28]. Aberrant methylation at both genes may contribute to ADHD etiology through neuroinflammation and dysregulation of glutamate signalling pathways for *LIME1* and *SPTBN2*, respectively.

The present study is the first of its kind to examine epigenetic by environment (Hg) interactions. While there is evidence from previous studies to suggest that the effect of Hg on neurobehavioural outcomes may be mediated by DNAm at some CpG sites, mediation is not the only way DNAm could be influencing relationships between Hg exposure and neurobehaviour [27, 29]. Epigenetic susceptibility or effect modification by DNAm, as is modelled in the present study, is highly under studied in the context of toxicant-associated health effects. In all children, we identified significant interactions between childhood Hg exposure and DNAm at three distinct CpG sites. Two of these sites (cg10396484 and cg21442182) were significant in models using urine Hg and CPT3 omissions errors scores, and the last (cg05093254) was significant in models using hair Hg and ISI change scores. We can conceptualize these interactions using a simplified interpretation that assumes all other variables including Hg levels remain constant. In this scenario, higher levels of DNAm at the significant locus would result in the effects of Hg on CPT3 performance being augmented when the interaction term is positive. In other words, if the main effect of Hg were positive, then we would predict an even larger increase in CPT3 scores with more DNAm, but if β_{Hg} were negative, a more pronounced reduction in CPT3 score would be expected (Supplemental Figures S5 and S6). Negative interaction terms suggest that with higher levels of DNAm, the effect of Hg on CPT3 performance is reduced. Meaning that if the main effect of Hg was positive, it would result in a less dramatic increase in CPT3 scores. Conversely, if the main effect of Hg was negative, it would likely result in a less pronounced decrease in CPT3 scores (Figure S7). These simplified scenarios do not account for the magnitudes of effect for the DNAm × Hg interaction term, or the main effects of Hg and DNAm which also need to be factored in. Lastly, as with any model, the effects of all covariates need to be considered for the most complete and accurate interpretation of results.

The majority of findings were observed in the gender stratified models, with 155 CpG sites significantly interacting with hair Hg levels in females and 3 CpG sites significantly interacting with urine Hg in males for the omissions errors and perseverations outcomes, respectively (Table 6–8). These findings suggest that meHg interacts with DNAm in their associations with symptoms of inattention in females, but not necessarily in males. Gender appears to be relevant to both DNAm and Hg exposure in their associations with ADHD-like symptoms separately, but also in how the two predictors interact with one another in these associations. These gender-specific differences could be a result of baseline differences in hormones, metabolism, enzymatic activity, immune response, or DNAm levels in males relative to females, which ultimately influences how organic and inorganic Hg respond to each unique environment and affects the body. Gender-disparities, such as those reported in the present study, are often reported in Hg

toxicity studies [25, 65–69]. Past research supports that males may be more susceptible to the neurotoxic and behavioural effects of meHg than females [65, 67–70]. The present study suggests that there are gender differences in epigenetic susceptibility to Hg. Future research is needed to support this claim.

Because DNAm has been implicated in previous research as a potential mediator in the relationship between Hg exposure and aberrant neurodevelopment and behaviour, it was important to consider the potential mediating role of DNAm at the sites of significant interaction that we identified [27, 29]. In a sensitivity analysis of mediation for these significant sites of interaction, there was no evidence of mediation in all children or in the males for any of the models (Supplemental Table S3 and S4). In the female models, of the 155 CpG sites we tested for mediation, only one displayed a significant ACME value (P-value $< .05$), suggesting its potential role as a mediator (Supplemental Tables S5). It is possible that this phenomenon occurred from chance alone, with the significant mediation result being an artefact of the significant interaction term, or vice versa. Alternatively, both mediation and interaction may be true at this site. The remaining 154 CpG sites exhibited no evidence of mediation (Supplemental Tables S5). Since DNAm and the outcome were measured at the same time, future research with repeat measures should explore the question of temporality and compare results from both mediation and interaction models to corroborate this new concept of epigenetic susceptibility. Through *in vitro* or *in vivo* experimentation, for example, one could quantify DNAm at the beginning of an experiment prior to any meHg exposure and then again following an exposure period. If DNAm levels did not change throughout the course of the experiment but still interact with meHg levels to influence the outcome, then it supports this concept of epigenetic susceptibility.

While epigenetic susceptibility has not yet been modelled in past research, several studies suggest genetic factors predispose children to neurotoxic effects of meHg exposure. Lozano *et al.*, for example, in the same study previously described, reported significant interaction effects for single nucleotide polymorphisms (SNPs) in three genes: the *GSTP1*, *BDNF*, and *APOE* [7]. In another study, a population of Portuguese children, comparable in age to those included in the present study, were subjected to several neuropsychological batteries, urinary Hg analysis, and genotyping for variants in 13 neurodevelopmentally relevant genes. Several associations between Hg exposure and neurobehaviour were significantly modified by genetic variation, but mostly in boys [71]. In addition to genetic variants, epigenetic factors have been implicated in the relationship between aberrant neurodevelopment and Hg exposure but are seldom modelled together much less through interaction [29, 72]. One study conducted by Cardenas *et al*, for instance, performed an EWAS to identify genes differentially methylated by prenatal Hg exposure (*PON1*, *WBP11P1*, and *TOR4A* were significant) and a subsequent analysis linking DNAm at these sites to cognitive performance in early childhood (modified by gender). While measures of Hg exposure, DNAm, and behavioural outcomes were all recorded, the authors did not assess the variables simultaneously [72].

The present study exhibits many strengths, as it addresses gaps in the literature and does so in an underrepresented Hispanic population. Furthermore, this research focuses on childhood exposures, which are important neurodevelopmental timepoints that are vulnerable to the influence of toxic exposures and aberrant DNAm. By modelling interaction between the two main predictors of interest, DNAm and Hg exposure, we assess a novel concept we termed epigenetic susceptibility. This has the potential to be used in future research including for other toxicants and disease outcomes. Significant DNAm–Hg interactions, as were identified in the present study, may serve as novel biomarkers of disease, refine risk assessment, and/or provide mechanistic insight that may be overlooked in assessments of either variable alone. DNAm is more malleable and dynamic in nature than its genetic counterpart, which may have mechanistic implications that can inform future therapeutic research. One notable weakness is the fact that DNAm was measured in blood, rather than brain, the target tissue of interest. While DNAm in biologically available blood is often correlated to DNAm in the brain, caution must be taken in interpretation. Mechanistic implications should be validated in *in vitro* or *in vivo* experiments. Another limitation is availability of data; childhood PUFA data (model 1) was not available for as many participants as adolescent PUFA data (model 2), limiting the sample size in analyses with model 1. PUFAs can potentially affect Hg levels through detoxifying pathways and support healthy brain development and function throughout one's life, making the variable at either timepoint potential confounders. Another weakness of the study is the temporality of the interacting variables, with Hg quantified in childhood and DNAm measured concurrently with the CPT3 outcomes in adolescence. Since Hg and DNAm measures were not available at both time points, we cannot substantiate the assumption that Hg levels and/or DNAm profiles remained similar over time. Future epigenetic susceptibility studies should consider the temporal relationship of the exposure and the DNAm variable and use concurrent measurements if available.

While the average hair and urine Hg levels in the children used this study were reported at levels between three and five times those of similarly aged populations in the USA, they are not nearly as high as Hg levels in children in populations with high seafood consumption, i.e. in the Faroe Islands or in Seychelles [5, 34, 61, 73]. Notably, average seafood intake in Mexico is comparable to that of the USA, consumption of meHg-rich species of fish, especially schoolshark and canned tuna, is more frequent in Mexico [74]. In a study by Basu *et al*, assessing mercury levels in common sources of seafood from Mexico City, 7 out of the 23 seafood species tested (including schoolshark) and 5 out of 9 brands of canned tuna exhibited mean Hg levels higher than the US EPA guidance level (0.3 μg/g) [34]. Still, the participants in this study exhibited a broad range of both low and high hair and urine Hg levels, representative of populations with varying levels of exposure across the world. Additionally, the ELEMENT participants' average CPT3 performance was comparable to that of populations of children of similar ages in different parts of the world with broad ranges of values [28, 75, 76]. A T-score of 50 (standard deviation $\pm$ 10) is regarded as average based on epidemiological evidence from children and adolescents in the USA [77].

Conclusion

This research used an innovative approach to examine relationships between childhood Hg exposure, adolescent DNAm, and adolescent CPT3 scores. In a cohort of Mexican children, we demonstrate associations between hair and urine Hg levels and four outcomes meant to assess four distinct aspects of attention: inattention, impulsivity, vigilance, and sustained attention. Lastly, we combine the principles of the first two models and considered the interaction of DNAm and Hg exposure on the CPT3 outcomes. Through this, we identified numerous significant interactions between DNAm and Hg exposure in all children and gender stratified models, suggesting that in some instances DNAm may influence the associations between Hg exposure and

ADHD-like behaviours. This innovative model of Epigenetic × Environment interaction is the first study of its kind and can be modified by future researchers interested in epigenetic susceptibility to toxicant effects.

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Supplementary Data

Supplementary data is available at *EnvEpig* online.

Conflict of interest. The authors wish to disclose that Dana Dolinoy has served as an expert witness in a legal case involving providing scientific expertise and testimony. We confirm that this role has not influenced the design, implementation, or reporting of the research presented in this manuscript. Apart from those disclosed, the authors have no other financial involvement or relevant affiliations with any organization or entity with a financial interest in or conflict with the subject matter or materials discussed in this manuscript.

Data availability

Epigenetic and demographic data from the ELEMENT study can be accessed through the NIH Human Health Exposure Analysis Resource data repository (doi: 10.36043/1431 392, 10.36043/1431 327 and 10.36043/1431 393). Access to additional data may be granted upon request from the ELEMENT research team.

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