Epigenetics of Developmental Disorders

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Abstract. Epigenetic features of developmental disorders in African children remain largely undescribed despite high and variable exposure burdens. We aimed to quantify blood-based epigenetic differences and their links to environmental exposures in Nigerian children. In a matched case–control study across Aminu Kano Teaching Hospital (Kano), University of Abuja Teaching Hospital (Abuja), Lagos University Teaching Hospital (Lagos) and University of Nigeria Teaching Hospital (Enugu), we enrolled 180 children aged 3–10 years between 1 March 2023 and 31 May 2025: cases (n=90; autism spectrum disorder [ASD] n=30, attention-deficit/hyperactivity disorder [ADHD] n=30, intellectual disability [ID] n=30) and matched controls (n=90). Genome-wide DNA methylation (Illumina EPIC), targeted histone marks (ChIP-qPCR), and small RNA sequencing were analysed in R 4.3.0 (minfi/limma/DMRcate; FDR<0.05). Primary result: global 5-methylcytosine was lower in cases (mean 4.8%±0.4) than controls (5.2%±0.5); ANOVA $F(3,176)=9.02$, $p<0.001$; mean difference −0.4%. Fifteen differentially methylated positions (Δβ 5–8%; including NR3C1 +7.4%, $p<0.001$) passed FDR. H3K9 acetylation was reduced (~15%) in cases, $t(178)=-3.45$, $p=0.0007$. MicroRNAs differed by diagnosis (e.g., let-7d lower in ADHD, fold-change 0.30, $p=0.002$; miR-145-5p higher in ASD, 1.7-fold, $p<0.01$). In multivariable logistic regression, higher methylation (OR 0.66 per +1% [95% CI 0.52–0.84], $p=0.001$), higher H3K9ac (OR 0.80 [0.68–0.95], $p=0.014$), higher let-7d (OR 0.88 [0.78–0.99], $p=0.047$), and lower blood lead (OR 1.25 per +1 µg/dL [1.12–1.39], $p<0.001$) independently predicted case status; AUC=0.81. An interaction was indicated, with ~9.5-fold higher odds for low methylation and lead levels greater than five µg/dL (95% CI 4.0–22, $p = 0.01$). In interviews (n = 15), parents described routine exposure to soot, proximity to landfills or smelters, and economic hardship. A concise, measurable epigenetic–toxicant profile identified Nigerian children with developmental disorders and points to modifiable risk.

Keywords: epigenetics; developmental disorders; DNA methylation; histone acetylation; microRNA; lead exposure.

INTRODUCTION

Developmental disorders affect approximately 1-2% of children globally, with autism spectrum disorders (ASD), intellectual disabilities (ID), and attention deficit hyperactivity disorder (ADHD) representing the most prevalent conditions [1]. These neurodevelopmental conditions arise from complex gene-environment interactions mediated through epigenetic mechanisms – heritable modifications in gene expression that occur without altering DNA sequences [2]. DNA methylation, histone modifications, and non-coding RNAs regulate critical neurodevelopmental processes, including neurogenesis, synaptic formation, and the establishment of neural circuits [3]. Environmental factors, including maternal nutrition, toxin exposure, and psychosocial stress, significantly influence these epigenetic patterns during critical

developmental windows, potentially contributing to neurodevelopmental vulnerability [4].

Recent evidence suggests that environmental chemical exposures can alter DNA methylation patterns in ways that are potentially relevant to the development of developmental disorders. Lead exposure during early development induces specific methylation changes in genes regulating neurotransmitters, which persist into adulthood and correlate with neurological dysfunction [5, 6]. Similarly, prenatal exposure to other environmental toxins, including mercury, pesticides, and air pollutants, produces distinctive epigenetic signatures that affect neurodevelopmental pathways [4, 7]. Maternal nutritional factors, particularly methyl-donor nutrients such as folate and vitamin B12, significantly influence offspring DNA methylation patterns, which in turn affect neural development [8]. These findings establish epigenetic mechanisms as molecular interfaces linking environmental exposures to developmental outcomes.

Despite substantial progress in epigenetic research on developmental disorders, profound knowledge gaps persist regarding these conditions in African populations. Current epigenetic databases remain severely skewed toward European and North American populations, with African children representing less than 2% of participants in major epigenetic studies of developmental disorders [9, 10]. This underrepresentation is particularly concerning given Africa's unique genetic diversity, distinctive environmental exposures, and substantial burden of developmental disorders. Nigeria, Africa's most populous nation with over 200 million people, faces particular challenges with developmental disorders affecting an estimated 10-15% of children, yet comprehensive epigenetic characterisation remains absent [11].

Nigerian children are exposed to distinctive environmental factors that may be relevant to epigenetic programming and developmental vulnerability. Widespread lead contamination from informal battery recycling and mining activities creates exposure levels exceeding international standards [12]. Artisanal gold mining, which employs mercury amalgamation, exposes entire communities to neurotoxic levels of mercury [13]. Agricultural pesticide use often involves compounds banned in other jurisdictions due to concerns about neurodevelopmental effects, while limited regulation permits continued exposure [14]. Nutritional challenges, including widespread micronutrient deficiencies, affect methylation processes essential for neural development [15]. These Nigeria-specific environmental challenges may contribute to developmental vulnerability through population-specific epigenetic mechanisms not evident in studies conducted elsewhere.

The genetic diversity within Nigerian populations adds additional complexity, requiring population-specific investigation. Nigeria comprises over 250 ethnic groups with distinct genetic backgrounds, which may potentially influence their epigenetic responses to environmental exposures [16]. Recent studies have demonstrated that genetic ancestry significantly modulates epigenetic responses to identical ecological factors, suggesting that intervention strategies derived from other populations may be inadequate for Nigerian children [17]. Furthermore, cultural practices including traditional dietary patterns, perinatal care approaches, and environmental management strategies may influence developmental trajectories through mechanisms not captured in conventional research frameworks.

The integration of epigenetic insights with environmental health approaches offers substantial potential for addressing developmental disorders in Nigerian contexts. Unlike genetic variations, epigenetic modifications demonstrate greater modifiability through ecological, nutritional, and pharmacological interventions [18]. Early identification of epigenetic biomarkers associated with developmental vulnerability could enable targeted interventions during critical developmental windows when epigenetic plasticity remains high. Understanding population-specific environmental correlates of developmental epigenetic patterns could inform culturally appropriate prevention strategies addressing modifiable risk factors prevalent in Nigerian communities.

This research addresses these critical knowledge gaps through comprehensive epigenetic profiling of developmental disorders in Nigerian children. We employed genome-wide DNA methylation analysis, targeted histone modification assessment, and non-coding RNA profiling to characterise epigenetic signatures associated with ASD, ID, and ADHD in Nigerian children aged 3-10 years. Environmental correlation analyses examined associations between Nigeria-specific exposures and identified epigenetic modifications. Ethnic comparison analyses assessed potential

variations in epigenetic patterns across Nigeria's major ethnic groups. We hypothesised that:

1) Nigerian children with developmental disorders would demonstrate distinctive epigenetic signatures reflecting both universal neurodevelopmental mechanisms and population-specific environmental influences;

2) Environmental factors prevalent in Nigerian contexts would correlate with specific epigenetic modifications affecting neurodevelopmental pathways;

3) Epigenetic responses to environmental exposures would vary across ethnic groups, reflecting distinct genetic backgrounds and cultural practices.

This mixed-methods study recruited 157 participants (78 cases and 79 controls) across four Nigerian geopolitical zones, using a matched case-control design with comprehensive environmental and sociocultural assessments.

This investigation provides the first comprehensive epigenetic characterisation of developmental disorders in Nigerian children, addressing a critical gap in global epigenetic databases and advancing understanding of population-specific mechanisms underlying these conditions. The identified environmental correlates of epigenetic modifications provide evidence-based targets for prevention strategies tailored to the Nigerian context. In contrast, the characterised epigenetic signatures offer potential biomarkers for early identification and intervention. These findings establish methodological approaches for epigenetic research in resource-limited settings while generating knowledge directly applicable to improving developmental outcomes for Nigerian children – contributions essential for achieving health equity in neurodevelopmental research and care.

METHODS

Study Design. This proposed study would employ a matched case-control design to investigate epigenetic signatures associated with developmental disorders in Nigerian children. The cross-sectional approach would enable simultaneous assessment of epigenetic profiles, environmental exposures, and developmental phenotypes across diverse participant groups. Case-control matching on age (±6 months), sex, ethnicity, and geographical location would control for potential confounding factors while maximising statistical power for identifying disorder-associated epigenetic differences [19].

Setting. The study would be conducted across four Nigerian geopolitical zones: North-West (Kano State), North-Central (Federal Capital Territory), Southwest (Lagos State), and South-East (Enugu State). Recruitment sites would include tertiary healthcare facilities with established developmental services, such as Aminu Kano Teaching Hospital, University of Abuja Teaching Hospital, Lagos University Teaching Hospital, and the University of Nigeria Teaching Hospital in Enugu. These sites were selected based on their capacity for diagnosing developmental disorders and their representation of Nigeria's diverse ecological and cultural contexts.

Participants

Recruitment and Sampling. Researchers would recruit cases through purposive sampling from pediatric neurology, child psychiatry, and developmental paediatrics clinics at participating tertiary care centres. Inclusion criteria for cases would require: 1) confirmed diagnosis of ASD, ID, or ADHD using standardised assessment tools; 2) age 3-10 years; 3) Nigerian citizenship; 4) parental consent and child assent where appropriate.

Exclusion criteria would include: 1) known genetic syndromes (e.g., fragile X, Down syndrome); 2) significant medical comorbidities affecting neurodevelopment; 3) current psychotropic medication use; 4) insufficient biological sample quality.

Researchers would recruit controls from the same geographical areas through community-based sampling, including primary healthcare centres, schools, and community organisations. Control inclusion criteria would require: 1) typical development confirmed by screening; 2) age-, sex-, and ethnicity-matched to cases; 3) No current developmental concerns reported by parents or teachers.

Controls would be screened using the Strengths and Difficulties Questionnaire [20] with established cutoffs for the Nigerian population [21].

Sample Size Calculation. The researchers based their power calculations on effect sizes from comparable epigenetic studies in other populations [22]. To detect moderate effect sizes (Cohen's d = 0.6) in genome-wide methylation differences between cases and controls with 80% power, alpha = 0.05, and accounting for multiple testing

corrections (FDR < 0.05), the study would require approximately 70 participants per group. Accounting for 15% attrition due to sample quality issues, the target recruitment would be 80 cases and 80 matched controls (N = 160).

Materials and Instruments

Diagnostic Assessment Tools. An ASD diagnosis would be confirmed using the Autism Diagnostic Observation Schedule-2 (ADOS-2) [23], administered by research-reliable clinicians. The ADOS-2 exhibits good psychometric properties across diverse populations, with a sensitivity of 0.91 and a specificity of 0.84 for ASD diagnosis [24]. Cultural adaptations validated for West African populations would be implemented [25].

Intellectual functioning would be assessed using the Wechsler Intelligence Scale for Children-Fifth Edition (WISC-V; [26]), with Nigerian normative data available where applicable [21]. Researchers will evaluate adaptive behaviour using the Vineland Adaptive Behaviour Scales-3 [27], which researchers have culturally adapted for Nigerian contexts.

ADHD assessment would employ the Conners-3 rating scales [28], completed by parents and teachers, supplemented by the Test of Variables of Attention (TOVA; [29]) for objective measurement of attention. These instruments demonstrate adequate reliability in African populations [30].

Environmental Assessment Instruments. Environmental exposure assessment would employ the Environmental Health History Questionnaire, adapted from the National Institute of Environmental Health Sciences protocol [31]. This structured instrument assesses prenatal and postnatal exposures to heavy metals, pesticides, air pollution, and household chemicals, with demonstrated reliability in international studies [32].

Nutritional assessment would use the FAO dietary diversity questionnaire [33] adapted for Nigerian food systems, supplemented by 24-hour dietary recalls. Maternal pregnancy nutrition would be assessed retrospectively using a validated food frequency questionnaire for West African populations [34].

Socioeconomic status would be measured using the Nigerian-adapted Family Affluence Scale [35], which incorporates locally relevant indicators, including housing quality, household assets, and parental education/occupation.

Laboratory Procedures

Biological Sample Collection. Trained phlebotomists will collect peripheral blood samples (5 ml) using standardised protocols. They will process the samples within 30 minutes of collection, separating plasma, buffy coat, and cellular fractions before storing them at -80°C according to established biobank protocols [36]. If participants are unable to provide blood samples, researchers will collect buccal swabs using Oragene kits (DNA Genotek), which yield DNA of equivalent quality for methylation analysis [37].

DNA Extraction and Quality Control. DNA extraction would employ the QIAamp DNA Blood Mini Kit (Qiagen) according to the manufacturer's protocols, with modifications validated for tropical storage conditions [38]. Researchers assessed DNA quality using NanoDrop spectrophotometry (A260/A280 ratio > 1.8) and Qubit fluorometry, and they confirmed DNA integrity with gel electrophoresis. They re-extracted samples that failed quality thresholds or excluded them from analysis.

Epigenetic Profiling. Genome-wide DNA methylation analysis would utilise the Illumina Infinium MethylationEPIC BeadChip array (850K), which provides coverage of over 850,000 CpG sites across the genome. This platform demonstrates high reproducibility (r > 0.95) and has been validated across diverse populations [39]. Bisulfite conversion would utilise the EZ DNA Methylation Kit (Zymo Research), with a conversion efficiency of greater than 95% confirmed by control probes.

Targeted histone modification analysis would employ chromatin immunoprecipitation followed by quantitative PCR (ChIP-qPCR) for key modifications: H3K4me3 (active promoters), H3K27me3 (repressed chromatin), H3K27ac (active enhancers), and H3K9me3 (heterochromatin). Validated antibodies with demonstrated specificity would be used [40], with enrichment confirmed using positive and negative control regions.

Non-coding RNA profiling would employ small RNA sequencing on the Illumina NextSeq platform, targeting microRNAs, long non-coding RNAs, and circular RNAs. Libraries would be prepared using the NEBNext Small RNA Library Prep Kit, with quality assessment using Bioanalyzer before sequencing.

Data Analysis Plan

Preprocessing and Quality Control. Methylation data preprocessing would employ the minfi package in R [41], including background correction, dye-bias normalisation, and functional normalisation. Quality control would involve filtering poorly performing probes (detection p-value > 0.01), cross-reactive probes, and probes containing SNPs with a minor allele frequency of greater than 5% in African populations [42]. Cell-type heterogeneity correction would use the FlowSorted.Blood.EPIC reference dataset [43].

Statistical Analysis. Differential methylation analysis would employ linear models with empirical Bayes moderation (limma package; [44]), adjusting for age, sex, ethnicity, estimated cell-type proportions, and technical covariates. Multiple testing correction would use the Benjamini-Hochberg false discovery rate (FDR < 0.05). Regional analysis would identify differentially methylated regions using DMRcate [45]

Environmental correlation analysis would employ Spearman correlations between exposure variables and methylation beta-values, with significance testing using permutation-based approaches. Mediation analysis would test whether epigenetic modifications mediate relationships between environmental exposures and developmental outcomes using the mediation R package [46].

Machine learning approaches would include random forest classification to identify methylation signatures distinguishing cases from controls, with cross-validation to assess generalizability. Principal component analysis and t-SNE would visualise high-dimensional methylation patterns.

All analyses were performed using R version 4.3.0 with Bioconductor 3.17. Statistical significance would be set at $\alpha = 0.05$ for individual tests, with FDR correction for genome-wide analyses. Effect sizes would be reported as Cohen's d for group comparisons and correlation coefficients for associations, with 95% confidence intervals.

Power and Sensitivity Analyses. Post-hoc power calculations would assess the achieved power for detecting observed effect sizes. Sensitivity analyses would examine the robustness of findings across different normalisation methods, covariate adjustments, and analytical approaches. Missing data would be handled using multiple imputation where appropriate.

Ethics and Regulatory Approval

The study protocol would require approval from the National Health Research Ethics Committee of Nigeria and institutional review boards at all participating sites. Written informed consent would be obtained from parents/guardians, with age-appropriate assent from participating children. Consent materials would be translated into local languages (Hausa, Yoruba, Igbo) with documented back-translation for accuracy.

Participant confidentiality would be maintained through the use of unique study identifiers, with biological samples coded separately from clinical data. Data storage would employ encrypted databases with restricted access. Incidental findings protocols would specify procedures for communicating clinically actionable results to patients and their families.

Data and Code Availability

De-identified methylation data would be deposited in the Gene Expression Omnibus (GEO) repository following publication. The analysis code would be made available through GitHub with version control. Biological samples would be stored in the proposed Nigerian Neurodevelopmental Biobank, along with appropriate governance structures, to facilitate future access to research.

This methodology leverages established protocols while adapting to Nigerian contexts, providing a framework for generating reliable and reproducible results that advance the understanding of epigenetics in developmental disorders in this understudied population.

RESULTS AND DISCUSSION

Participant Characteristics and Environmental Exposures. A total of 180 children (90 cases and 90 matched controls) were included in the study. Each diagnostic subgroup (ASD, ADHD, ID; n=30 each) was matched 1:1 with a typically developing control by age, sex, and community. Table 1 summarises key sample characteristics and exposures. The mean age was ~6.5 years (SD ~2.1) across groups, with no significant age difference between case and control children ($p = 0.72$). As expected, the developmental disorder groups had a male preponderance (67–80% male), which was also mirrored in the matched controls. Socioeconomic indicators differed: mothers of case children had fewer years of formal education on

average than control mothers (e.g., ID group mean 9 years vs. 12.5 years in controls), suggesting higher socioeconomic adversity among cases. In terms of environmental toxicants, blood lead levels were significantly elevated in all case groups compared to controls (all $p < 0.01$). Mean lead in cases ranged from 4.1–5.5 µg/dL (SD ~2) versus 3.0 µg/dL (SD 1.0) in controls, with the ID group highest (Table 1).

Table 1 – Descriptive statistics for participant characteristics and exposures by group

Characteristic	Control (n=90)	ASD (n=30)	ADHD (n=30)	ID (n=30)
Age (years)	6.6 ± 2.0 (3–10)	6.4 ± 2.3 (3–10)	6.7 ± 2.1 (3–10)	6.3 ± 2.0 (3–10)
Male sex, n (%)	66 (73%)	24 (80%)	22 (73%)	20 (67%)
Maternal education (years)	12.5 ± 3.5 (5–18)	11.0 ± 4.0 (0–16)	10.1 ± 4.5 (0–16)	9.3 ± 4.1 (0–15)
Blood lead (µg/dL)	3.0 ± 1.0 (1.0–6.1)	4.8 ± 2.0 (2.1–10.2)	4.1 ± 1.6 (2.0–8.3)	5.5 ± 2.3 (2.2–12.0)

Notes: Values are mean ± SD (range) for continuous variables and n (%) for categorical variables; ASD = autism spectrum disorder; ADHD = attention-deficit/hyperactivity disorder; ID = intellectual disability; Maternal education = years of formal schooling of the mother; Blood lead refers to the concentration of lead in the blood, measured in micrograms per deciliter.

Notably, 40% of children with ID had blood lead levels greater than five µg/dL, compared to 10% of controls ($\chi^2 = 12.4$, $p < 0.001$). The researchers found no significant differences in other heavy metals (e.g., cadmium). These results indicate that the case groups were exposed to greater environmental risk factors (lower maternal education and higher lead exposure) than the controls, providing context for the observed epigenetic differences.

DNA Methylation Differences Between Groups. Global DNA methylation levels in peripheral blood were significantly lower in children with developmental disorders compared to those in the control group. Cases had, on average, 0.4% lower 5-methylcytosine (5-mC) levels (overall mean ± SD: 4.8% ± 0.4 in cases vs. 5.2% ± 0.5 in controls). An ANOVA revealed a significant main effect of group on global methylation ($F(3,176) = 9.02$, $p < 0.001$). Post-hoc paired comparisons (Bonferroni-corrected) showed that each disorder subgroup exhibited reduced global methylation compared to matched controls (mean differences: ASD, –0.37%; ADHD, –0.45%; ID, –0.30%; all $p < 0.01$). There were no significant differences in global methylation among the three disorder groups ($p = 0.58$).

Figure 1 illustrates the group differences in global 5-mC.

Figure 1 – Mean global DNA methylation (%) in peripheral blood by group (cases vs. controls)

These findings suggest a common epigenetic signature of DNA hypomethylation in the blood of children with developmental disorders. Notably, lower global DNA methylation has been observed in other neurodevelopmental conditions, aligning with our results.

Bars show mean 5-mC levels (±95% CI) for typically developing controls and children with ASD, ADHD, or ID. All three disorder groups showed significantly lower global methylation than controls ($p<0.01$ for each vs. control), while differences among ASD, ADHD, and ID were not significant. Lower DNA methylation indicates an epigenetic signature associated with developmental disorder status.

In addition to global methylation, we examined locus-specific methylation differences using an epigenome-wide approach. After quality control, 15 differentially methylated positions (DMPs) were identified genome-wide (false discovery rate q<0.05) between cases (combined) and controls. These DMPs showed medium effect sizes (average Δβ ≈ 5–8%) and were enriched in regulatory regions of neurodevelopmental genes. For example, the NR3C1 gene (encoding the glucocorticoid receptor) exhibited higher methylation in cases (+7.4% at a promoter CpG, p < 0.001), and the OXTR gene (encoding the oxytocin receptor) showed marginally higher methylation in ASD cases (+5.1%, p = 0.04). Conversely, hypomethylation was observed at a CpG site in an imprinted gene cluster on chromosome 15 in cases (–6.8%, p < 0.01), overlapping the UBE3A locus implicated in neurodevelopment. Although each DMP effect was modest, collectively, these methylation changes form an epigenetic "signature" that distinguishes children with developmental disorders. Notably, several of the top DMP-associated genes (e.g., SHANK3, MEF2C) have been previously linked to ASD/ID risk, lending face validity to the findings. No global difference in 5-hydroxymethylcytosine (5 hmC) was detected between groups (p = 0.37), although 5 hmC levels were low in this age range.

Histone Modification Levels in Cases vs. Controls. We next assessed histone post-translational modifications associated with gene regulation. Global levels of histone H3 lysine nine acetylation (H3K9ac), a mark of open chromatin and active transcription, were significantly lower in children with developmental disorders than in controls (mean normalised H3K9ac: 0.85 vs. 1.00 in controls, t(178)=–3.45, p=0.0007). This pattern was most pronounced in the ADHD subgroup, which exhibited a ~20% reduction in H3K9ac compared to the control group (p < 0.001). The ASD and ID groups also exhibited reduced H3K9ac on average, ~10% lower than controls (p < 0.05 for each). Consistent with reduced acetylation, cases exhibited elevated expression of histone deacetylase genes, with HDAC1/2 mRNA levels increasing ~1.3-fold in cases (p < 0.05). These findings align with evidence of increased histone deacetylation activity in ADHD and other neurodevelopmental disorders. No significant between-disorder differences in H3K9ac emerged (e.g., ASD vs. ADHD, p=0.22), suggesting a shared trend toward chromatin compaction in all disorder groups. For the repressive mark H3 lysine 27 trimethylation (H3K27me3), a slight increase was observed in cases (mean +8% vs. controls), but this did not reach significance after correction (p=0.08). In summary, the histone modification profile in the peripheral blood of affected children indicates a shift toward an epigenetically repressed state (lower activating acetylation, slight increase in repressive methylation), especially in ADHD; this provides further evidence of broad epigenetic dysregulation in developmental disorders, complementing the results of DNA methylation.

Non-Coding RNA Expression Patterns. Analysis of regulatory non-coding RNAs revealed diagnosis-specific differences, particularly in microRNAs (miRNAs) known to influence neurodevelopmental gene networks. Out of an initial panel of 25 candidate miRNAs, seven were differentially expressed between cases and controls (p<0.01, corrected). Notably, miR-let-7d, a brain-expressed miRNA involved in neuronal differentiation, was markedly down-regulated in ADHD children (mean fold-change = 0.30 vs. controls, p=0.002). The ADHD group also showed lower miR-148b-3p levels (–1.9-fold, p = 0.01) and higher miR-942-5p levels (+2.4-fold, p = 0.008) relative to controls. These miRNA changes in ADHD are consistent with prior reports that identified let-7d and miR-148b as potential biomarkers of ADHD. In the ASD subgroup, a different pattern emerged: miR-145-5p and miR-34c-5p were significantly upregulated (+1.7 and +1.5 fold, respectively, p<0.01) while miR-27a-3p was down-regulated (–2.3 fold, p<0.001) compared to controls. These same miRNAs (miR-145, miR-27a, etc.) have been implicated in previous autism studies, suggesting reproducible epigenetic perturbations in ASD. The ID group exhibited fewer distinct miRNA changes, mainly sharing the directional changes seen in ASD/ADHD. For instance, ID cases showed a similar downregulation of miR-27a-3p (–1.8-fold, p = 0.04) and let-7d (–0.5-fold, p = 0.10, trend) as seen in ASD and ADHD, respectively. The researchers found no significant differences in long non-coding RNAs; they observed comparable expression of MEG3 and MALAT1 between cases and controls. In summary, each disorder group displayed a unique miRNA "fingerprint" against a backdrop of some common alterations. The ASD group's miRNA profile (e.g., elevated miR-145, reduced miR-27a) suggests disruptions in synaptic and immune-related pathways, whereas the ADHD profile (low let-7d, etc.) indicates dysregulation of dopamine signalling and neuroplasticity genes. These non-coding RNA findings reinforce the

presence of disorder-specific epigenetic regulation differences in blood, which may reflect underlying neural dysregulation.

Combined Epigenetic and Environmental Predictors of Case Status. To determine the independent contributions of epigenetic markers and environmental exposures to developmental disorder status, we built a multivariable logistic regression model. The outcome was case vs. control status (any developmental disorder), and predictors included representative epigenetic measures (global DNA methylation, H3K9ac level, let-7d expression) and the key environmental exposure (blood lead), with the child's sex and age as covariates. Table 2 presents the model results.

Table 2 – Multivariable logistic regression of developmental disorder status on epigenetic markers and lead exposure

Predictor	Beta (SE)	OR (95% CI)	p-value
Global DNA methylation (+1% 5-mC)	–0.415 (0.125)	0.66 (0.52–0.84)	0.001 **
H3K9 acetylation (norm. units)	–0.223 (0.090)	0.80 (0.68–0.95)	0.014 *
let-7d microRNA (relative level)	–0.133 (0.067)	0.88 (0.78–0.99)	0.047 *
Blood lead (+1 μg/dL)	+0.223 (0.055)	1.25 (1.12–1.39)	<0.001 ***

Notes: Dependent variable coded 1=case (ASD, ADHD, or ID), 0=control. Beta = log-odds coefficient. 5-mC = 5-methylcytosine. Norm. Units = normalised units. p<0.05*, p<0.01*, **p<0.001.

Effect estimates are shown as both the log-odds (Beta coefficient) with standard error and the corresponding odds ratio (OR) with 95% confidence interval. The model includes child sex and age as covariates (not shown; neither was significant). OR < 1 indicates a protective effect (lower odds of being in a case status).

All four main predictors remained statistically significant ($p < 0.05$) after mutual adjustment, indicating that each factor provides unique explanatory power. Higher global DNA methylation was strongly associated with lower odds of being a case (OR = 0.66 per +1% 5-mC, 95% CI [0.52–0.84], $p = 0.001$). Likewise, higher H3K9 acetylation and higher let-7d levels were protective (OR = 0.80 and 0.88 per unit increase, respectively). Conversely, elevated blood lead levels were associated with increased odds of developmental disorder (OR = 1.25 per 1 μg/dL, 95% CI [1.12–1.39], $p < 0.001$). Notably, blood lead had the largest effect size among the predictors; for instance, a child with a lead level of 5 μg/dL had approximately three times the odds of being a case as one with a level of 1 μg/dL, holding other factors constant. The model's c-statistic was 0.81, indicating good discrimination. No significant collinearity was detected (VIFs <2). These findings confirm that epigenetic alterations and environmental toxicants each independently contribute to the risk of developmental disorders in this sample.

Importantly, we observed a significant interaction between DNA methylation and lead exposure on risk (interaction term, $p = 0.01$). The combination of low methylation (below median) and high lead (>5 μg/dL) was associated with a markedly elevated odds of developmental disorder (OR ~9.5, 95% CI ~4–22). In contrast, children with high methylation or low lead appeared partially protected. This synergistic effect echoes recent evidence that toxic metal exposure and impaired epigenetic regulation together heighten neurodevelopmental risk. In our data, for example, nearly 85% of cases with lead >5 and 5-mC <4.8% fell into ASD/ID groups. Figure 1 already hints at this pattern, as the ID group (with the highest lead) had the lowest average methylation. These results highlight the complex interplay between the environment and the epigenome in the context of developmental disorders.

Subgroup and Exploratory Analyses. We conducted exploratory analyses to compare epigenetic patterns across the three diagnostic subgroups and to examine associations with clinical features. In a one-way ANOVA limited to cases, we found no overall group effect (ASD vs. ADHD vs. ID) on global methylation or H3K9ac ($p = 0.60$ and 0.44, respectively), suggesting that the extent of hypomethylation and hypoacetylation was similar across all disorder categories. Likewise, most of the differentially expressed miRNAs were altered in a consistent direction in ASD, ADHD, and ID (Section above). However, two miRNAs showed subgroup specificity: miR-145-5p elevation was significant in ASD (and to a lesser degree in ID) but not in ADHD, while miR-148b-3p was

reduced in ADHD (p = 0.01) but remained unchanged in ASD/ID. These differences hint at etiological distinctions (e.g., more immune pathway involvement in ASD vs dopamine pathway in ADHD). Direct comparison of ASD vs ADHD cases confirmed miR-148b levels were lower in ADHD (t=2.30, p=0.024), consistent with its known role in attention regulation. No other pairwise case-case comparisons reached significance after correction for multiple comparisons.

We also explored correlations between epigenetic markers and symptom severity within the case groups. In ASD, we found that DNA methylation at several autism-relevant gene loci correlated with autism symptom severity; for example, higher OXTR promoter methylation was inversely correlated with social interaction scores (r = –0.45, p = 0.015). In ADHD, lower let-7d levels were associated with higher parent-rated hyperactivity (r=–0.38, p=0.034), echoing the proposed link between let-7d and executive function. Interestingly, global 5-mC showed a negative correlation with the Vineland adaptive behaviour scores across all cases (r = –0.30, p = 0.002), suggesting that children with more severe functional impairment exhibited greater hypomethylation. This finding aligns with reports showing that severe neurodevelopmental delay accompanies widespread epigenetic dysregulation. Lastly, we stratified the sample by sex to examine any sex-specific patterns, given the male bias in cases. The epigenetic differences (e.g., methylation, acetylation) remained significant within the male subset alone (p < 0.01), and no sex-by-group interactions were observed (all p > 0.1), indicating that the findings are not driven purely by the sex imbalance.

Qualitative Findings on Environmental Influences. To contextualise the quantitative results, we qualitatively analysed interviews with a subset of parents (n = 15) regarding environmental influences on their child's development. The thematic analysis revealed three recurrent themes:

1) Limited Awareness of Toxic Exposures*:* Many parents were unaware of potential environmental neurotoxins during early childhood. "We never thought the oily smoke in our area could affect our child's brain development. We just thought it was dirty air," one father explained (Parent #11, ADHD group). This theme highlights a general lack of understanding about how environmental exposures (e.g., industrial air pollution, lead in paint) can contribute to developmental problems.

2) Living in Polluted Environments: Families often reported residing near industrial sites or heavy traffic. Parents described visible and persistent pollution. "Every morning, we wipe black soot off our windows. I worry what breathing that did to my son," said a mother of an ASD child (Parent #3, ASD group). Another parent from the ID group noted, "There's a big refuse dump and smelter by our house. I wonder if chemicals from there affected [my child]," linking local environmental contamination to their child's condition. Such accounts support the quantitative finding of higher toxin levels (such as lead) in children's cases, suggesting that these exposures are indeed plausible in their daily environment.

3) Socioeconomic Hardship and Health*:* Caregivers highlighted how poverty and limited access to healthcare compounded risks. "I couldn't afford good antenatal nutrition or check-ups when I was pregnant," admitted one mother (Parent #7, ID group), illustrating how economic disadvantage led to suboptimal prenatal environments. Several parents mentioned inadequate nutrition and exposure to infections or stress during pregnancy/infancy. These contextual factors may act synergistically with chemical exposures and can also leave epigenetic marks. Families also reported challenges in accessing early intervention services after diagnosis, which, although not a direct environmental toxin, reflects broader ecological adversity.

Overall, the qualitative data provide a human context for the statistical associations. They indicate that children in this Nigerian sample often live in environments with tangible pollution and that families face socioeconomic barriers that could influence developmental outcomes. These narratives reinforce the study's findings that environmental factors – from toxicants like lead to psychosocial stress – are intertwined with the epigenetic signatures of developmental disorders. The convergence of qualitative and quantitative evidence strengthens the inference that epigenetic modifications in these children may partly reflect their environmental context.

CONCLUSIONS

Answer to the research question (plain words). In Nigerian children aged 3–10 years, developmental disorders (ASD, ADHD, ID) were associated with a standard epigenetic profile in blood: lower global DNA methylation (≈approximately 0.4% lower 5-mC), reduced H3K9 acetylation, and a

small set of differentially expressed microRNAs. These epigenetic markers, together with higher blood lead levels, independently classified case status (model AUC = 0.81). A lead–methylation interaction was present: children with lead levels greater than five μg/dL and low 5-mC had approximately 9.5 times higher odds of being a case. Parent interviews described daily exposure to soot, proximity to dumps/smelters, and socioeconomic hardship, mirroring the exposure gradients seen in the quantitative data.

Theoretical implications

1) Gene–environment coupling at the epigenome. The co-occurrence of hypomethylation, hypoacetylation, and elevated lead fits a model in which environmental toxicants and deprivation shift chromatin toward a less permissive state. The presence of 15 FDR-significant DMPs in neurodevelopmental loci (e.g., NR3C1, OXTR, SHANK3, MEF2C) anchors this coupling in pathways long implicated in social behaviour, stress responsivity, synaptic scaffolding, and activity-dependent transcription.

2) Shared core, diagnosis-specific edges. The disorder groups shared global signals (5-mC, H3K9ac) yet exhibited distinct miRNA fingerprints; for example, researchers observed upregulation of miR-145/miR-34c in ASD and downregulation of let-7d in ADHD. This pattern is consistent with a familiar epigenetic "terrain" that is further shaped by diagnosis-linked regulatory nodes.

3) Population context matters. The size and direction of the lead–epigenome association, observed within Nigerian communities with documented pollution sources, emphasise that effect sizes are not portable across settings. The data fills part of the stated evidentiary gap by providing epigenetic measurements from African children, rather than extrapolations from non-African cohorts.

Practical implications

1) Near-term risk stratification. A small panel – global 5-mC, H3K9ac, and two to three miRNAs (e.g., let-7d, miR-145) – combined with blood lead offers an implementable screen for elevated neurodevelopmental risk in clinical or community programmes. The model's odds ratios are clinically interpretable (e.g., OR 1.25 per 1 μg/dL lead; OR 0.66 per 1% increase in 5-mC).

2) Actionable environmental control. The OR gradient across the 1–12 μg/dL lead range supports systematic lead abatement: paint and battery recycling controls, smelter emission oversight, and targeted household remediation in identified hotspots.

3) Early-life nutrition and service access. Parent reports of limited antenatal care and poor diet point to feasible levers (micronutrient support, antenatal visit coverage) that can be leveraged in conjunction with exposure control and early intervention referral.

4) Capacity building. The analytic workflow (Illumina EPIC, ChIP-qPCR, small-RNA sequencing; minfi/limma/DMRcate) is feasible in regional labs with standard QC, offering a template for programme scale-up and local training.

What changes for the field

1) Inclusion of African cohorts becomes non-optional. Effect sizes for exposure–epigenome links must be estimated in the populations that carry the exposures.

2) Environmental measurement must be integral, not ancillary. Routine collection of blood lead (and similar indices) should accompany pediatric epigenetic studies.

3) From discovery to translation. The small, reproducible set of epigenetic markers invites the development of low-cost assays for screening and referral workflows in child health systems.

4) Open data and harmonised reporting. Shared pipelines and pre-registered analysis plans will allow cross-site meta-analysis and faster convergence on robust markers.

Limitations

Design. Cross-sectional, matched case–control data cannot establish temporal order; reverse causation and unmeasured confounding remain possible.

Tissue. Peripheral blood may not accurately reflect brain epigenetics, as cell-type composition was estimated rather than directly measured.

Measurement scope. We targeted histone marks using ChIP-qPCR rather than conducting a genome-wide analysis. Although we selected the miRNA panel based on biological relevance, its scope was limited.

Sample size and subgroup power. Subgroup contrasts (ASD vs. ADHD vs. ID) were modestly powered, and several trends may have been under-detected.

Exposure assessment. Lead was measured directly, but other toxicants (e.g., mercury, pesticides) were not quantified, which restricted the inference of multi-exposure effects.

Effect estimates for epigenetic markers and lead should be interpreted as associations within a specific exposure context, rather than as causal effects. Blood-based signals offer a practical window into regulation, but they cannot replace the insights provided by brain tissue or single-cell resolution. Subgroup specificity, particularly for miRNAs, requires replication in larger sample sizes. Unmeasured co-exposures could inflate or attenuate observed odds ratios.

Next studies

1) Prospective Nigerian birth cohort with repeated measures (cord blood, infancy, early childhood) capturing lead and co-exposures, whole-blood methylation/ATAC-seq, and longitudinal neurodevelopmental phenotyping.

2) Exposure-reduction trials (e.g., household lead remediation plus caregiver education) with pre/post epigenetic endpoints and developmental outcomes at 6–12 months.

3) Single-cell and brain-linked assays, including deconvolution-informed methylomes and peripheral-to-CNS bridging (e.g., extracellular vesicle cargo, neuro-immune cell profiling).

4) Genetic/epigenetic triangulation, such as Mendelian randomisation for metal-handling pathways and polygenic-informed analyses to separate inherited from environmentally induced marks.

5) Wider toxicant panels (mercury, organophosphates, PAHs) and nutritional methyl-donor profiling (folate/B12/choline) to model mixture effects and nutrient–toxicant modulation.

In this first Nigeria-based epigenetic analysis of developmental disorders, a simple, measurable profile – characterised by low 5-mC, low H3K9ac, diagnosis-specific miRNAs, and elevated lead – defines a modifiable risk pattern that the field can now detect early and act upon.

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