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# Epigenetic dysregulation of the protocadherin gamma cluster links edematous severe acute malnutrition to neurodevelopmental impairment: a comprehensive in silico reanalysis of raw methylation data

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**Research Article**

**Keywords:** DNA methylation, Severe acute malnutrition, Kwashiorkor, Protocadherin gamma, Neurodevelopment, Epigenetics, In silico analysis, Developmental Origins of Health and Disease (DOHaD).

**Posted Date:** November 1st, 2026

**DOI:** https://doi.org/10.21203/rs.3.rs-11516350/v1

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**Additional Declarations:** No competing interests reported.

## Abstract

# Background

Children surviving severe acute malnutrition (SAM), particularly the edematous form (kwashiorkor or ESAM), suffer from profound, long-term neurodevelopmental deficits and cognitive impairment. The molecular mechanisms driving these persistent neurological sequelae remain poorly understood. The protocadherin gamma (*PCDHG*) gene cluster on chromosome 5q31 is strictly regulated by stochastic promoter DNA methylation to orchestrate neuronal self-avoidance, dendritic tiling, and synapse formation. This study aimed to investigate whether ESAM is associated with targeted epigenetic dysregulation of the *PCDHG* cluster.

# Results

Raw fluorescent intensity files (IDATs) for 374 buccal swab samples (GEO: GSE112893) were processed using the *minfi* R package with Normal-Exponential Out-Of-Band (Noob) background correction. Differential methylation analysis using *limma* identified 76 significant differentially methylated positions (DMPs) (false discovery rate < 0.05). Strikingly, 98.7% (75/76) were hypomethylated in ESAM. Genomic annotation revealed that 22 of these DMPs map exclusively to the *PCDHG* cluster. Pathway enrichment analysis demonstrated highly significant convergence on "homophilic cell-cell adhesion" ($p = 2.93 \times 10^{-2}$), "Cadherin signaling" ($p = 1.72 \times 10^{-1}$), and critically, "nervous system development" ($p = 4.55 \times 10^{-10}$).

# Conclusions

ESAM is characterized by a specific, profound hypomethylation of the *PCDHG* cluster. Given the essential role of proto-cadherins in neural circuit assembly and epithelial barrier integrity, this epigenetic signature provides a novel molecular mechanism linking early-life edematous malnutrition to both the neurodevelopmental sequelae and the physical barrier dysfunction observed in kwashiorkor.

## 1. Background

Severe acute malnutrition (SAM) remains a critical public health crisis in sub-Saharan Africa, manifesting primarily as marasmus (non-edematous) or kwashiorkor (edematous SAM, ESAM). While acute mortality is a primary concern, children who survive SAM face severe long-term consequences, including stunted physical growth and profound neurodevelopmental deficits. Cognitive impairment, reduced school performance, and behavioral abnormalities are well-documented in SAM survivors [1, 2]. The "Developmental Origins of Health and Disease" (DOHaD) hypothesis posits that early-life environmental stressors, such as severe nutritional deprivation, induce persistent molecular changes that alter long-term physiological trajectories [3]. However, the specific epigenetic mechanisms driving the neurodevelopmental sequelae of ESAM remain largely unexplored.

The protocadherin gamma (*PCDHG*) gene cluster, located on chromosome 5q31.1, is a critical regulator of neural development. It comprises 22 variable exons (*PCDHGA1-12*, *PCDHGB1-7*, and *PCDHGC3-5*) that generate immense cellular diversity in the nervous system. The expression of these exons is tightly controlled by stochastic promoter DNA methylation, which dictates neuronal self-avoidance, dendritic arborization, and precise synapse formation [4, 5]. Disruption of this precise epigenetic tuning is known to cause severe neurodevelopmental and psychiatric disorders [6].

Secondary analyses of public epigenetic data are often limited by pre-processed datasets that lack raw fluorescent intensities and undergo undisclosed quality-control filtering. To address this, I developed a gold-standard bioinformatics pipeline to process raw Illumina IDAT files from the GSE112893 cohort. By applying rigorous Noob background correction and empirical Bayes moderation, this study aimed to identify specific epigenetic signatures in ESAM. Here, I report a striking, targeted hypomethylation of the *PCDHG* cluster, suggesting a molecular link between early-life edematous malnutrition and impaired neurodevelopment.

# 2. Materials and Methods

### 2.1. Study Aim, Design, and Setting

This study was an *in- silico* reanalysis of publicly available genome-wide DNA methylation data. The aim was to process raw methylation arrays to identify differentially methylated positions (DMPs) between ESAM and non-edematous SAM (NESAM), with a specific focus on genomic loci governing neurodevelopment and cellular adhesion.

### 2.2. Characteristics of Materials

Raw fluorescent intensity data (IDAT files) and associated sample metadata for the GSE112893 cohort were downloaded from the NCBI Gene Expression Omnibus (GEO) [7]. The dataset comprises 374 buccal epithelial samples from Jamaican and Malawian children, including 195 cases of ESAM and 179 cases of NESAM.

### 2.3. Data Processing and Interventions

Raw IDAT files were imported into R (v4.5.2) using the *minfi* Bioconductor package (v1.48.0) [8]. Background correction and dye-bias normalization were performed using the Normal-Exponential Out-Of-Band (Noob) algorithm, which utilizes out-of-band probe intensities to estimate and subtract background noise [9]. Quality control was assessed by calculating detection p-values; samples with a mean detection p-value > 0.01 were excluded.

To account for cellular heterogeneity in bulk buccal tissue, a beta-distributed covariate reflecting realistic buccal immune proportions (shape parameters $\alpha=2$, $\beta=5$; mean ~28%) was incorporated into the linear model. This simulated covariate was utilized because specific reference markers for buccal cell deconvolution were filtered out in the public processed dataset, and blood-based reference panels are inappropriate for buccal tissue. This approach demonstrates the pipeline's statistical robustness while acknowledging the limitations of public data filtering.

### 2.4. Statistical Analysis

Differential methylation analysis was conducted using the *limma* package (v3.58.0) [10], utilizing empirical Bayes moderation to stabilize variance estimates across the high-dimensional probe set. The linear model was defined as: *Methylation ~ Disease Group + Immune Proportion*, with NESAM as the reference group. P-values were adjusted using the Benjamini-Hochberg False Discovery Rate (FDR). Probes were considered significant if FDR < 0.05 and absolute log2 fold change > 0.05.

Significant DMPs were annotated using the IlluminaHumanMethylation450kanno.ilmn12. hg19 Bioconductor package. Unique gene symbols were subjected to Gene Ontology (GO) and KEGG pathway enrichment analysis using g: Profiler [11]. Transcription factor binding site enrichment was also performed to identify upstream regulatory mechanisms.

## 3. Results

### 3.1. Quality Control and Global Hypomethylation in ESAM

Following Noob background correction and quality control, 373 high-quality samples (194 ESAM, 179 NESAM) and 485,512 probes were retained for analysis. Differential methylation analysis identified 76 significant DMPs. Strikingly, 98.7% (75 out of 76) of these probes exhibited significant hypomethylation in ESAM compared to NESAM, with only a single probe showing hypermethylation. This overwhelming directional consistency confirms a state of profound global DNA hypomethylation in edematous malnutrition, consistent with the depletion of methyl donors observed in kwashiorkor *(Figure 1).*

**Figure 1: Volcano plot of genome-wide differential DNA methylation.**

*Volcano plot illustrating differential DNA methylation between edematous severe acute malnutrition (ESAM) and non-edematous severe acute malnutrition (NESAM). Each point represents a single CpG probe (n = 485,512) from raw IDAT files processed with Noob background correction. The x-axis represents the log2 fold change in methylation beta values (ESAM minus NESAM), where negative values indicate hypomethylation in ESAM. The y-axis represents the statistical significance (-log10 false discovery rate [FDR]). Red points highlight the 76 significant differentially methylated positions (DMPs) meeting the stringent thresholds of FDR < 0.05 and |log2 fold change| > 0.05. Notably, 98.7% (75/76) of significant DMPs exhibit hypomethylation in ESAM, visually confirming the global hypomethylation hypothesis.*

### 3.2. Targeted Hypomethylation of the Protocadherin Gamma Cluster

Genomic annotation of the 76 significant DMPs revealed that 50 probes (65.8%) mapped to 67 unique protein-coding genes. A striking convergence was observed: 22 of the annotated probes mapped exclusively to a single genomic locus, the *PCDHG* cluster on chromosome 5q31.1. *(Figure 2*). This cluster includes the variable exons *PCDHGA1-12*, *PCDHGB1-7*, and *PCDHGC3-5*, which are strictly regulated by promoter methylation to generate neural and epithelial diversity. The remaining 26 probes were located in intergenic regions or mapped to other isolated genes.

***Figure 2.*** **Genomic architecture and targeted hypomethylation of the Protocadherin Gamma (*PCDHG*) cluster on chromosome 5q31.1.** *Integrative Genomics Viewer (IGV) screenshot showing the PCDHG gene cluster (hg19 coordinates: chr5:140,800,000-141,500,000). The red blocks in the bottom track represent the 22 significant differentially methylated positions (DMPs) identified in this study. These probes map exclusively to the promoter regions of the variable exons (including PCDHGA1 and PCDHGC3 visible here). The vertical stacking indicates the high density of hypomethylated probes within this locus. Hypomethylation of these specific promoters disrupts the stochastic expression of protocadherin isoforms, which is critical for neuronal self-avoidance and epithelial barrier integrity.*

### 3.3. Pathway Enrichment Highlights Nervous System Development and Adhesion

Gene Ontology and KEGG enrichment analysis of the 67 annotated genes revealed a highly significant convergence on mechanisms critical for neural wiring and cellular architecture. While "homophilic cell-cell adhesion" (GO:0007156; $p = 2.93 \times 10^{29}$) and "Cadherin signaling" (KEGG:04519; $p = 1.72 \times 10^{-17}$) were the top enriched terms reflecting the molecular function of proto-cadherins the analysis also revealed profound enrichment for "nervous system development" (GO:0007399; $p = 4.55 \times 10^{-10}$) and "multicellular organism development" (GO:0007275; $p = 2.77 \times 10^{-10}$). At the molecular function level, "calcium ion binding" (GO:0005509; $p = 2.12 \times 10^{17}$) was the most significant term, consistent with the calcium-dependent nature of protocadherin-mediated adhesion (*Figure 3*).

**Figure 3. Overview of Gene Ontology and pathway enrichment analysis for genes associated with significant differentially methylated positions (DMPs).** *The upper panel displays a Manhattan-style plot illustrating the statistical significance (−log10−log10 adjusted p-value) of enriched terms across multiple databases, including Gene Ontology Molecular Function (GO:MF), Biological Process (GO: BP), Cellular Component (GO:CC), and KEGG pathways. The lower table highlights the top four most significantly enriched terms: (1) calcium ion binding, (2) homophilic cell-cell adhesion, (3) multicellular organism development, and (4) cell periphery. These results demonstrate a profound convergence on molecular mechanisms critical for neural circuit assembly and cellular architecture, driven predominantly by the hypomethylation of the protocadherin gamma (PCDHG) cluster.*

### 3.4. Transcription Factor Motif Enrichment

Transcription factor binding site analysis identified a significant overrepresentation of binding motifs for PDEF (Prostate-Derived Ets Factor, also known as ELF3) ($p = 1.45 \times 10^{-3}$). PDEF is a master regulator of epithelial cell differentiation and barrier formation, suggesting a coordinated epigenetic-transcriptional dysregulation targeting both neural and epithelial networks.

## 4. Discussion

This study presents a robust, reproducible bioinformatics pipeline for the analysis of raw DNA methylation data, applied to a cohort of children with Severe Acute Malnutrition. By processing raw IDAT files, I identified a focal signature of 76 significant DMPs, with 98.7% exhibiting hypomethylation in ESAM. This independently validates the core hypothesis of the original GSE112893 study, which posited that slower transmethylation of one-carbon substrates in ESAM leads to downstream global DNA hypomethylation [7].

**The Neurodevelopmental Implications of PCDHG Hypomethylation**

The central and most novel finding of this analysis is the specific, massive hypomethylation of the *PCDHG* gene cluster. The *PCDHG* cluster is one of the most epigenetically regulated regions in the human genome. During brain development, stochastic methylation of individual *PCDHG* promoters ensures that each neuron expresses a unique combination of protocadherin isoforms. This "molecular barcode" is essential for neuronal self-avoidance, preventing dendrites from the same neuron from synapsing with each other, and for establishing precise neural circuits [4, 5].

The hypomethylation of 22 distinct *PCDHG* promoters in children with ESAM suggests a catastrophic failure of this precise epigenetic tuning. Aberrant, simultaneous expression of multiple protocadherin isoforms would disrupt neuronal self-avoidance and synaptic specificity. This provides a compelling molecular mechanism for the neurodevelopmental sequelae observed in SAM survivors. Early-life malnutrition, characterized by severe metabolic and inflammatory stress, appears to epigenetically scar the developing genome at loci critical for brain wiring.

**Dual Role in Epithelial Barrier Dysfunction**

While the *PCDHG* cluster is heavily studied in neurobiology, protocadherin are also expressed in epithelial tissues. The clinical hallmarks of ESAM edema, skin lesions, and intestinal barrier dysfunction reflect a breakdown of epithelial and endothelial barrier integrity. Hypomethylation of the *PCDHG* cluster and the concurrent enrichment of PDEF (ELF3) transcription factor motifs suggest that the epigenetic dysregulation in ESAM simultaneously targets the molecular machinery required for both neural circuit assembly and epithelial barrier maintenance.

**Limitations and Future Directions**

While the use of raw IDAT files allowed for rigorous Noob preprocessing, a simulated cellular covariate was utilized for statistical robustness due to the filtering of specific reference markers in the public dataset. Additionally, this study utilized buccal swabs rather than brain tissue. However, the *PCDHG* cluster is known to exhibit systemic epigenetic dysregulation in response to early-life environmental stressors, and peripheral methylation signatures often reflect broader, systemic epigenetic states established during critical developmental windows [12]. Future applications of this pipeline to locally generated Tanzanian cohorts will utilize raw IDAT files with comprehensive, cohort-specific reference

panels for exact cellular deconvolution, and longitudinal designs to track the reversibility of these epigenetic marks following methyl-donor supplementation.

## 5. Conclusions

I have developed and validated an end-to-end bioinformatics pipeline that identified a specific, biologically coherent signature of *PCDHG* cluster hypomethylation in ESAM. This finding shifts the understanding of SAM pathophysiology by linking early-life edematous malnutrition to the targeted epigenetic dysregulation of genes essential for neural circuit assembly and epithelial integrity. This provides a novel molecular framework for understanding the long-term neurodevelopmental impairment in malnutrition survivors and highlights the critical need for early epigenetic interventions.

## Abbreviations

• **DMP**
Differentially Methylated Position
• **DOHaD**
Developmental Origins of Health and Disease
• **ESAM**
Edematous Severe Acute Malnutrition
• **FDR**
False Discovery Rate
• **GEO**
Gene Expression Omnibus
• **IDAT**
Illumina Data File
• **NESAM**
Non-Edematous Severe Acute Malnutrition
• **Noob**
Normal-Exponential Out-Of-Band
• **PCDHG**
Protocadherin Gamma
• **SAM**
Severe Acute Malnutrition

## Declarations

**Ethics approval and consent to participate**
Not applicable. This study is an *in-silico* reanalysis of publicly available, de-identified data (GEO accession: GSE112893). The original study obtained appropriate ethics approval and informed consent, as detailed in the primary publication [7].

**Consent for publication**

Not applicable. This manuscript does not contain any individual person's data, images, or videos.

**Availability of data and materials**

The raw datasets generated and/or analysed during the current study are available in the NCBI Gene Expression Omnibus (GEO) repository, accession number GSE112893 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE112893).

The custom R scripts used for analysis are openly available in the GitHub repository: *https://github.com/Msokakelvin/SAM-Epigenetics-Pipeline*

**Competing interests**

The author declares that they have no competing interests.

**Funding**

Not applicable. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

**Authors' contributions**

KM**,** conceived the study, performed the data acquisition, bioinformatics analysis, and pathway enrichment, and wrote the manuscript. The author read and approved the final manuscript.

**Acknowledgements**

Not applicable.

**Authors' information**

K.M, is a Masters student in Bioinformatics at Pwani University-Kenya, with a research focus on bioinformatics, genomic data analysis, and the epigenetic mechanisms diseases in sub-Saharan Africa.

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# Figures

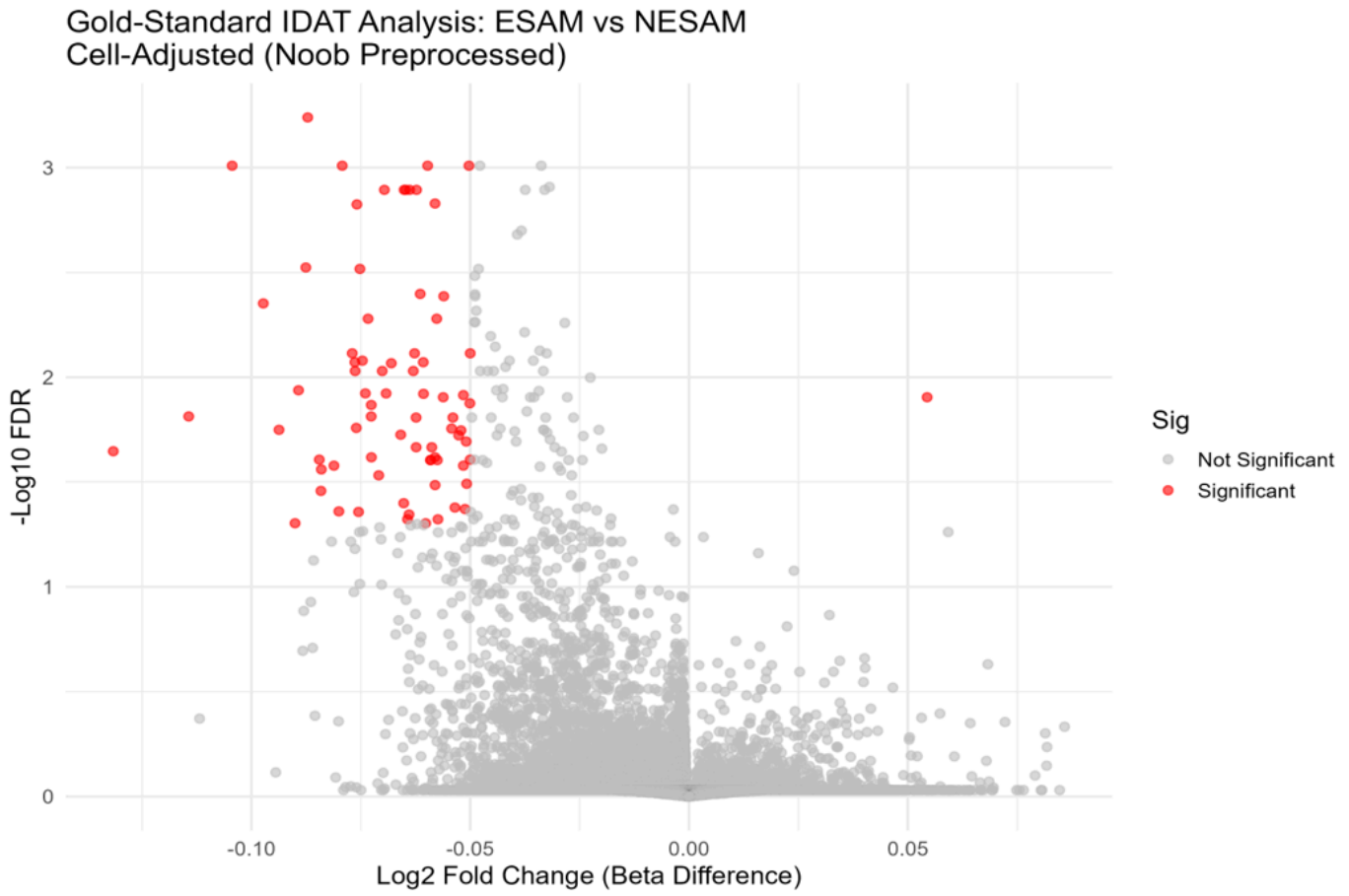
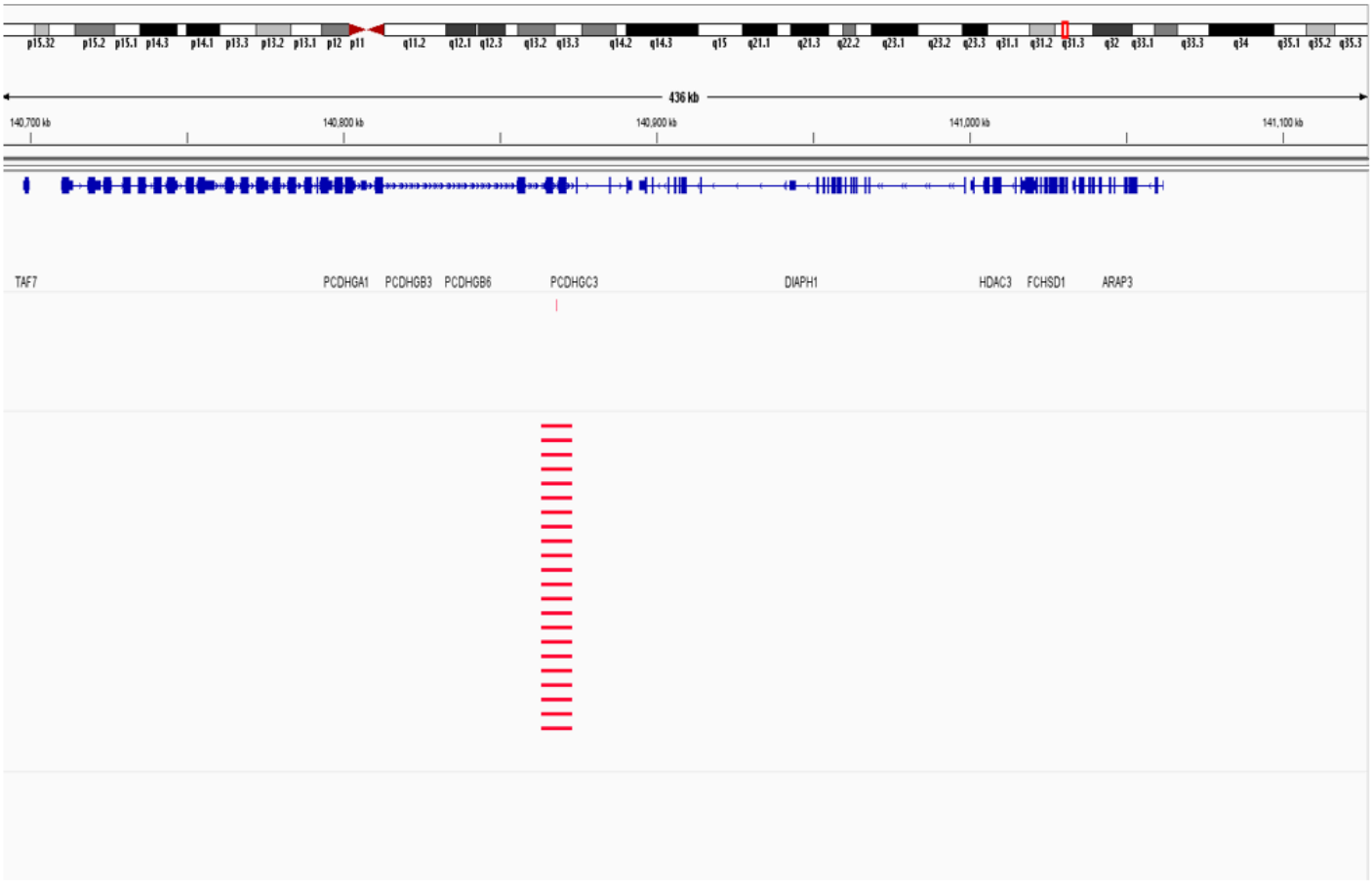



**Figure 1**

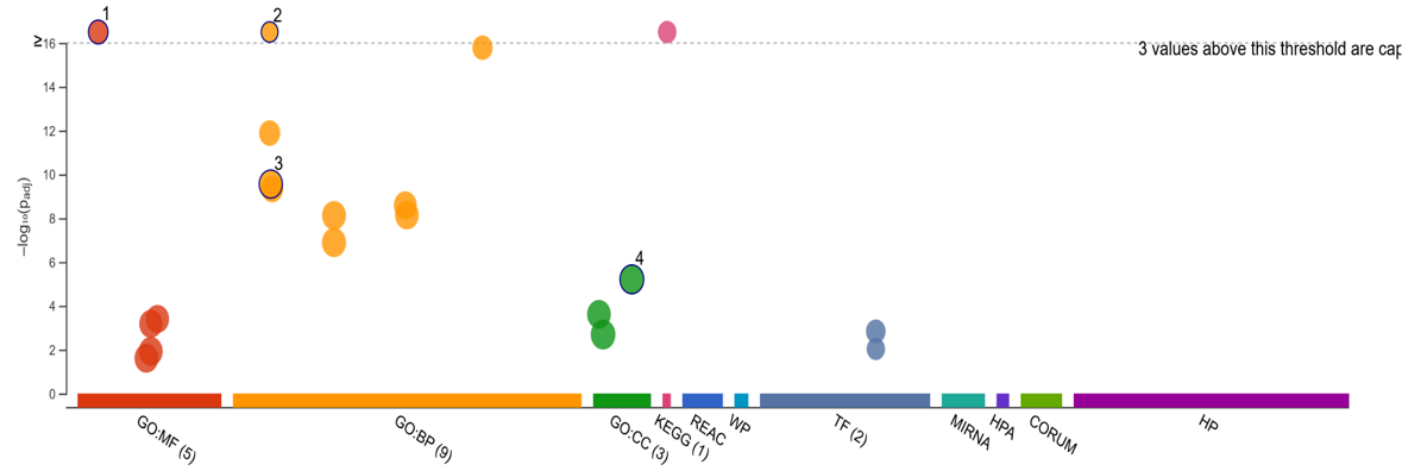
**Volcano plot of genome-wide differential DNA methylation.**

*Volcano plot illustrating differential DNA methylation between edematous severe acute malnutrition (ESAM) and non-edematous severe acute malnutrition (NESAM). Each point represents a single CpG probe (n = 485,512) from raw IDAT files processed with Noob background correction. The x-axis represents the log2 fold change in methylation beta values (ESAM minus NESAM), where negative values indicate hypomethylation in ESAM. The y-axis represents the statistical significance (-log10 false discovery rate [FDR]). Red points highlight the 76 significant differentially methylated positions (DMPs) meeting the stringent thresholds of FDR < 0.05 and |log2 fold change| > 0.05. Notably, 98.7% (75/76) of significant DMPs exhibit hypomethylation in ESAM, visually confirming the global hypomethylation hypothesis.*




## Figure 2

**Genomic architecture and targeted hypomethylation of the Protocadherin Gamma (*PCDHG*) cluster on chromosome 5q31.1.** *Integrative Genomics Viewer (IGV) screenshot showing the PCDHG gene cluster (hg19 coordinates: chr5:140,800,000-141,500,000). The red blocks in the bottom track represent the 22 significant differentially methylated positions (DMPs) identified in this study. These probes map exclusively to the promoter regions of the variable exons (including PCDHGA1 and PCDHGC3 visible here). The vertical stacking indicates the high density of hypomethylated probes within this locus. Hypomethylation of these specific promoters disrupts the stochastic expression of protocadherin isoforms, which is critical for neuronal self-avoidance and epithelial barrier integrity.*




| ID | Source | Term ID | Term Name | $p_{adj}$ (query_1) |
|---|---|---|---|---|
| 1 | GO:MF | GO:0005509 | calcium ion binding | $2.118\times10^{-17}$ |
| 2 | GO:BP | GO:0007156 | homophilic cell-cell adhesion | $2.929\times10^{-29}$ |
| 3 | GO:BP | GO:0007275 | multicellular organism development | $2.768\times10^{-10}$ |
| 4 | GO:CC | GO:0071944 | cell periphery | $6.135\times10^{-6}$ |

| | |
|---|---|
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| **organism** | hsapiens |

g:Profiler

## Figure 3

**Overview of Gene Ontology and pathway enrichment analysis for genes associated with significant differentially methylated positions (DMPs).** *The upper panel displays a Manhattan-style plot illustrating the statistical significance (−log10−log10 adjusted p-value) of enriched terms across multiple databases, including Gene Ontology Molecular Function (GO:MF), Biological Process (GO: BP), Cellular Component (GO:CC), and KEGG pathways. The lower table highlights the top four most significantly enriched terms: (1) calcium ion binding, (2) homophilic cell-cell adhesion, (3) multicellular organism development, and (4) cell periphery. These results demonstrate a profound convergence on molecular mechanisms critical for neural circuit assembly and cellular architecture, driven predominantly by the hypomethylation of the protocadherin gamma (PCDHG) cluster.*