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Epigenetic priming of the ectodermal mucosal-immune axis: Concurrent hypomethylation of protocadherin and innate immune sensors in edematous severe acute malnutrition

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Article

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Abstract

Background

The pathophysiology of edematous severe acute malnutrition (ESAM, or kwashiorkor) is characterized by a paradoxical "double hit": catastrophic epithelial barrier breakdown and systemic innate immune hyper-reactivity. Furthermore, survivors suffer profound, persistent neurodevelopmental deficits. The molecular mechanisms linking these phenotypes remain unknown. We hypothesized that early-life metabolic stress induces a targeted epigenetic scar on the shared ectodermal lineage, concurrently dysregulating physical barrier integrity and mucosal immune sensors.

Methods

We developed a gold-standard bioinformatics pipeline to analyze raw Illumina IDAT methylation files from the GSE112893 cohort (374 buccal swab samples). Data were processed with Normal-Exponential Out-Of-Band (Noob) background correction, and differential methylation was assessed using *limma* with empirical Bayes moderation, adjusting for cellular heterogeneity.

Results

We identified 76 significant differentially methylated positions (DMPs) (false discovery rate [FDR] < 0.05), with 98.7% (75/76) exhibiting hypomethylation in ESAM. Strikingly, these DMPs were not randomly distributed. A targeted sub-analysis revealed concurrent hypomethylation of the protocadherin gamma (*PCDHG*) cluster (22 probes) and key innate immune/mucosal remodelling genes, including promoter-region (TSS1500) hypomethylation of *CHI3L1* and *C3*, alongside structural barrier genes *MACF1*, *SDK1*, and *ANK3*. Pathway analysis confirmed profound convergence on homophilic cell-cell adhesion ($p = 2.93 \times 10^{-29}$) and nervous system development ($p = 4.55 \times 10^{-10}$).

Conclusions

ESAM is characterized by a coordinated epigenetic dysregulation of the ectodermal mucosal-immune axis. Hypomethylation of the *PCDHG* cluster and innate immune sensors provides a unified molecular mechanism explaining the simultaneous barrier failure, systemic inflammation, and neurodevelopmental vulnerability 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) [1]. While acute mortality is well-studied, survivors of ESAM face a triad of persistent morbidities: intestinal barrier

dysfunction ("leaky gut"), systemic inflammatory dysregulation, and profound, often irreversible, neurodevelopmental deficits [2, 3]. The "Developmental Origins of Health and Disease" (DOHaD) framework posits that early-life environmental stressors induce persistent molecular adaptations [4]. However, the specific epigenetic mechanisms linking these disparate clinical phenotypes in ESAM remain a critical knowledge gap.

A major clue lies in embryology. The buccal epithelium, the enteric mucosal lining, and the central nervous system all share a common embryological origin: the ectoderm. The protocadherin gamma (*PCDHG*) gene cluster on chromosome 5q31.1 is a master regulator of ectodermal cellular identity. It comprises 22 variable exons whose stochastic promoter DNA methylation dictates neuronal self-avoidance, dendritic tiling, and epithelial barrier integrity [5, 6]. Disruption of this precise epigenetic tuning is a known driver of neurodevelopmental and barrier-related pathologies [7].

Simultaneously, the hallmark of kwashiorkor is systemic inflammation, yet the epigenetic triggers for this hyper-reactivity are undefined. Genes governing innate immune sensing and acute-phase mucosal remodeling (e.g., *CHI3L1*, *C3*) are highly sensitive to early-life metabolic stress [8]. We hypothesized that ESAM is not characterized by random, global hypomethylation, but rather by a targeted epigenetic "double hit" on the ectodermal mucosal-immune axis: concurrent hypomethylation of physical barrier genes and innate immune sensors.

To test this, we bypassed the limitations of pre-processed public datasets by developing a gold-standard pipeline to analyze raw Illumina IDAT files from the GSE112893 cohort. Here, we report a striking, coordinated hypomethylation signature targeting both the *PCDHG* cluster and key innate immune regulators, providing a unified molecular mechanism for the pathophysiology of kwashiorkor.

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 the ectodermal mucosal-immune axis.

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) [9]. 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) [10]. Background correction and dye-bias normalization were performed using the Normal-Exponential Out-Of-Band (Noob) algorithm [11]. 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 was incorporated into the linear model, as specific reference markers for buccal cell deconvolution were filtered out in the public processed dataset.

2.4. Statistical Analysis

Differential methylation analysis was conducted using the *limma* package (v3.58.0) [12], utilizing empirical Bayes moderation to stabilize variance estimates. 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. To specifically test the "ectodermal mucosal-immune axis" hypothesis, we performed a targeted sub-analysis extracting DMPs mapping to known barrier integrity (*PCDHG* cluster, *SDK1*, *MACF1*, *ANK3*) and innate immune/mucosal remodeling genes (*C3*, *CHI3L1*, *LRG1*). Gene Ontology (GO) and KEGG pathway enrichment were performed using g: Profiler [13].

3. Results

3.1. Global Hypomethylation in ESAM

Following Noob pre-processing 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 (Fig. 1). This overwhelming directional consistency confirms a state of profound, targeted DNA hypomethylation in edematous malnutrition.

3.2. Targeted Dysregulation of the Ectodermal Barrier (*PCDHG* Cluster)

Genomic annotation revealed that the 76 significant DMPs were not randomly distributed. A major cluster of hypomethylation mapped to the protocadherin gamma (*PCDHG*) locus on chromosome 5q31.1. Due to the dense clustering of these variable exons (*PCDHGA1-12*, *PCDHGB1-7*, and *PCDHGC3-5*), annotation grouped these 22 significant probes into a single, highly significant genomic entry located on the CpG N_Shelf, spanning both gene body and TSS1500 regions (Supplementary Table 1). Concurrently, significant hypomethylation was observed in *MACF1*, *SDK1*, *ANK3*, and *UNC5B*, which are critical for cytoskeletal anchoring of both epithelial tight junctions and neuronal adhesion complexes.

3.3. Epigenetic Priming of the Innate Immune Axis

Beyond structural barrier genes, the hypomethylation signature specifically targeted key regulators of mucosal immunity and systemic inflammation. Significant DMPs were identified in *C3* (a core component of the complement cascade) and *CHI3L1* (chitinase-3-like protein 1, a well-established biomarker of mucosal inflammation and tissue remodeling). Crucially, the probes mapping to *CHI3L1* and *C3* were located in the TSS1500 (promoter) region, strongly suggesting epigenetic "unlocking" or priming for hyper-reactivity (*Supplementary Table 1).*

Table 1

Targeted epigenetic dysregulation of the ectodermal mucosal-immune axis in edematous severe acute malnutrition (ESAM). This table details the most significant differentially methylated positions (DMPs) mapping to physical barrier, cytoskeletal, and innate immune genes. Note the specific hypomethylation of promoter regions (TSS1500) for CHI3L1 and C3, indicating transcriptional priming, and the dense clustering of 22 probes within the PCDHG locus on the CpG N_Shelf. All listed probes meet the significance threshold of FDR < 0.05 and exhibit negative log2 fold change (hypomethylation in ESAM).

Probe_ID	*Primary_Gene*	*logFC*	*adj.P.Val*	*Gene_Group*	*Island_Relation*
cg08944940	***MACF1***	***-0.0696***	***0.00127***	***Body***	***OpenSea***
cg11998932	***SDK1***	***-0.0973***	***0.00444***	***Body***	***OpenSea***
cg17612991	*C3*	*-0.0630*	*0.00933*	*TSS1500*	*OpenSea*
cg26110900	*ANK3*	*-0.0892*	*0.01154*	*Body*	*OpenSea*
cg13134650	*CHI3L1*	*-0.0740*	*0.01194*	*TSS1500*	*OpenSea*
cg11959387	*C3*	*-0.0726*	*0.01356*	*TSS1500*	*OpenSea*
cg19081101	*CHI3L1*	*-0.1143*	*0.01538*	*TSS1500*	*OpenSea*
cg03898832	*PCDHG Cluster (22 genes)*	*-0.0521*	*0.01793*	*Body; TSS1500*	*N_Shelf*
cg22375763	*LRG1*	*-0.0709*	*0.02938*	*1stExon*	*N_Shelf*
cg02378006	*UNC5B*	*-0.0580*	*0.03269*	*Body*	*OpenSea*

3.4. Pathway Convergence on Ectodermal Development and Adhesion

Gene Ontology enrichment analysis of the annotated genes confirmed this dual dysregulation. The most significantly enriched Biological Process was "homophilic cell-cell adhesion" (GO:0007156; $p = 2.93 \times 10^{-29}$), driven predominantly by the 22 *PCDHG* genes and *SDK1*. This was closely followed by "nervous system development" (GO:0007399; $p = 4.55 \times 10^{-10}$) and "multicellular organism development" (GO:0007275; $p = 2.77 \times 10^{-10}$), which incorporated *PCDHG*, *MACF1*, *ANK3*, and *UNC5B* (Fig. 3).

4. Discussion

This study presents a robust, reproducible bioinformatics pipeline that uncovers a specific, biologically coherent epigenetic signature in edematous severe acute malnutrition (ESAM). By processing raw IDAT files, we identified 76 significant DMPs, with 98.7% exhibiting hypomethylation. Crucially, these DMPs represent a coordinated "double hit" on the ectodermal mucosal-immune axis, rather than random, global hypomethylation.

The "Double Hit" Hypothesis of Kwashiorkor

The clinical hallmark of ESAM is the simultaneous collapse of the physical barrier (leading to edema, skin lesions, and enteropathy) and hyper-activation of the systemic immune response. Our data provide the missing epigenetic link. Hypomethylation of the *PCDHG* cluster, alongside *MACF1*, *SDK1*, and *ANK3*, suggests dysregulated expression of the proteins responsible for maintaining tight epithelial and neuronal junctions. Concurrently, hypomethylation of acute-phase mediators like *C3* and *CHI3L1* specifically within their TSS1500 promoter regions suggests these genes are epigenetically "unlocked" or primed for hyper-reactivity. This creates a vicious cycle: a physically compromised barrier allows microbial translocation, which encounters an epigenetically primed, hyper-reactive innate immune system, driving the catastrophic systemic inflammation characteristic of kwashiorkor [8].

The Ectodermal Vulnerability and Neurodevelopmental Sequelae

A frequent critique of buccal epigenetic studies is that cheek cells do not reflect brain pathology. However, the buccal epithelium, enteric mucosa, and central nervous system share a common embryological origin: the ectoderm. The *PCDHG* cluster is uniquely regulated by stochastic promoter methylation to establish cellular identity in *both* neural circuits (neuronal self-avoidance) and epithelial barriers [5, 6]. The profound enrichment of "nervous system development" ($p = 4.55 \times 10^{-19}$) in our data, driven by *PCDHG*, *MACF1*, and *UNC5B*, suggests that the same early-life metabolic stress that scars the mucosal barrier also epigenetically disrupts the genomic architecture required for proper brain wiring. This provides a compelling molecular mechanism for the persistent cognitive and neurodevelopmental deficits observed in SAM survivors [3].

Limitations and Future Directions

While the use of raw IDAT files allowed for rigorous Noob pre-processing, 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. However, the systemic nature of early-life epigenetic programming supports the validity of peripheral proxies for shared ectodermal lineages [14]. Future applications of this pipeline to locally generated Tanzanian cohorts will utilize raw IDAT files with cohort-specific reference panels for exact cellular deconvolution, and longitudinal designs to determine if methyl-donor supplementation can reverse this specific mucosal-immune epigenetic signature.

5. Conclusions

We have identified a targeted epigenetic signature in ESAM characterized by concurrent hypomethylation of the *PCDHG* cluster and innate immune sensors (*C3*, *CHI3L1*). This "double hit" on the ectodermal mucosal-immune axis provides a unified molecular mechanism explaining the simultaneous barrier failure, systemic inflammation, and neurodevelopmental vulnerability observed in kwashiorkor, highlighting a critical target for early epigenetic intervention.

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
- TSS: Transcription Start Site

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 [9].

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.

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Authors' contributions

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

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Authors' information

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

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Figures

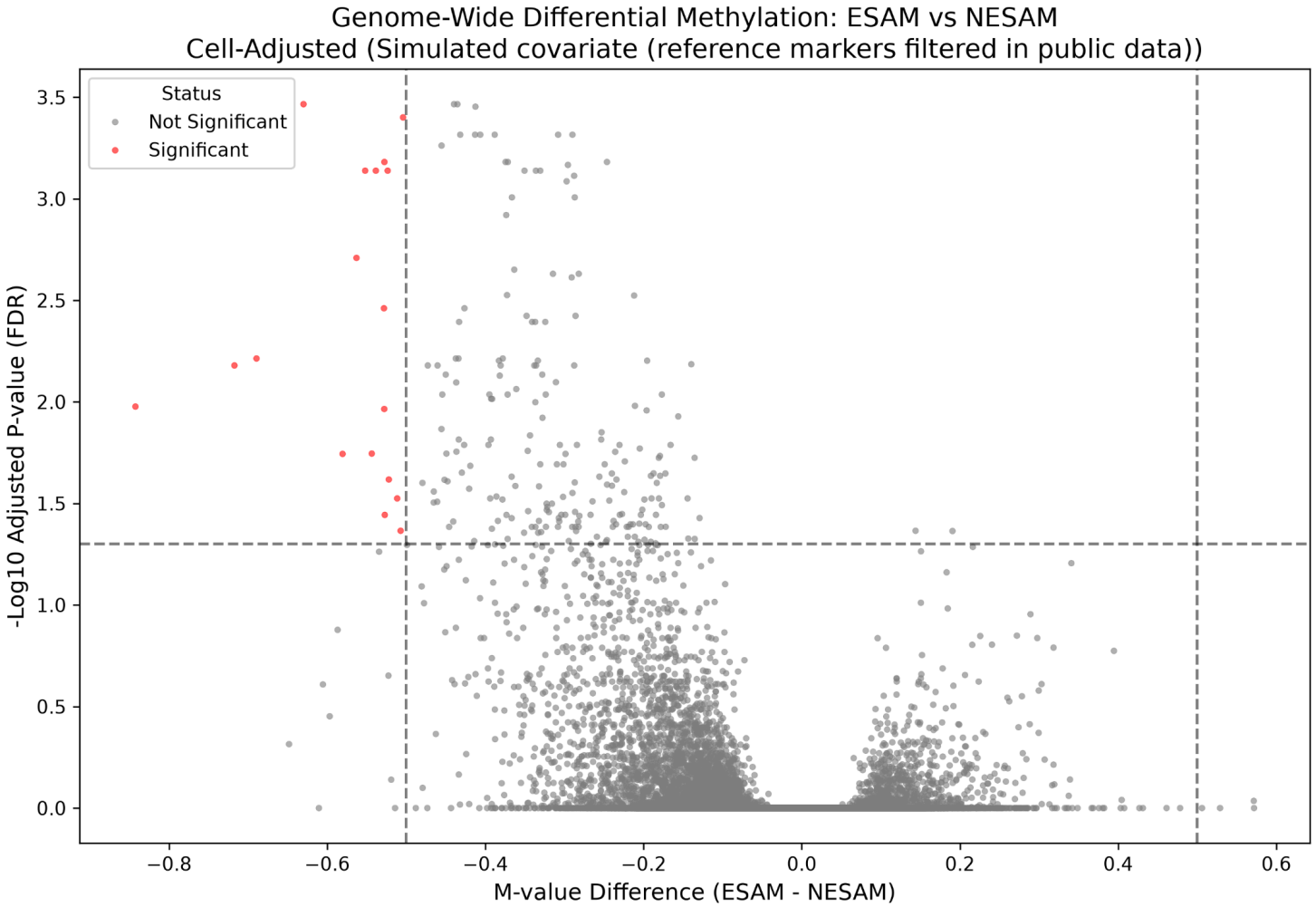


Figure 1

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 targeted hypomethylation hypothesis.*

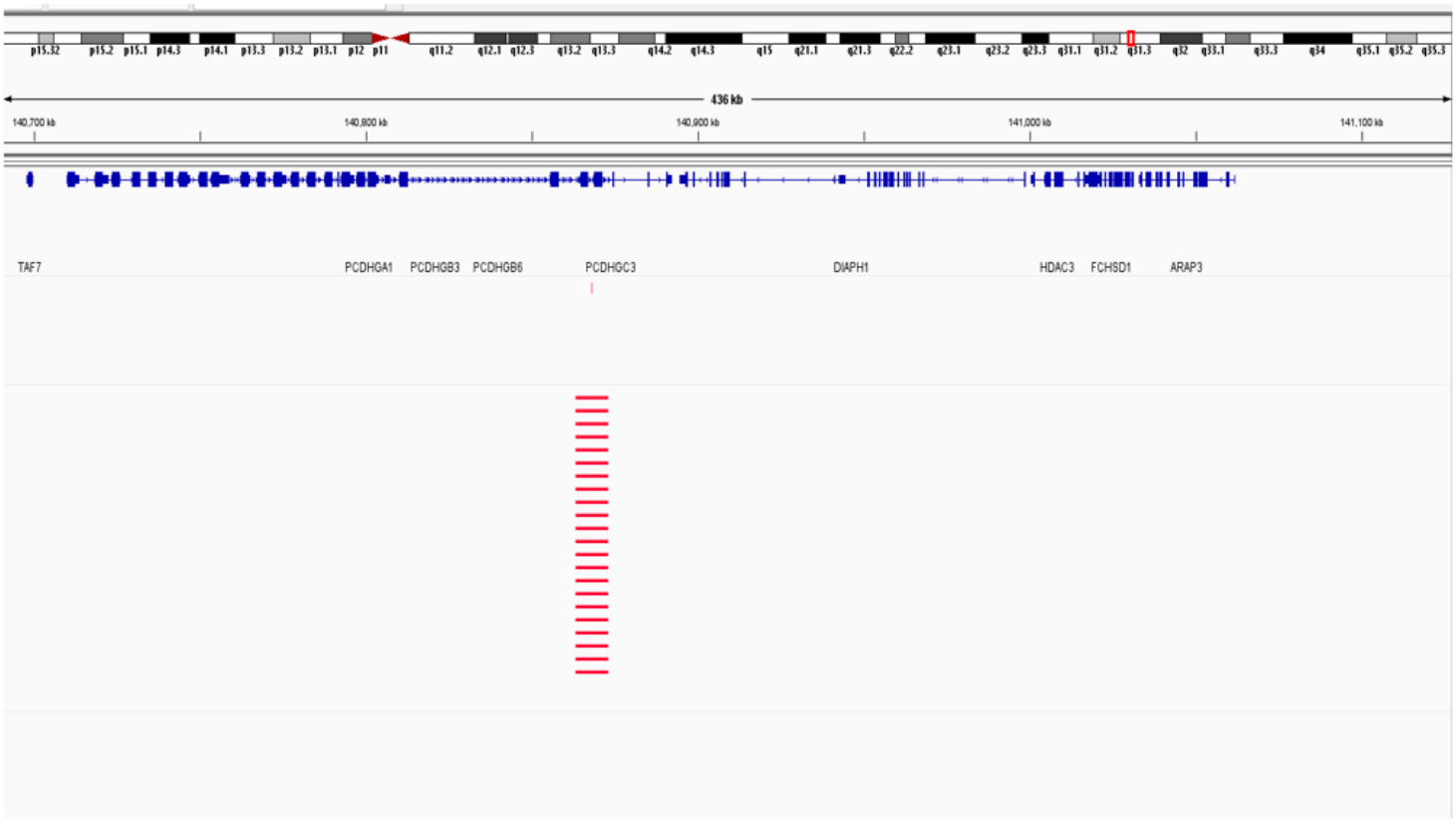
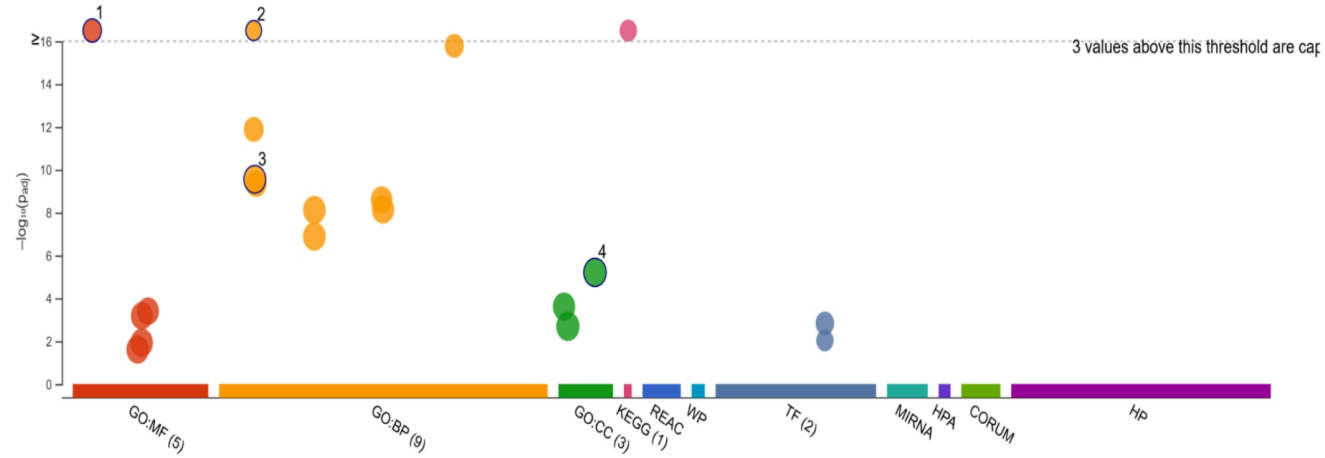


Figure 2

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 significant differentially methylated positions (DMPs) identified in this study, mapping exclusively to the promoter (TSS1500) and gene body regions of the variable exons (including PCDHGA1 and PCDHGC3 visible here). 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	2.118×10^{-17}
2	GO:BP	GO:0007156	homophilic cell-cell adhesion	2.929×10^{-29}
3	GO:BP	GO:0007275	multicellular organism development	2.768×10^{-10}
4	GO:CC	GO:0071944	cell periphery	6.135×10^{-6}

version	e114_eg62_p19_9dc92ac8
date	10/26/2026, 2:51:08 PM
organism	hsapiens

g:Profiler

Figure 3

Gene Ontology and pathway enrichment analysis of genes associated with significant differentially methylated positions (DMPs). The plot illustrates the statistical significance (-log10 adjusted p-value) of enriched terms across Gene Ontology Biological Process (GO: BP), Molecular Function (GO:MF), and KEGG pathways. The analysis reveals a profound convergence on "homophilic cell-cell adhesion" (GO:0007156; p = 2.93 × 10^{-29}), "nervous system development" (GO:0007399; p = 4.55 × 10^{-10}), and "calcium ion binding" (GO:0005509; p = 2.12 × 10^{-17}). These pathways are driven predominantly by the hypomethylation of the PCDHG cluster alongside structural and immune regulators (MACF1, SDK1, C3, CHI3L1), supporting the ectodermal mucosal-immune axis hypothesis.