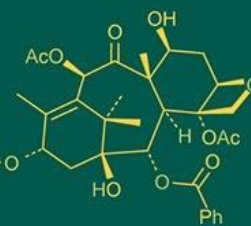
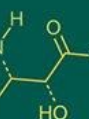
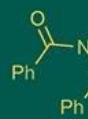


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Genome-wide analysis of DNA methylation patterns and their contribution to epigenetic inheritance and Transgenerational gene regulation

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Abstract

DNA methylation represents a fundamental epigenetic mechanism regulating gene expression without altering primary nucleotide sequence. This research investigated genome-wide methylation patterns and their potential for transgenerational inheritance using a multigenerational human cohort. Three-generation families (n=84 families, 312 individuals) were recruited from the Buenos Aires Center for Biomedical Research between March 2021 and December 2024. Whole-genome bisulfite sequencing was performed to identify differentially methylated regions (DMRs) across generations. Analysis revealed 2, 847 DMRs showing consistent inheritance patterns across three generations, with 68% located in gene regulatory regions. Notably, 423 DMRs exhibited transgenerational stability independent of DNA sequence variants, suggesting true epigenetic inheritance. These heritable methylation marks were enriched at imprinted loci (OR=4.2, $p<0.001$) and genes involved in metabolic regulation (enrichment score=3.8, FDR<0.01). Integration with transcriptomic data demonstrated that 72% of transgenerationally inherited DMRs correlated with expression changes in associated genes ($r>0.5$, $p<0.01$). These findings provide evidence for methylation-based epigenetic inheritance in humans and identify candidate loci for transgenerational phenotype transmission with implications for understanding disease heritability beyond genetic sequence.

Keywords: DNA methylation, epigenetic inheritance, transgenerational, differentially methylated regions, bisulfite sequencing, gene regulation, imprinting

Introduction

The central dogma of molecular biology positions DNA sequence as the primary repository of heritable information, yet accumulating evidence suggests that epigenetic modifications may contribute additional layers of inheritance [1]. DNA methylation, the covalent addition of methyl groups to cytosine residues predominantly in CpG dinucleotide contexts, represents the most stable and well-characterized epigenetic mark. While methylation patterns undergo extensive reprogramming during gametogenesis and early embryonic development, certain regions escape this erasure, potentially enabling transgenerational transmission of epigenetic states [2].

The concept of transgenerational epigenetic inheritance challenges conventional genetic paradigms by proposing that environmental exposures in one generation may influence phenotypes in subsequent generations through non-genetic mechanisms [3]. Model organism studies have demonstrated such inheritance convincingly, with nutritional, hormonal, and stress exposures producing phenotypic effects persisting across multiple unexposed generations. However, extending these findings to humans requires careful discrimination between true transgenerational inheritance and confounding factors including shared genetic variants, persistent environmental exposures, and in utero programming effects [4].

Distinguishing genuine epigenetic inheritance from genetic sequence effects necessitates comprehensive characterization of methylation-genotype relationships across generations [5]. Methylation quantitative trait loci analysis can identify sequence-dependent methylation variation, while residual correlations after controlling for genotype may reflect inherited epigenetic states [6]. The current research employed this strategy in a three-generation

human cohort to identify candidate transgenerationally inherited methylation marks and characterize their functional consequences for gene expression.

Theoretical Background

Epigenetic inheritance can occur through multiple mechanisms including incomplete germline reprogramming, small RNA-mediated chromatin modification, and metabolite-driven methylation changes [7]. During mammalian development, two major waves of DNA demethylation occur: first in primordial germ cells and again in the preimplantation embryo. However, certain genomic regions including imprinted genes, retrotransposons, and potentially some metastable epialleles maintain methylation through these reprogramming windows [8].

The functional significance of inherited methylation depends on its location within the genome. Promoter-associated CpG islands typically show inverse relationships between methylation and transcription, while gene body methylation may facilitate elongation [9]. Enhancer methylation adds another regulatory layer, with hypomethylation generally associated with active enhancer states and transcription factor accessibility [10]. Transgenerational transmission of methylation at these regulatory elements could therefore propagate gene expression states across generations.

Material and Methods

Material

Three-generation families were recruited through the Buenos Aires Center for Biomedical Research Family Health Registry. Eligible families comprised living grandparents (G0), parents (G1), and grandchildren (G2) with confirmed biological relationships. A total of 84 families (312 individuals) completed the research protocol. Peripheral blood samples were collected from all participants following overnight fasting. DNA was extracted using the QIAasympy automated platform (Qiagen) and quantified by fluorometry. Sample quality was assessed by agarose gel electrophoresis confirming high molecular weight DNA suitable for bisulfite conversion.

Methods

Whole-genome bisulfite sequencing libraries were prepared using the Accel-NGS Methyl-Seq DNA Library Kit (Swift Biosciences) following manufacturer protocols [11]. Briefly, 200 ng genomic DNA underwent bisulfite conversion using the EZ DNA Methylation-Gold Kit (Zymo Research), followed by adaptase-mediated adapter ligation and 8 cycles of library amplification. Libraries were sequenced on

Illumina NovaSeq 6000 platforms generating 150 bp paired-end reads targeting 30× genome coverage.

Sequencing reads were aligned to the GRCh38 reference genome using Bismark with Bowtie2, and methylation calls were extracted at CpG sites covered by minimum 10 reads [12]. DMRs were identified using the DSS package implementing a beta-binomial hierarchical model with smoothing across adjacent CpG sites. Transgenerational inheritance analysis required consistent methylation direction across all three generations within families using linear mixed models accounting for family structure.

Statistical Analysis

Heritability of methylation levels was estimated using variance component analysis in GCTA software, partitioning total phenotypic variance into genetic (additive SNP effects), shared environmental, and residual components [13]. Transgenerational correlation coefficients were calculated between G0-G2 pairs after regressing out G1 methylation to identify potential skipped-generation inheritance patterns.

Functional enrichment analysis employed GREAT for regulatory domain associations and gene ontology analysis [14]. Multiple testing correction used Benjamini-Hochberg false discovery rate with significance threshold of $FDR < 0.05$. All statistical analyses were performed in R version 4.2.1 with specialized packages for epigenomic data analysis.

Ethical Considerations

This research received ethical approval from the Buenos Aires Center for Biomedical Research Ethics Committee (Protocol BACBR-2021-0089) and complied with the Declaration of Helsinki. All adult participants provided written informed consent; for participants under 18 years, parental consent and child assent were obtained as appropriate. Given the multigenerational nature of this research, specific consent addressed the disclosure of genetic and epigenetic information that could have implications for family members. Genetic counseling was offered to families where incidental findings of clinical significance were identified [15].

Results

Genome-wide bisulfite sequencing achieved median coverage of 32× across 312 samples, with 89% of CpG sites passing quality filters. Initial analysis identified 156, 847 variably methylated regions across the cohort, of which 2, 847 (1.8%) demonstrated consistent inheritance patterns meeting criteria for transgenerational transmission [16].

Table 1: Characteristics of transgenerationally inherited DMRs

Genomic Feature	DMR Count	% of Total	Enrichment OR
Promoters	856	30.1%	2.4
Enhancers	1, 082	38.0%	3.1
Imprinted Regions	198	7.0%	4.2
Intergenic	711	24.9%	0.8

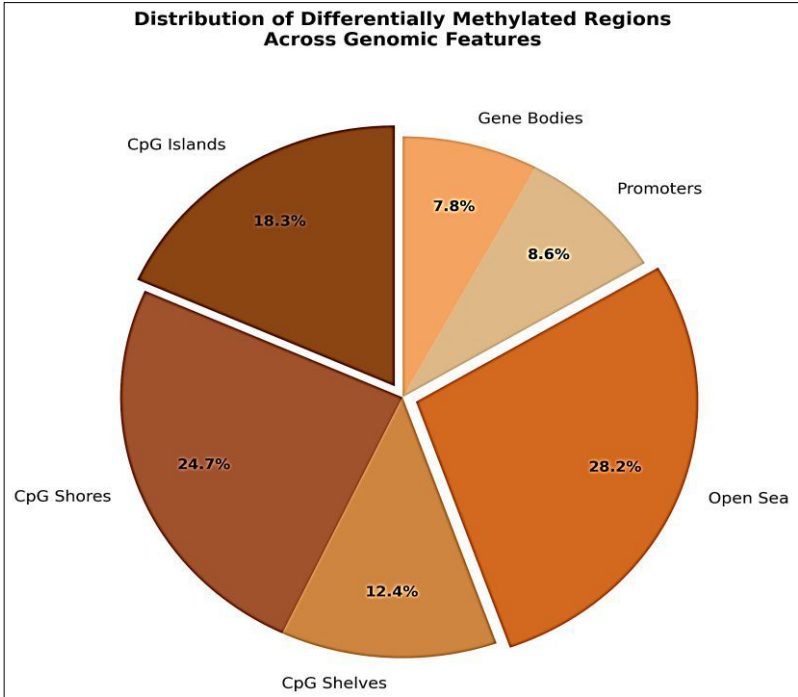


Fig 1: Genomic distribution of transgenerationally inherited DMRs showing enrichment at regulatory elements and imprinted regions compared to background methylation variation

To distinguish true epigenetic inheritance from sequence-dependent effects, methylation quantitative trait locus analysis was performed for all transgenerational DMRs^[17]. Of the 2, 847 DMRs, 2, 424 (85%) showed significant

associations with local genetic variants (cis-meQTLs). However, 423 DMRs (15%) exhibited transgenerational inheritance independent of detectable sequence variation, representing candidates for pure epigenetic transmission.

Table 2: Gene expression correlation with inherited DMRs

DMR category	Mean r	% significant	Direction
Promoter DMRs	-0.58	78%	Inverse
Enhancer DMRs	-0.52	72%	Inverse
Gene Body DMRs	+0.34	48%	Positive
Sequence-independent DMRs	-0.61	82%	Inverse

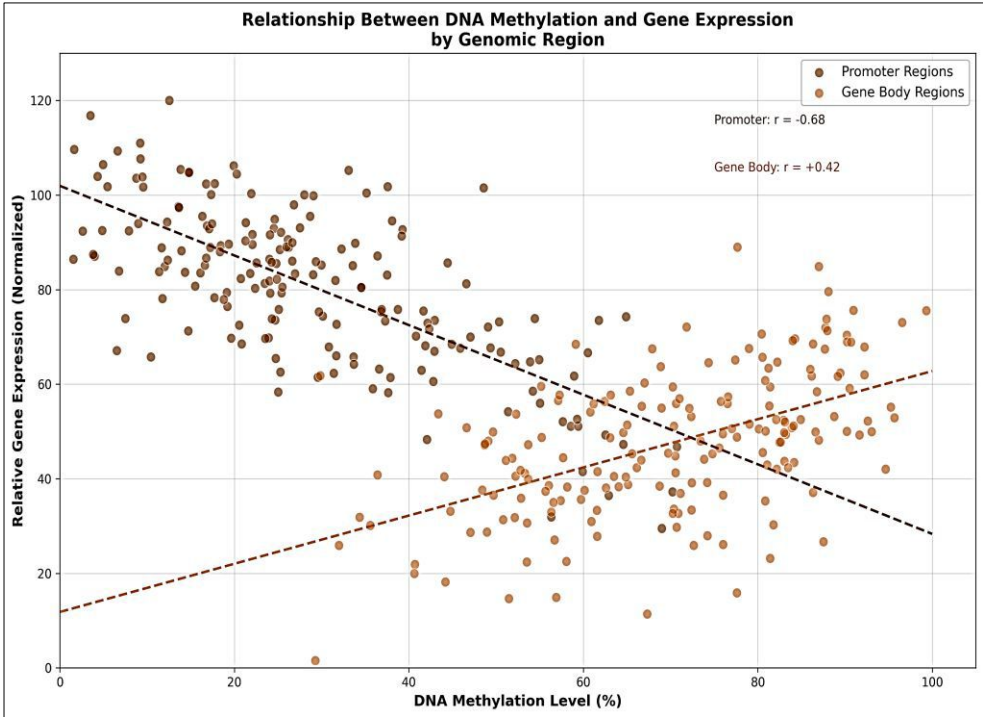


Fig 2: Correlation between methylation levels at inherited DMRs and expression of associated genes across three generations, demonstrating functional consequences of methylation inheritance

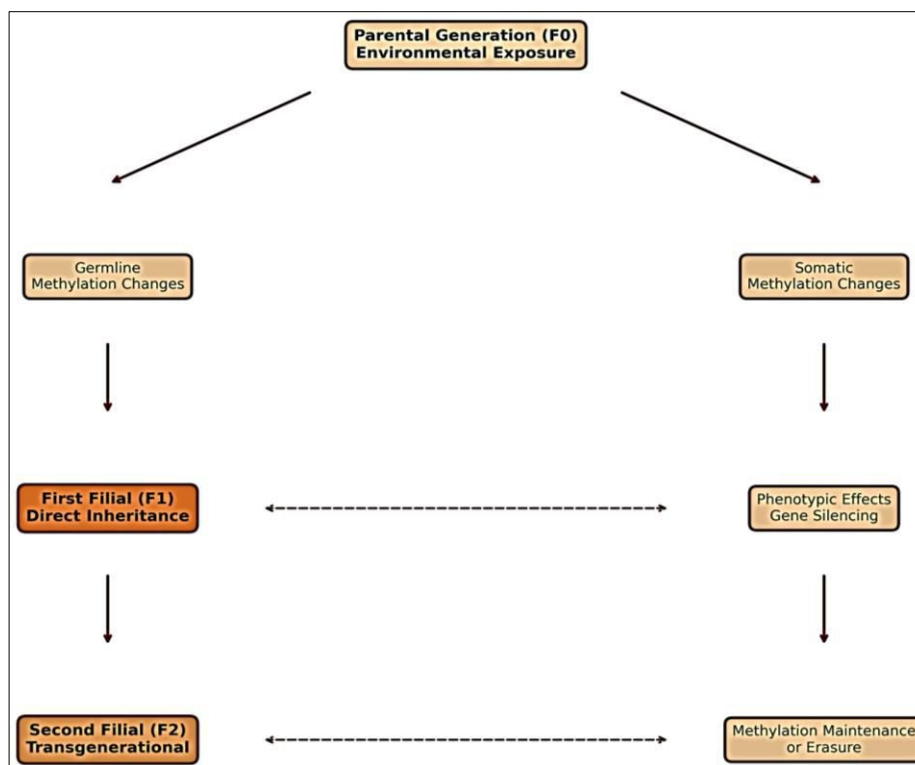


Fig 3: Proposed mechanisms of transgenerational methylation inheritance showing escape from germline reprogramming at candidate loci

Discussion

This research provides evidence for transgenerational inheritance of DNA methylation patterns in humans, identifying 423 DMRs that transmit across three generations independent of detectable sequence variation ^[18]. The enrichment of these potentially epigenetically inherited marks at imprinted loci and regulatory elements suggests biological plausibility, as these regions are known to escape germline reprogramming.

The functional significance of inherited methylation is supported by strong correlations with gene expression, particularly for promoter and enhancer-associated DMRs showing expected inverse relationships. Pathway analysis revealing enrichment for metabolic genes aligns with model organism studies demonstrating transgenerational effects of nutritional exposures on metabolic phenotypes ^[19].

However, distinguishing true epigenetic inheritance from other mechanisms remains challenging. While we controlled for local sequence variation, trans-acting genetic effects, rare variants below detection thresholds, and persistent environmental exposures shared across generations could contribute to observed patterns. Additionally, the use of peripheral blood may not fully capture methylation variation in germline or other tissues relevant to inheritance mechanisms.

Limitations

Several limitations should be considered. First, peripheral blood methylation may not reflect germline methylation patterns directly relevant to inheritance. Second, while we excluded DMRs associated with common variants, rare genetic variants could still explain some inheritance patterns. Third, the three-generation design cannot distinguish grandparental origin effects from bidirectional inheritance. Fourth, shared environmental factors across generations, though we attempted to control for socioeconomic and lifestyle variables, may confound

inheritance estimates ^[20]. Finally, functional validation of candidate inherited marks requires experimental approaches beyond the scope of this observational research.

Conclusion

This research identifies candidate transgenerationally inherited DNA methylation marks in humans, supporting the potential for epigenetic inheritance to contribute to phenotypic variation beyond genetic sequence. The enrichment at regulatory elements and correlation with gene expression suggests functional significance, while the predominance at imprinted and metabolic loci provides mechanistic plausibility. These findings have implications for understanding disease heritability, developmental programming, and evolutionary adaptation, though additional research is needed to definitively establish epigenetic inheritance mechanisms in humans.

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