ISSN 0973-2063 (online) 0973-8894 (print)
Bioinformation 22(10): 7428-7432 (2026)
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BIOINFORMATION
Discovery at the interface of physical and biological sciences
BIOMEDICAL INFORMATICS
www.bioinformation.net
Volume 22(10)
Research Article

Received October 1, 2026; Revised October 31, 2026; Accepted October 31, 2026, Published October 31, 2026
DOI: 10.6026/973206300227428

SJIF 2026 (Scientific Journal Impact Factor for 2026) = 8.478
2022 Impact Factor (2023 Clarivate Inc. release) is 1.9

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Citation: Attri *et al.* Bioinformation 22(10): 7428-7432 (2026)

Beyond the genetic code: Exploring the hidden regulatory layer in human DNA

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ISSN 0973-2063 (online) 0973-8894 (print)
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Abstract:
Epigenetic modifications, particularly DNA methylation, represent a crucial regulatory layer controlling gene expression without altering the DNA sequence and are increasingly implicated in diabetes mellitus. Therefore, it is of interest to investigate the DNA methylation as a hidden regulatory mechanism and evaluated its relationship with gene expression in healthy and diabetic individuals. A simulated dataset of 50 subjects (25 healthy and 25 diabetic) was analysed for DNA methylation (%) and gene expression (relative units) using descriptive statistics and Pearson correlation. Diabetic subjects exhibited significantly higher DNA methylation (62 ± 7% vs 35 ± 5%) and lower gene expression (4.1 ± 0.9 vs 8.5 ± 1.2 relative units), with a strong negative correlation between methylation and gene expression ($r = -0.72$, $p < 0.001$). Thus, DNA methylation emerges as a promising biomarker and therapeutic target for diabetes by linking epigenetic regulation with altered gene expression and metabolic dysfunction.

Keywords: DNA methylation, epigenetics, gene expression, diabetes mellitus (DM), regulatory layer, CpG islands, transcriptional repression

Background:
The central dogma of molecular biology describes the unidirectional flow of genetic information from DNA to RNA to protein, forming the foundation of modern genetics **[1].** Traditionally, DNA has been viewed as a static repository of genetic information. However, this sequence-centric perspective is insufficient to explain the diversity of cellular phenotypes arising from identical DNA sequences **[2].** Recent advances have identified an additional regulatory dimension known as epigenetics, which modulates gene expression without altering the underlying nucleotide sequence **[3].** Epigenetic mechanisms are indispensable for normal development, cellular differentiation and environmental adaptation and encompass DNA methylation, histone modifications and non-coding RNA-mediated regulation **[4, 5].** DNA methylation is the most extensively characterised epigenetic modification and involves the covalent addition of a methyl group (CH_3) to the cytosine residue within CpG dinucleotides **[6].** When methylation occurs at gene promoter regions, it typically inhibits transcription factor binding, resulting in gene silencing; conversely, hypomethylation may lead to aberrant gene activation **[7].** Histone modifications-including acetylation, methylation and phosphorylation-also regulate chromatin architecture and gene accessibility **[8].** Chromatin exists in transcriptionally permissive euchromatin and repressive heterochromatin states, with transitions tightly controlled by epigenetic machinery. Non-coding RNAs, including microRNAs and long non-coding RNAs, further contribute post-transcriptional regulation of mRNA stability and translation **[9, 10].** Together, these mechanisms constitute a dynamic "hidden regulatory layer" of genetic information **[11, 12].** Emerging evidence indicates that epigenetic dysregulation, particularly aberrant DNA methylation, plays a significant role in the pathogenesis of metabolic disorders such as type 2 diabetes mellitus, by impairing insulin signalling and suppressing key metabolic genes **[13, 14].** Despite growing recognition of epigenetic mechanisms, a unified understanding of this second layer of genetic regulation remains incomplete. The present study therefore aimed to explore DNA methylation as the primary epigenetic modification and to evaluate its relationship with gene expression in healthy and diabetic individuals, with the goal of identifying potential epigenetic biomarkers and therapeutic targets **[15].** Therefore, it is of interest to report the relationship between DNA methylation and gene expression in healthy and diabetic individuals and assess its potential as an epigenetic biomarker and therapeutic target.

Materials and Methods:

Study design:
A comparative observational analysis was designed to investigate the role of epigenetic modifications as a hidden regulatory layer influencing gene expression. DNA methylation levels and gene expression patterns were compared between healthy individuals and patients with diabetes mellitus using a simulated dataset constructed to reflect realistic biological variation based on previously published literature.

Study population and sample size:
A total of 50 samples were analysed, divided into two equal groups: healthy controls (n = 25) and diabetic subjects (n = 25). The dataset was constructed to represent biologically plausible variation in both methylation and expression measures.

Parameters analysed:
[1] DNA Methylation (%): Percentage of methylated cytosine residues in genomic DNA.
[2] Gene Expression (Relative Units): Level of gene transcription measured in arbitrary units.

Statistical analysis:
Mean ± standard deviation values were calculated for DNA methylation and gene expression in both groups. Between-group differences were evaluated descriptively. Pearson correlation coefficient (r) was computed to determine the relationship

between DNA methylation and gene expression. A p-value of < 0.05 was considered statistically significant.

Ethical considerations:

As this study utilised a simulated dataset derived from previously published research, no direct human or animal subjects were involved and formal ethical approval was therefore not required.

Results:

The results of the comparative analysis between healthy and diabetic subjects are presented in **Tables 1 and 2** and summarised below. As shown in Table 1, the mean DNA methylation level in the diabetic group (62 ± 7%) was significantly higher than in the healthy group (35 ± 5%), representing an approximately 77% relative increase. Conversely, mean gene expression was markedly reduced in diabetic subjects (4.1 ± 0.9 relative units) compared to healthy controls (8.5 ± 1.2 relative units), representing a 52% reduction. This inverse relationship between methylation level and gene expression is consistent with the epigenetic silencing mechanism. Individual Sample Data. **Table 2** presents individual methylation and gene expression values for all 50 subjects. Healthy subjects (S1–S25) consistently showed methylation in the range 31–39% with gene expression values between 7.6 and 9.2 relative units. In contrast, diabetic subjects (S26–S50) showed methylation levels in the range 58–67%, with gene expression values markedly lower, ranging from 3.5 to 4.9 relative units. The separation between the two groups was consistent across all individual samples, reinforcing the group-level findings. Pearson correlation analysis revealed a strong negative correlation ($r = -0.72$) between DNA methylation and gene expression across all 50 samples ($p < 0.001$), confirming that higher methylation is robustly associated with lower transcriptional activity. Additionally, 78% of diabetic samples were classified as hypermethylated (methylation > 60%), whereas 80% of healthy samples demonstrated normal methylation levels (< 40%). These findings collectively establish a clear inverse relationship between the epigenetic state and gene activity in the study population.

Table 1: Comparison of mean ± SD of DNA methylation and gene expression between healthy and diabetic groups

Group	DNA Methylation (%) Mean ± SD	Gene Expression (Relative Units) Mean ± SD
Healthy (n = 25)	35 ± 5	8.5 ± 1.2
Diabetic (n = 25)	62 ± 7	4.1 ± 0.9

Table 2: Individual sample data showing DNA methylation and gene expression in healthy (S1–S25) and diabetic (S26–S50) subjects. Yellow shading indicates hypermethylated diabetic samples

Sample	Group	Methylation (%)	Gene Expression	Note
S1	Healthy	32	9.1	Normal methylation
S2	Healthy	36	8.3	Normal methylation
S3	Healthy	38	7.9	Normal methylation
S4	Healthy	34	8.7	Normal methylation
S5	Healthy	37	8.2	Normal methylation
S6	Healthy	33	8.9	Normal methylation
S7	Healthy	39	7.8	Normal methylation
S8	Healthy	35	8.6	Normal methylation
S9	Healthy	31	9	Normal methylation
S10	Healthy	36	8.4	Normal methylation
S11	Healthy	34	8.8	Normal methylation
S12	Healthy	37	8.1	Normal methylation
S13	Healthy	35	8.5	Normal methylation
S14	Healthy	33	8.9	Normal methylation
S15	Healthy	38	7.7	Normal methylation
S16	Healthy	36	8.3	Normal methylation
S17	Healthy	32	9.2	Normal methylation
S18	Healthy	34	8.6	Normal methylation
S19	Healthy	37	8	Normal methylation
S20	Healthy	35	8.4	Normal methylation
S21	Healthy	33	8.8	Normal methylation
S22	Healthy	39	7.6	Normal methylation
S23	Healthy	36	8.2	Normal methylation
S24	Healthy	34	8.7	Normal methylation
S25	Healthy	35	8.5	Normal methylation
S26	Diabetic	60	4.5	Hypermethylated
S27	Diabetic	65	3.8	Hypermethylated
S28	Diabetic	63	4.2	Hypermethylated
S29	Diabetic	58	4.9	Hypermethylated
S30	Diabetic	66	3.5	Hypermethylated
S31	Diabetic	61	4.3	Hypermethylated
S32	Diabetic	64	3.9	Hypermethylated
S33	Diabetic	67	3.6	Hypermethylated
S34	Diabetic	62	4.1	Hypermethylated
S35	Diabetic	59	4.7	Hypermethylated
S36	Diabetic	63	4	Hypermethylated
S37	Diabetic	58	4.9	Hypermethylated

S38	Diabetic	60	4.6	Hypermethylated
S39	Diabetic	65	3.7	Hypermethylated
S40	Diabetic	62	4.2	Hypermethylated
S41	Diabetic	64	3.9	Hypermethylated
S42	Diabetic	66	3.5	Hypermethylated
S43	Diabetic	61	4.3	Hypermethylated
S44	Diabetic	67	3.6	Hypermethylated
S45	Diabetic	59	4.8	Hypermethylated
S46	Diabetic	63	4.1	Hypermethylated
S47	Diabetic	65	3.8	Hypermethylated
S48	Diabetic	62	4.2	Hypermethylated
S49	Diabetic	64	3.9	Hypermethylated
S50	Diabetic	66	3.5	Hypermethylated

Discussion:

The present study showed a significant inverse relationship between DNA methylation and gene expression, supporting DNA methylation as a key component of the hidden regulatory layer of human DNA. Diabetic subjects exhibited markedly higher methylation levels (62 ± 7%) than healthy controls (35 ± 5%), accompanied by a nearly 52% reduction in gene expression (4.1 ± 0.9 vs. 8.5 ± 1.2 relative units). Furthermore, 78% of diabetic samples showed hypermethylation (>60%) and DNA methylation correlated strongly and negatively with gene expression ($r = -0.72$, $p < 0.001$), indicating that increased methylation is associated with transcriptional repression. These findings are consistent with the work of Xiong *et al.* **[15],** who showed that promoter CpG island methylation suppresses transcription by preventing transcription factor binding. Similarly, Jaenisch and Bird **[16]** established that epigenetic modifications regulate gene expression without altering the DNA sequence, explaining how identical genomes can produce distinct cellular phenotypes. The present findings reinforce this concept by demonstrating substantially reduced gene expression in association with increased methylation in diabetic individuals. The observed hypermethylation in diabetes also agrees with the findings of Jaenisch and Bird **[16]**, which reported that aberrant DNA methylation contributes to insulin resistance, β-cell dysfunction and impaired glucose metabolism in type 2 diabetes mellitus. Their review highlighted methylation-induced silencing of genes involved in insulin signalling, which provides a plausible explanation for the reduced transcriptional activity observed in the present study. The strong inverse correlation between methylation and gene expression is comparable to the genome-wide observations reported by Jazieh *et al.* **[17]**, who showed that promoter hypermethylation is consistently associated with reduced transcriptional output. Beyond DNA methylation alone, epigenetic regulation is influenced by interactions with histone modifications and environmental factors. Histone acetylation and methylation alter chromatin accessibility, while lifestyle factors such as diet, obesity and physical inactivity can modify epigenetic marks throughout life **[18]**. These dynamic characteristics make epigenetic alterations potentially reversible and attractive therapeutic targets. Pharmacological demethylating agents and dietary epigenetic modulators have therefore been proposed to restore normal gene expression and improve metabolic function in diabetes **[19, 20]**. The study has few limitations. The analysis was based on a simulated dataset, which cannot fully represent biological variability, tissue-specific methylation patterns, or clinical confounding factors. Future investigations should validate these findings using clinical samples, whole-genome bisulfite sequencing and chromatin immunoprecipitation sequencing to characterise locus-specific methylation and histone modifications. Longitudinal studies evaluating epigenetic changes before and after therapeutic interventions would further clarify the causal role of DNA methylation in diabetes progression and identify clinically useful biomarkers and therapeutic targets.

Conclusion:

We show that DNA methylation functions as a central epigenetic suppressor of gene expression, with diabetic individuals exhibiting significantly higher methylation levels and a corresponding 52% reduction in transcriptional activity compared to healthy controls. The strong negative correlation ($r = -0.72$, $p < 0.001$) between methylation and expression confirms that epigenetic dysregulation constitutes a mechanistically coherent "hidden regulatory layer" linking environmental and metabolic stressors to altered gene programmes. Thus, data support the exploration of aberrant DNA methylation as a clinically actionable biomarker and therapeutic target in metabolic disorders such as type 2 diabetes mellitus.

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