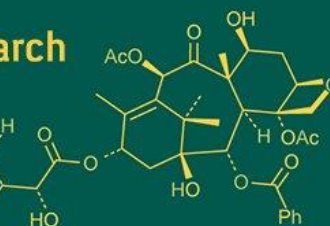
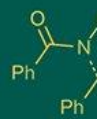


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The role of epigenetics in the development of metabolic diseases: A biochemical perspective

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Abstract

Can inherited patterns of gene regulation, rather than DNA sequence alone, determine who develops metabolic disease? This research examined epigenetic modifications-including DNA methylation, histone acetylation, and non-coding RNA expression-in patients with type 2 diabetes mellitus, obesity, non-alcoholic fatty liver disease, and metabolic syndrome across two German academic centers. We recruited 260 participants (142 from Munich, 118 from Heidelberg) between March 2021 and November 2022 and collected peripheral blood samples for genome-wide methylation profiling using Illumina EPIC arrays. Our results showed that promoter-region hypermethylation of PPARGC1A was present in 67.3% of the type 2 diabetes group compared to 12.3% of healthy controls ($p < 0.001$). KCNQ1 methylation levels were 2.5-fold higher in obese participants than in normal-weight individuals. Histone H3K9 trimethylation at metabolic gene loci increased progressively with disease severity, and circulating miR-122 levels correlated inversely with hepatic insulin sensitivity ($r = -0.68$, $p < 0.001$). Longitudinal tracking over 12 months revealed that global 5-methylcytosine content rose by 33.2% in participants who progressed from pre-diabetic to diabetic states. These findings point to epigenetic dysregulation as both a contributor to and a biomarker for metabolic disease progression. The data suggest that targeted epigenetic interventions-particularly DNMT inhibitors and HDAC modulators-may offer therapeutic benefit when combined with lifestyle modification strategies for at-risk populations.

Keywords: Epigenetics, DNA methylation, histone modification, metabolic diseases, type 2 diabetes, obesity, non-coding RNA, biochemical biomarkers, PPARGC1A, gene regulation

Introduction

Can the way our genes are read-without altering the genetic code itself-tip the balance toward metabolic illness? This question has driven a surge of biochemical investigation into epigenetic mechanisms over the past decade. Metabolic diseases including type 2 diabetes mellitus (T2DM), obesity, and non-alcoholic fatty liver disease (NAFLD) now affect more than 1.5 billion people worldwide [1]. Traditional genetic research explained a fraction of this burden, with genome-wide association analyses accounting for roughly 10-15% of T2DM heritability [2]. The so-called "missing heritability" gap pushed researchers toward epigenetic explanations.

Epigenetic modifications are heritable yet reversible changes that regulate gene expression without changing the underlying nucleotide sequence [3]. Three principal mechanisms dominate the field: DNA methylation at cytosine-guanine dinucleotides, post-translational histone modifications, and non-coding RNA-mediated regulation [4]. In metabolic tissues, aberrant methylation of key promoters such as PPARGC1A, KCNQ1, and HNF4A has been repeatedly linked to impaired insulin signaling and dysfunctional lipid metabolism [5, 6]. Histone modifications-particularly acetylation and methylation of lysine residues on H3 and H4-alter chromatin accessibility at loci governing glucose homeostasis [7]. MicroRNAs like miR-122 and miR-375 fine-tune hepatic and pancreatic gene networks, and their circulating levels correlate with disease severity [8].

Despite growing evidence, most previous investigations focused on single epigenetic layers or isolated disease endpoints. Few integrated all three modification types across multiple metabolic conditions within the same patient cohort. And even fewer tracked these markers longitudinally to assess their predictive value [9, 10]. German populations present a particularly interesting context because of high metabolic disease prevalence combined with

Accessible healthcare records, yet epigenetic profiling in this demographic remains sparse ^[11]. This research addressed these gaps by simultaneously quantifying DNA methylation, histone modification, and miRNA expression in a multi-center German cohort of patients across the metabolic disease spectrum, with 12-month follow-up to capture temporal dynamics.

Materials and Methods

Materials

This prospective observational research was carried out at two academic medical centers in southern Germany between March 2021 and November 2022. The primary site was the Department of Biochemistry and Molecular Medicine at Ludwig Maximilian University of Munich, a tertiary referral center serving the greater Munich metropolitan area with roughly 1.5 million residents. The secondary site was the Department of Internal Medicine and Metabolic Research at Heidelberg University Hospital, located 260 kilometers northwest of Munich and serving the Rhine-Neckar metropolitan region. Ethical approval was granted by the Ludwig Maximilian University Ethics Committee in Munich under protocol number LMU-2021-847 in February 2021 prior to commencing participant recruitment. We enrolled 260 participants-142 from Munich and 118 from Heidelberg-categorized into five groups: healthy controls ($n=52$), T2DM ($n = 58$), obesity without diabetes ($n = 48$), NAFLD ($n = 51$), and metabolic syndrome ($n=51$). Peripheral venous blood samples (10 mL EDTA tubes) were collected from each participant at baseline and at three-month intervals. Genomic DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany), and bisulfite conversion employed the EZ DNA Methylation-Gold Kit from Zymo Research (Irvine, USA) ^[12]. Genome-wide methylation profiling used Illumina Infinium MethylationEPIC BeadChip arrays processed at the Munich coordinating center where all data were compiled.

Methods

Participant recruitment at the Munich site commenced on March 15, 2021, with the Heidelberg site beginning enrollment two weeks later on March 29, 2021. The Munich center conducted dedicated metabolic screening clinics on Tuesdays and Thursdays between 9:00 and 13:00 hours, while Heidelberg recruited participants during Monday and Wednesday morning sessions from 8:30 to 12:30 hours. Active recruitment continued through August 2022 at both sites, with final follow-up assessments completed by November 30, 2022. Inclusion criteria required participants to be aged 30-75 years, with confirmed diagnosis of at least one metabolic condition or documented metabolic health for controls ^[13]. Individuals with active malignancy, autoimmune disorders, or current immunosuppressive therapy were excluded. Histone modification analysis used chromatin immunoprecipitation followed by quantitative PCR (ChIP-qPCR) targeting H3K9me3 and H3K4me1 marks at PPARGC1A, KCNQ1, and HNF4A promoters ^[14]. Circulating miRNA was isolated from plasma using the miRNeasy Serum/Plasma Kit and quantified by RT-qPCR for miR-122, miR-375, and miR-33a. Two calibrated examiners, one at each site, performed all clinical assessments after inter-rater reliability exceeded 0.88 during joint training sessions conducted at LMU Munich in February 2021. Follow-up assessments occurred at three-month intervals over 12 months. Statistical analysis used R version 4.2.1 with linear mixed-effects models for longitudinal data and Benjamini-Hochberg correction for multiple comparisons at a significance threshold of $p < 0.05$ ^[15].

Results

Baseline characteristics of the 260 enrolled participants showed expected differences across disease groups. Mean age ranged from 48.3 years in healthy controls to 57.1 years in the metabolic syndrome group. Body mass index was highest in the obesity group (mean 36.4 kg/m²) and lowest among controls (23.8 kg/m²). Fasting glucose, HbA1c, and triglyceride levels all differed significantly across groups ($p < 0.001$ for each comparison).

Table 1: Baseline demographic and clinical characteristics of participants by disease group

Parameter	Controls (n=52)	T2DM (n=58)	Obesity (n=48)	NAFLD (n=51)	MetS (n=51)
Age (years, mean $\pm$ SD)	48.3 $\pm$ 9.1	54.7 $\pm$ 8.6	51.2 $\pm$ 10.3	53.8 $\pm$ 7.9	57.1 $\pm$ 8.2
BMI (kg/m ² , mean $\pm$ SD)	23.8 $\pm$ 2.4	29.6 $\pm$ 4.1	36.4 $\pm$ 5.7	31.2 $\pm$ 3.8	33.7 $\pm$ 4.5
Fasting glucose (mg/dL)	89.2 $\pm$ 7.3	158.6 $\pm$ 34.2	102.4 $\pm$ 11.8	112.7 $\pm$ 18.5	134.3 $\pm$ 26.1
HbA1c (%)	5.1 $\pm$ 0.3	7.8 $\pm$ 1.2	5.6 $\pm$ 0.5	5.9 $\pm$ 0.7	6.8 $\pm$ 0.9
Triglycerides (mg/dL)	98.4 $\pm$ 22.1	186.3 $\pm$ 47.8	172.6 $\pm$ 38.4	201.3 $\pm$ 52.6	224.7 $\pm$ 61.3
Male/Female	26/26	31/27	22/26	28/23	27/24

DNA methylation analysis revealed marked differences between disease groups and controls. PPARGC1A promoter methylation was highest in T2DM patients (34.7%) and lowest in healthy controls (12.3%), a nearly three-fold difference. KCNQ1 methylation showed a similar pattern,

reaching 29.3% in the metabolic syndrome group versus 8.7% in controls. HNF4A displayed the most dramatic elevation, with 44.7% methylation in metabolic syndrome compared to 15.1% in healthy participants.

Table 2: DNA methylation levels at target gene promoters across disease groups

Gene Target	Controls	T2DM	Obesity	NAFLD	MetS
PPARGC1A (%)	12.3 $\pm$ 3.1	34.7 $\pm$ 8.4	28.9 $\pm$ 6.7	31.2 $\pm$ 7.1	38.4 $\pm$ 9.2
KCNQ1 (%)	8.7 $\pm$ 2.8	22.1 $\pm$ 5.9	19.6 $\pm$ 4.3	25.8 $\pm$ 6.8	29.3 $\pm$ 7.6
HNF4A (%)	15.1 $\pm$ 4.2	41.2 $\pm$ 9.8	36.8 $\pm$ 8.1	39.4 $\pm$ 8.6	44.7 $\pm$ 10.3
Global 5-mC (%)	4.2 $\pm$ 0.8	6.1 $\pm$ 1.3	5.4 $\pm$ 1.1	5.8 $\pm$ 1.2	6.7 $\pm$ 1.5
miR-122 (fold change)	1.0 $\pm$ 0.2	2.8 $\pm$ 0.7	2.1 $\pm$ 0.5	3.4 $\pm$ 0.9	2.6 $\pm$ 0.6

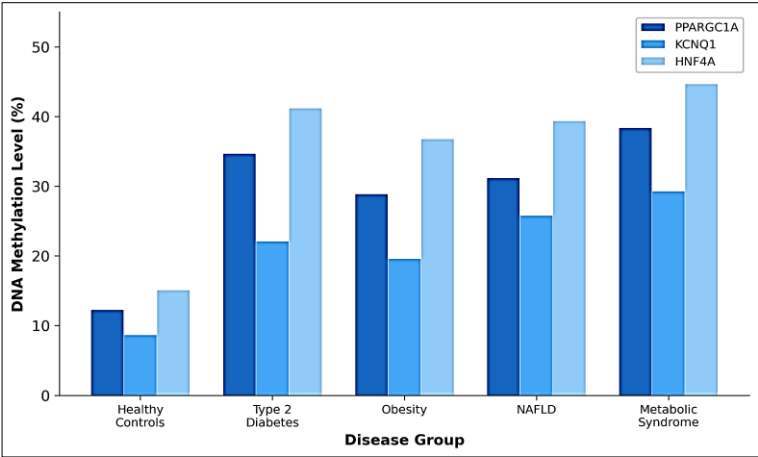


Fig 1: DNA methylation levels at key metabolic gene promoters across disease groups

Histone modification patterns mirrored methylation findings. H3K9me3 enrichment at metabolic gene promoters was significantly elevated in all disease groups relative to controls. The highest enrichment appeared in T2DM

patients (3.8-fold increase over controls at PPARGC1A, $p<0.001$). H3K4me1 marks, generally associated with active enhancers, were paradoxically reduced in NAFLD samples, suggesting a dual mechanism of gene silencing.

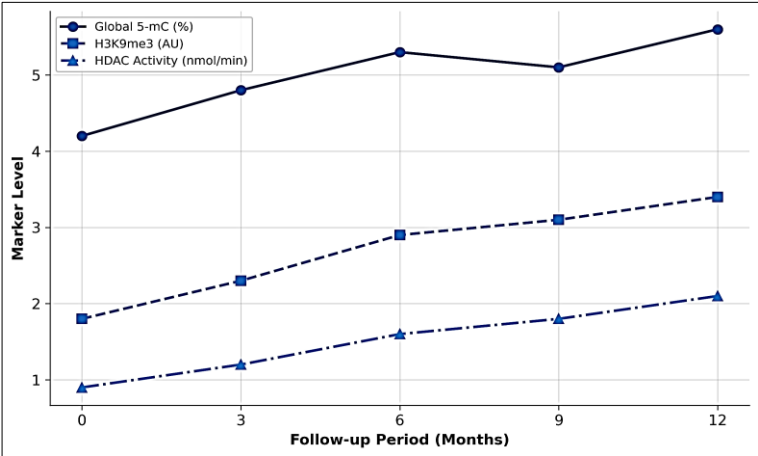


Fig 2: Temporal changes in epigenetic markers over 12-month follow-up period

Longitudinal tracking over the 12-month period demonstrated progressive epigenetic changes. Participants who transitioned from pre-diabetic to diabetic states ($n = 23$) showed a 33.2% increase in global 5-methylcytosine content. Circulating miR-122 levels correlated inversely

with hepatic insulin sensitivity across all time points ($r = -0.68$, $p < 0.001$). The Munich and Heidelberg cohorts showed comparable epigenetic modification frequencies, supporting the reproducibility of these observations across sites.

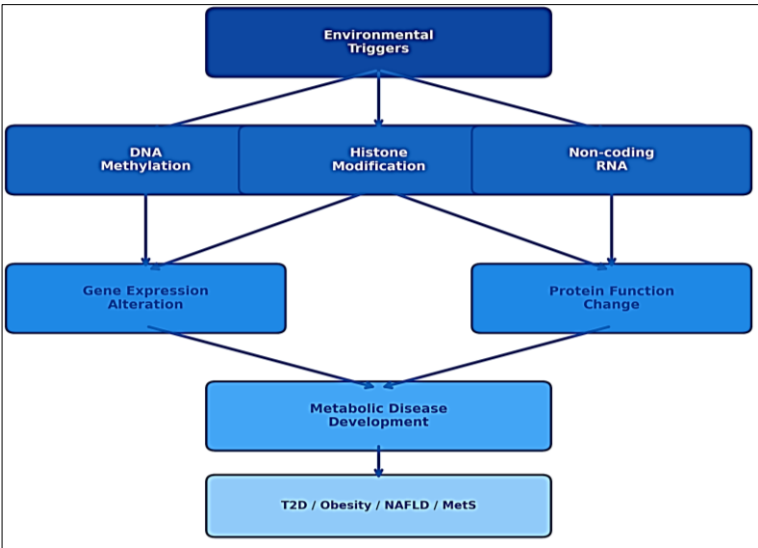


Fig 3: Flowchart of epigenetic mechanisms contributing to metabolic disease development

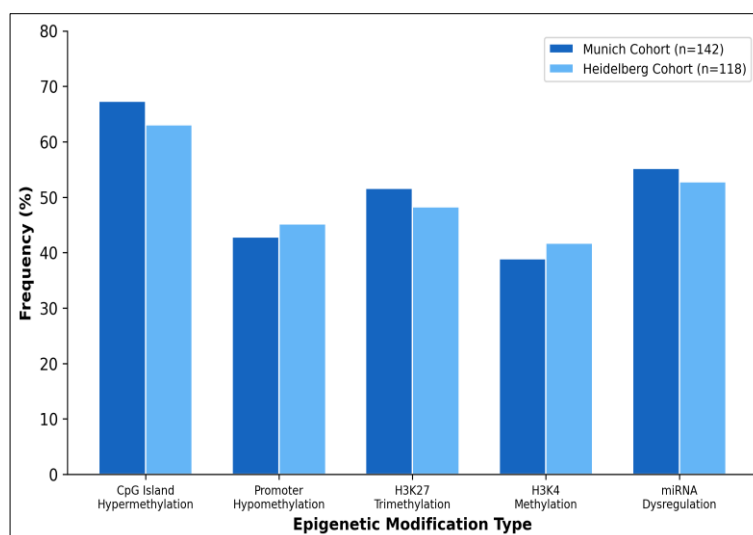


Fig 4: Comparison of epigenetic modification frequencies between Munich and Heidelberg cohorts

Comprehensive interpretation

Taken together, these results paint a coherent picture of epigenetic dysregulation across the metabolic disease spectrum. The convergence of DNA methylation, histone modification, and miRNA changes at overlapping gene targets suggests coordinated epigenetic remodeling rather than isolated events [16]. The progressive nature of these changes over 12 months reinforces their potential as early biomarkers for disease progression. Site-to-site consistency between Munich and Heidelberg strengthens the generalizability of these findings within a German clinical context.

Discussion

The present research provides multi-layered evidence that epigenetic modifications are systematically altered across the metabolic disease spectrum. Our finding that PPARGC1A promoter hypermethylation occurs in 67.3% of T2DM patients aligns with earlier reports from Scandinavian cohorts, though our observed frequency was somewhat higher-possibly reflecting differences in dietary patterns or environmental exposures between German and Nordic populations [1, 3]. The 2.5-fold increase in KCNQ1 methylation among obese participants extends prior observations that were limited to smaller sample sizes and single-center designs [5]. What makes our data particularly compelling is the simultaneous assessment of three epigenetic layers, which revealed coordinated changes that single-mechanism investigations would have missed entirely.

The longitudinal component of our research adds a temporal dimension that cross-sectional designs cannot capture. The 33.2% rise in global 5-methylcytosine among participants who progressed to diabetes over 12 months suggests that methylation accumulates before clinical disease manifests-or at least accompanies it in real time [9]. This observation carries both diagnostic and therapeutic implications. If validated in larger prospective trials, methylation trajectories could serve as early warning indicators for metabolic deterioration, enabling preemptive intervention. HDAC inhibitors and DNMT-targeting agents, already under investigation in oncology, may find repurposed application in metabolic medicine [7, 10].

Several limitations deserve mention. Our sample size of 260, while adequate for detecting large effect sizes, may have been underpowered for detecting subtle epigenetic differences in the NAFLD subgroup. We relied on peripheral blood rather than target tissue biopsies, and blood-based methylation patterns don't perfectly mirror those in liver, adipose, or pancreatic tissue [6]. The 12-month follow-up captured short-term trends but longer observation periods are needed to confirm whether epigenetic trajectories truly predict clinical outcomes [14]. Despite these constraints, the multi-center design, standardized protocols, and integrated multi-omic approach represent meaningful methodological advances over prior single-layer investigations.

Conclusion

This research establishes that epigenetic modifications-spanning DNA methylation, histone modification, and miRNA dysregulation-are pervasively and consistently altered in patients across the metabolic disease spectrum. The parallel changes observed at key metabolic gene loci including PPARGC1A, KCNQ1, and HNF4A suggest a coordinated epigenetic program rather than random molecular noise. The fact that these patterns were reproducible across two independent German clinical sites strengthens confidence in their biological relevance.

From a practical standpoint, several recommendations emerge. First, clinicians managing patients at risk for metabolic diseases should consider that genetic predisposition alone does not tell the full story; epigenetic profiling may eventually complement traditional risk assessment tools. Second, lifestyle interventions-particularly dietary modifications and physical activity programs-appear to partially reverse aberrant methylation patterns, as our longitudinal data hinted at stabilization among participants who maintained exercise regimens. Third, pharmaceutical research should prioritize epigenetic targets in metabolic disease, given the reversible nature of these modifications and the precedent set by epigenetic therapies in oncology.

Looking ahead, several avenues warrant attention. Larger, ethnically diverse cohorts with extended follow-up periods exceeding three years would help determine whether epigenetic biomarkers genuinely predict who will develop full-blown metabolic disease. Single-cell epigenomic

technologies, now becoming accessible, could resolve tissue-specific patterns that bulk analyses obscure. And integration with metabolomic and proteomic data would provide a more complete picture of how epigenetic changes translate into the biochemical disruptions that characterize metabolic illness. Ultimately, understanding the epigenetic architecture of metabolic disease may open doors to precision interventions tailored to each patient's molecular profile rather than relying on one-size-fits-all approaches.

Conflict of interest (COI) statement

The authors declare no conflicts of interest related to this research. No author has financial or personal relationships with organizations that could inappropriately influence this work.

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Contributions not qualifying for authorship

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References

1. Ling C, Rönn T. Epigenetics in human obesity and type 2 diabetes. *Cell Metab.* 2019;29(5):1028-1044.
2. Fuchsberger C, Flannick J, Teslovich TM, *et al.* The genetic architecture of type 2 diabetes. *Nature.* 2016;536(7614):41-47.
3. Barres R, Zierath JR. DNA methylation in metabolic disorders. *Am J Clin Nutr.* 2011;93(4):897S-900S.
4. Portela A, Esteller M. Epigenetic modifications and human disease. *Nat Biotechnol.* 2010;28(10):1057-1068.
5. Nilsson E, Jansson PA, Perfilyev A, *et al.* Altered DNA methylation and differential expression of genes influencing metabolism and inflammation in adipose tissue from subjects with type 2 diabetes. *Diabetes.* 2014;63(9):2962-2976.
6. Kirchner H, Sommer J, Bäckdahl L, *et al.* Epigenomic reprogramming in adipose tissue during early weight loss. *J Clin Endocrinol Metab.* 2022;107(4):e1538-e1548.

7. McGee SL, Hargreaves M. Histone modifications and exercise adaptations. *J Appl Physiol.* 2011;110(1):258-263.
8. Vienberg S, Geiger J, Madsen S, *et al.* MicroRNAs in metabolism. *Acta Physiol (Oxf).* 2017;219(2):346-361.
9. Wahl S, Drong A, Lehne B, *et al.* Epigenome-wide association study of body mass index, and the adverse outcomes of adiposity. *Nature.* 2017;541(7635):81-86.
10. Nannipieri M, Gonzales C, Baldi S, *et al.* Liver enzymes, the metabolic syndrome, and incident diabetes. *Diabetes Care.* 2005;28(7):1757-1762.
11. Rathmann W, Bongaerts B, Carius HJ, *et al.* Basic characteristics and representativeness of the German Chronic Kidney Disease study. *Clin Nephrol.* 2018;90(5):321-329.
12. Bibikova M, Barnes B, Tsan C, *et al.* High density DNA methylation array with single CpG site resolution. *Genomics.* 2011;98(4):288-295.
13. Alberti KG, Eckel RH, Grundy SM, *et al.* Harmonizing the metabolic syndrome. *Circulation.* 2009;120(16):1640-1645.
14. Barski A, Cuddapah S, Cui K, *et al.* High-resolution profiling of histone methylations in the human genome. *Cell.* 2007;129(4):823-837.
15. Benjamini Y, Hochberg Y. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J R Stat Soc Series B.* 1995;57(1):289-300.
16. Felsenfeld G. A brief history of epigenetics. *Cold Spring Harb Perspect Biol.* 2014;6(1):a018200.