Review Article

The Impact of Epigenetics on the Pathophysiology of Type 2 Diabetes and Associated Nephropathic Complications

Amit Mukherjee, Tanusree Debbarman, Basu D. Banerjee[1], Sheelu S. Siddiqi

Rajiv Gandhi Centre for Diabetes and Endocrinology, J N Medical College and Hospital, Aligarh Muslim University, Aligarh, Uttar Pradesh, [1]Department of Elementology and Toxicology, Hamdard University, New Delhi, India

Abstract

Type 2 diabetes (T2D) is a long-term metabolic condition that presents considerable health challenges globally. As the disease progresses, the interplay between genetic, environmental, and lifestyle factors becomes increasingly evident, leading to complications. Epigenetics has emerged as a critical area of research, providing insights into how these factors can modify the expression and cellular behavior without altering the underlying DNA sequence. Various epigenetic mechanisms, including DNA methylation, histone modifications, chromatin remodeling, and non-coding RNA regulation, drive cell dysfunction, inflammation, and fibrosis, aggravating diabetes and its complications. Amongst all the complications diabetic kidney disease (DKD) also known as diabetic nephropathy (DN), is a significant microvascular complication often regarded as a silent killer, as early diagnosis remains highly complicated. This review investigates various epigenetic modifications associated with T2D and DKD, employing a database search strategy incorporating the PICO framework method to ensure comprehensive coverage of relevant literature. Advancements in epigenome profiling provide valuable insights into the functional outcomes and chromatin states of cells impacted by T2D. Understanding epigenetics thus emphasizes its crucial role in the development and progression of T2D and transition to DKD, while also highlighting the potential reversibility of epigenetic modifications and potency as a biomarker for predicting DKD. More extensive research is needed to identify specific epigenetic mechanisms involved in DKD to further refine predictive models and therapeutic strategies. This unified exploration of significant epigenetic modifications offers a focused analysis of how these alterations influence the trajectory of disease and presents new avenues for therapeutic intervention.

Keywords: Epigenetics, diabetic kidney disease (DKD), diabetic nephropathy (DN), DNA methylation, histone modification, micro-RNA, type 2 diabetes (T2D)

Introduction

The development of type 2 diabetes mellitus (T2D) is influenced by genetics to a significant extent. A family history of T2D increases the risk of developing the disease.[1] This realization has prompted a vigorous pursuit of genetic factors contributing to the etiology of T2D. Although genome-wide association studies have identified several loci associated with the risk of T2D,[2] genetic factors alone are unable to explain the development and progression of the disease and associated complications.[3] Diabetic kidney disease (DKD), or diabetic nephropathy (DN), is a microvascular complication of diabetes often called a 'silent killer'. It is the leading cause of end-stage renal disease (ESRD), profoundly affecting the quality of life.[4]

A decade ago, initial epigenetic studies investigating the epigenetic changes in specific candidate genes of pancreatic islets and skeletal muscle in individuals with T2D revealed altered DNA methylation patterns,[5] supporting a role for epigenetics in diabetes. Furthermore, chronic hyperglycemia-induced metabolic disorders, oxidative stress, and hemodynamic changes are largely driven by epigenetic variations rather than solely genetic variations in diabetic

Address for correspondence: Prof. Sheelu S. Siddiqi, Rajiv Gandhi Centre for Diabetes and Endocrinology, J N Medical College and Hospital, AMU, Aligarh, Uttar Pradesh, India.
E-mail: shafiqsheeluims@gmail.com

Submitted: 03-Feb-2024 **Revised:** 13-Oct-2024
Accepted: 11-Nov-2024 **Published:** 30-Dec-2024

How to cite this article: Mukherjee A, Debbarman T, Banerjee BD, Siddiqi SS. The impact of epigenetics on the pathophysiology of type 2 diabetes and associated nephropathic complications. Indian J Endocr Metab 2024;28:569-78.

Supplementary material available online: https://journals.lww.com/indjem/

Access this article online

Quick Response Code:

Website: https://journals.lww.com/indjem/

DOI: 10.4103/ijem.ijem_43_24

individuals.[6] Research indicates that even with strict glycemic control, diabetes complications can still develop because of prior high glucose exposure, a condition known as metabolic memory. This effect is linked to epigenetic alterations at promoter regions, implicating epigenetic modifications in the prolonged pathological processes of diabetes.[7]

Since then, advancements in technology and growing interest in T2D epigenetics have propelled research in this field, highlighting the need to understand these modifications for the pathogenesis of diabetic kidney disease (DKD) and identify predictive and therapeutic targets. This review aims to emphasize the need for more epigenetic research in DKD, as early diagnosis is still challenging and less explored compared to complications like diabetic retinopathy. By focusing on this critical area, we can identify novel biomarkers and therapeutic targets for better patient outcomes.

Methods

Identification of source

A comprehensive literature search was conducted to gather relevant studies and reviews on the role of epigenetics in the pathophysiology of T2D and DKD. The search was performed across PubMed and Web of Science databases. Keywords used in the search included 'epigenetics', 'type 2 diabetes', 'diabetic nephropathy', 'diabetic kidney disease', 'DNA methylation', 'histone modification', 'non-coding RNAs', and 'pathophysiology of diabetic kidney disease and diabetic nephropathy'. The search was limited to articles published in English from 2000 to 2023.

Inclusion and exclusion criteria

Articles were selected based on their relevance to the subject matter.

Inclusion criteria included:

a. Original articles and reviews focused on epigenetic mechanisms in T2D and DKD.
b. Studies involving human, animal, or *in vitro* models.
c. Articles published in peer-reviewed journals.

Exclusion criteria included:

a. Articles not focused on epigenetic mechanisms or unrelated to T2D or DKD.
b. Studies with insufficient data or unclear conclusions.
c. Non-peer-reviewed sources like conference abstracts or editorials.

Data extraction and synthesis

Relevant data were extracted from selected studies, including information on the type of epigenetic modifications (e.g. DNA methylation, histone modification, non-coding RNA), the specific genes or pathways involved, and reported clinical or experimental outcomes. The extracted data were organized into thematic categories for structured synthesis, and discrepancies in interpretation were resolved through discussion among the authors.

Method of evaluation

The quality of the included literature was critically evaluated, prioritizing the high-quality papers with substantial citations, featuring precise statistical analysis and accurate methodology.

Fundamental epigenetic mechanisms

Heritable information not encoded in DNA but can be passed down during cell division or from parent to offspring—is known as epigenetics. This includes incorporating histone variations and covalent modifications of histones and nucleotides [Figure 1].

In recent decades, pioneering research has established significant connections between epigenetic processes and the pathogenesis of T2D. Fundamental epigenetic mechanisms, highlighting the key epigenetic dysregulations in T2D-associated vascular complications, and treatment approaches are crucial for gaining insights into the impact of epigenetics on our current knowledge of T2D.[8]

DNA in the chromatin is tightly linked with histone proteins, which help package and organize DNA into the smallest structural units called nucleosomes. Eukaryotic chromosomal DNA starts its organization with nucleosomes and then a succession of higher-order structures, eventually forming the condensed chromosome visible under a light microscope. The key epigenetic mechanisms are outlined below.

Histone modification and chromatin remodeling

Histones are highly basic proteins in the chromatin of all eukaryotic cells. They are enriched with arginine and lysine, constituting approximately 25% of the amino acid residues.[9] Eukaryotic histones are classified into five major classes based on their molecular weight and amino acid composition.

Histones can undergo enzymatic modifications such as acetylation, methylation, glycosylation, ubiquitination, sumoylation, etc. These modifications impact various properties of histones, including net electric charge, shape, and the structural and functional characteristics of chromatin.[10] Consequently, these modifications play a critical role in regulating transcription.[9] X-ray diffraction analysis of crystallized nucleosome cores shows that each nucleosome comprises eight histone molecules with wrapped DNA.[11]

Amino-terminal tails extending from each histone are the sites for most of the histone modifications, and these tails play a crucial role in establishing interactions between nucleosomes in the chromatin.[12] The accurate positioning of nucleosome cores contributes to the regulation of gene expression.[12] Chromatin remodeling refers to the modification of nucleosomes in transcribed DNA by multiprotein complexes, facilitating the functioning of RNA polymerase during transcription.[13] There are two broad categories of complexes involved in chromatin modification or remodeling. One of these categories consists of the enzyme histone acetyltransferases (HATs) that transfer acetyl groups to specific lysine in nucleosomal histones, facilitating chromatin modification.[12] Multiple research has established a significant correlation between histone acetylation and enhanced

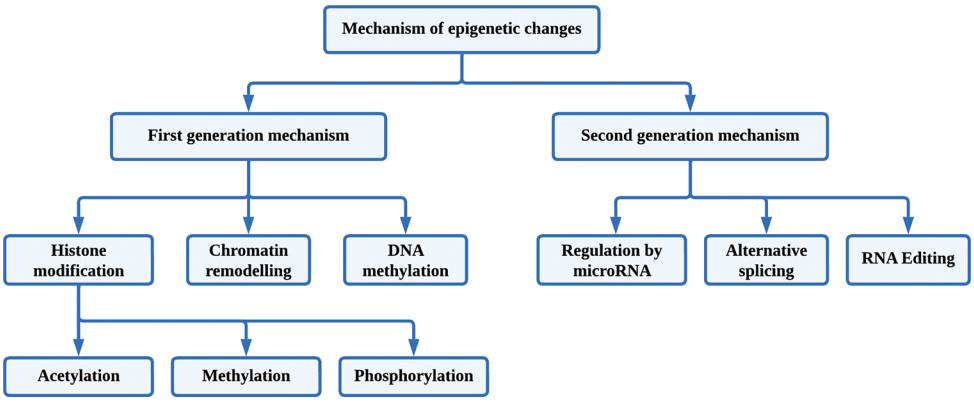

Figure 1: Schematic diagram of epigenetic changes.[12]

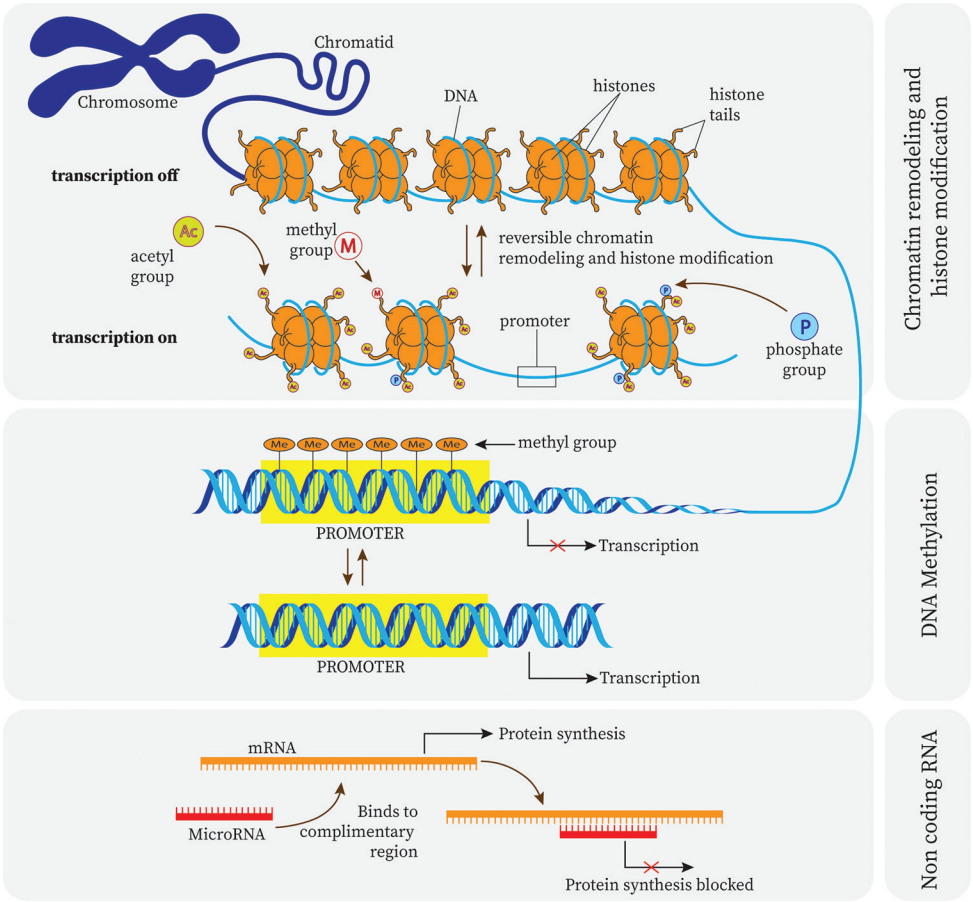

Figure 2: Basic epigenetic mechanisms for the regulation of gene expression

gene expression. This correlation might be attributed to the acetyl groups' ability to loosen the interaction between DNA and histone octamers in nucleosomes [Figure 2], potentially leading to increased transcription.[14] In addition to chromatin-modifying complexes, kinases, enzymes that transfer phosphate groups, could potentially be involved in the process.[12]

Another type of complex can interfere with the structure of nucleosomes associated with the gene's promoter. The SWI/SNF complex, consisting of at least eight proteins, regulates transcription by moving histone octamers along the DNA associated with nucleosomes. This repositioning of nucleosomes by the SWI/SNF complex enables transcription factors to interact with the DNA, activating gene expression.[15,16]

Active chromatin can also undergo reverse remodeling, leading to its transition into inactive chromatin. This process involves two biochemical modifications to histones in nucleosomes: deacetylation (carried out by histone deacetylases, or HDACs) and methylation (catalyzed by histone methyl transferases, or HMTs). These modifications result in transcriptionally inactive chromatin.[17–19]

DNA methylation

Chemical modifications of nucleotides also play a crucial role in gene regulation [Figure 2]. The majority of methylated cytosines are typically observed in pairs with the structure 5′-mCpG-3′ and its complementary sequence 3′-GpCm-5′.[20]

CpG dinucleotides are not evenly distributed throughout the genome. Instead, there are specific segments of DNA that have a higher density of CpG dinucleotides compared to other regions. These segments are referred to as CpG islands. CpG islands are predominantly found near the transcription start sites in the human genome, with an estimated count of approximately 30,000 islands. CpG islands are often unmethylated or have low methylation levels, which promotes transcription.[21]

miRNA

RNA interference (RNAi) involves small RNA molecules called short interfering RNAs (siRNAs) or microRNAs (miRNAs). These molecules, around 21 to 28 base pairs long, are produced from larger double-stranded RNA molecules by enzymes called Dicer. In the cytoplasm, siRNAs and miRNAs are incorporated into ribonucleoprotein particles called RNA-Induced Silencing Complexes (RISCs). Within the RISC, the double-stranded RNA is unwound, and one strand is eliminated. The remaining single strand of RNA then binds to specific messenger RNA (mRNA) molecules, preventing gene expression. RISCs contain proteins from the Argonaute family, and perfect or near-perfect base-pairing between the RNA in the RISC and the target mRNA can lead to mRNA cleavage. Imperfect base pairing inhibits the translation of the mRNA.

Epigenetics and pathogenesis of T2D and its progression to DKD

A multitude of research has established a strong correlation between epigenetic modification and the development and progression of T2D, revealing critical implications for its microvascular complications, especially DKD. Figure 3 illustrates the etiology of epigenetic risk factors associated with the development of the T2D. The objective of this review is to investigate the integrated role of epigenetic signatures in T2D that may serve as predictors for the progression to DKD, thereby addressing the interconnectedness of these two closely linked conditions.

Research has primarily focused on DNA methylation of candidate genes associated with T2D, such as *PPARGC1A, PDX1, INS,* and *GLP1R* in human pancreatic islets.[22–25] Islets from individuals diagnosed with T2D exhibit elevated levels of DNA methylation accompanied by reduced expression of these important genes, which are implicated in compromised insulin secretion [Figure 4]. High glucose levels and increased glycated hemoglobin (HbA1c) have been shown to directly enhance the DNA methylation of these genes, establishing a potential mechanistic pathway linking hyperglycemia to renal complications.[23,24]

The advent of high-throughput platforms like Illumina's Infinium arrays enabled the simultaneous analysis of the methylation of approximately 27,000 and 450,000 CpG sites, respectively, in pancreatic islets from T2D and non-diabetic donors. This comprehensive approach identifies altered DNA methylation in 1,649 CpG sites in islets from T2D cases compared to controls, with differential expression observed in 102 genes related to insulin secretion.[26]

Further exploration into clonal β cells demonstrated that decreased methylation leads to increased expression of *CDKN1A*, which encodes a cyclin-dependent kinase inhibitor that regulates progression to the G1 phase of the cell cycle, ultimately resulting in reduced β cell proliferation.[26] This relationship suggests that altered insulin secretion is intricately linked to epigenetic changes within pancreatic islets. Notably, DNA methylation variations of CpG islands of T2D candidate genes, identified through genome-wide association studies (GWASs), also include *ADCY5, FTO, HHEX, IRS1, KCNQ1, PPARG,* and *TCF7L2*.[26]

To acquire a comprehensive understanding of epigenome, whole genome investigations of various epigenetic marks are required. Techniques such as whole-genome bisulfite

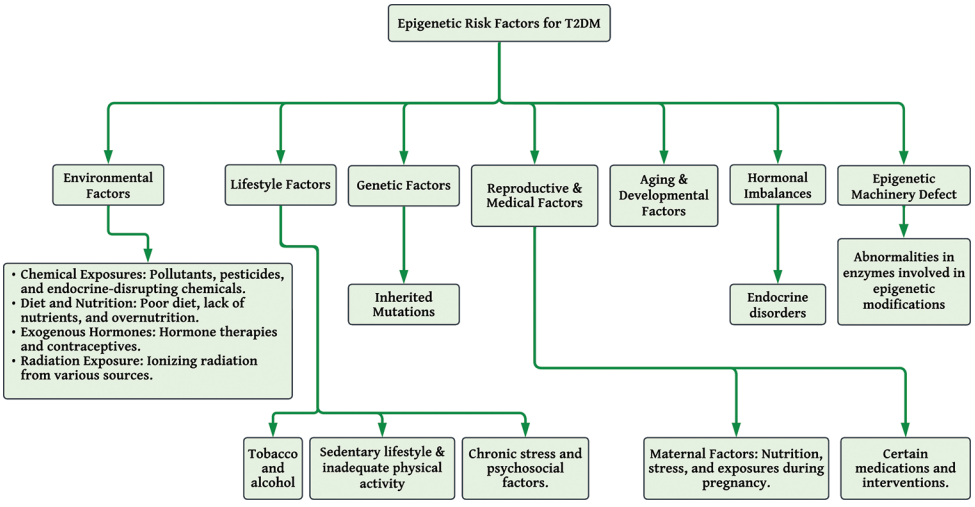

Figure 3: Epigenetic risk factors associated with type 2 diabetes[24,37]

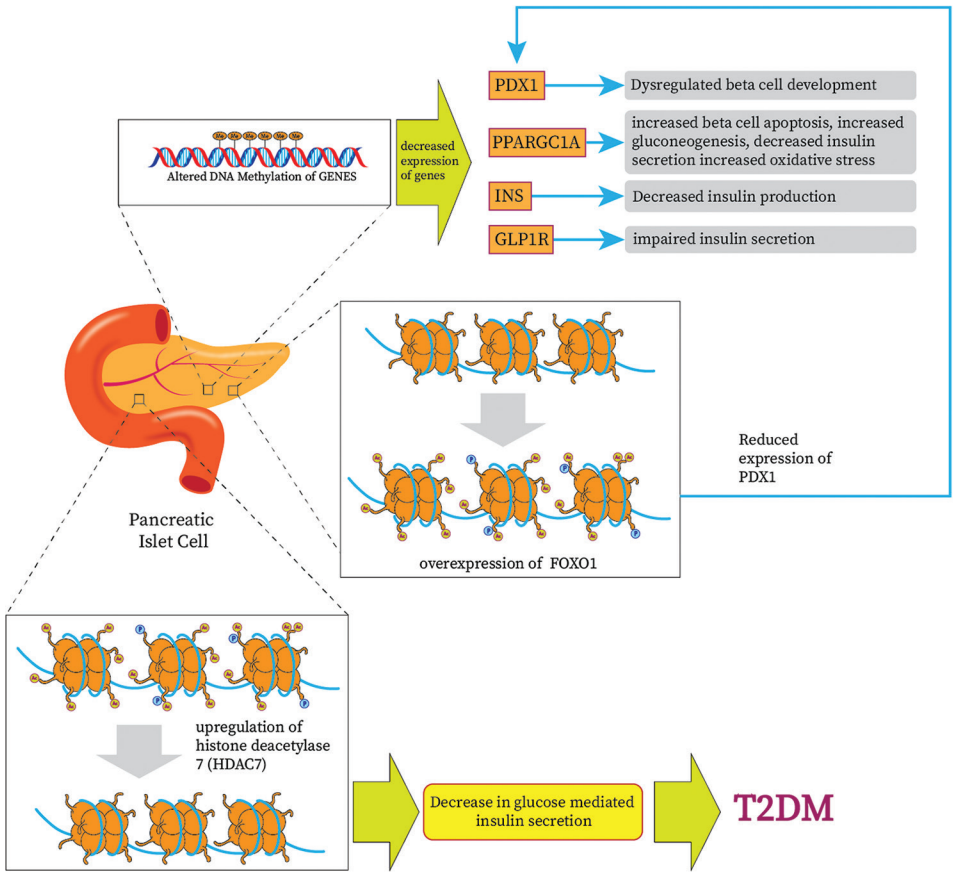

Figure 4: Epigenetics of impaired insulin production in T2D

sequencing (WGBS) for DNA methylation, chromatin immunoprecipitation sequencing (ChIP-seq) for histone modifications, and the assay for transposase-accessible chromatin sequencing (ATAC-seq) for chromatin accessibility offer profound insights into diverse aspects of the epigenome and how alterations in epigenetic marks influence the pathogenesis of T2D[27] and DKD.[28]

The research identified 457 genes, including *NR4A3*, *PARK2, PID1,* and *SOCS2,* with differentially methylated regions (DMRs), resulting in altered expression and impaired insulin secretion in T2D islets. This evidence illustrates a strong connection between epigenetic modifications and islet dysfunction [Figure 5], emphasizing the necessity of examining these changes in the context of DKD progression. Islet methylation levels were also associated with specific histone marks, indicating that combinations of different epigenetic marks regulate gene activity and chromatin structure. A reduction in glucose-mediated insulin secretion was seen in the human pancreatic cells of people with T2D when histone deacetylase 7 (HDAC7) was upregulated.[29] Conversely, acetylation of the *FOXO1* gene, which regulates *PDX1*, disrupts glucose homeostasis and beta-cell formation.[30]

MicroRNAs (miRNA) have been increasingly recognized for their pivotal role in the pathogenesis of T2D and the potential progression to DKD.[31] Studies on rodent models indicate that higher expression of *miR-200c* in islets from T2D donors results in decreased glucose-mediated insulin production relative to nondiabetic counterparts.[32] Additionally, *MiR-375* overexpression is also associated with diminished insulin secretion in both rat and human islet cells.[33] *MiR-124a* expression in T2D may affect beta cell function, insulin secretion, and target genes involved in glucose-sensing and insulin exocytosis, potentially leading to beta cell dysfunction,[34] indicating that such epigenetic regulators may have predictive value in understanding the transition from T2D to DKD.

Beyond direct methylation effects, the inhibition of monocarboxylate transporter 1 (MCT1) expression in pancreatic beta cells has been indirectly controlled through miRNAs like miR-29a and miR-29b.[35] Research has showcased that miRNA (MiR-506-3p) dysregulation also contributes to insulin resistance, primarily through signaling pathways such as IRS1/PI3K/AKT.[36]

Most epigenetic research on T2D has primarily focused on pancreatic islets, with a limited number of studies exploring the role of epigenetics in other cell types [Supplementary Table 1]. However, research has shown that epigenetic mechanisms are also linked to T2D in non-islet cells, like histone modifications in monocytes cultured under normal and high glucose conditions.[38]

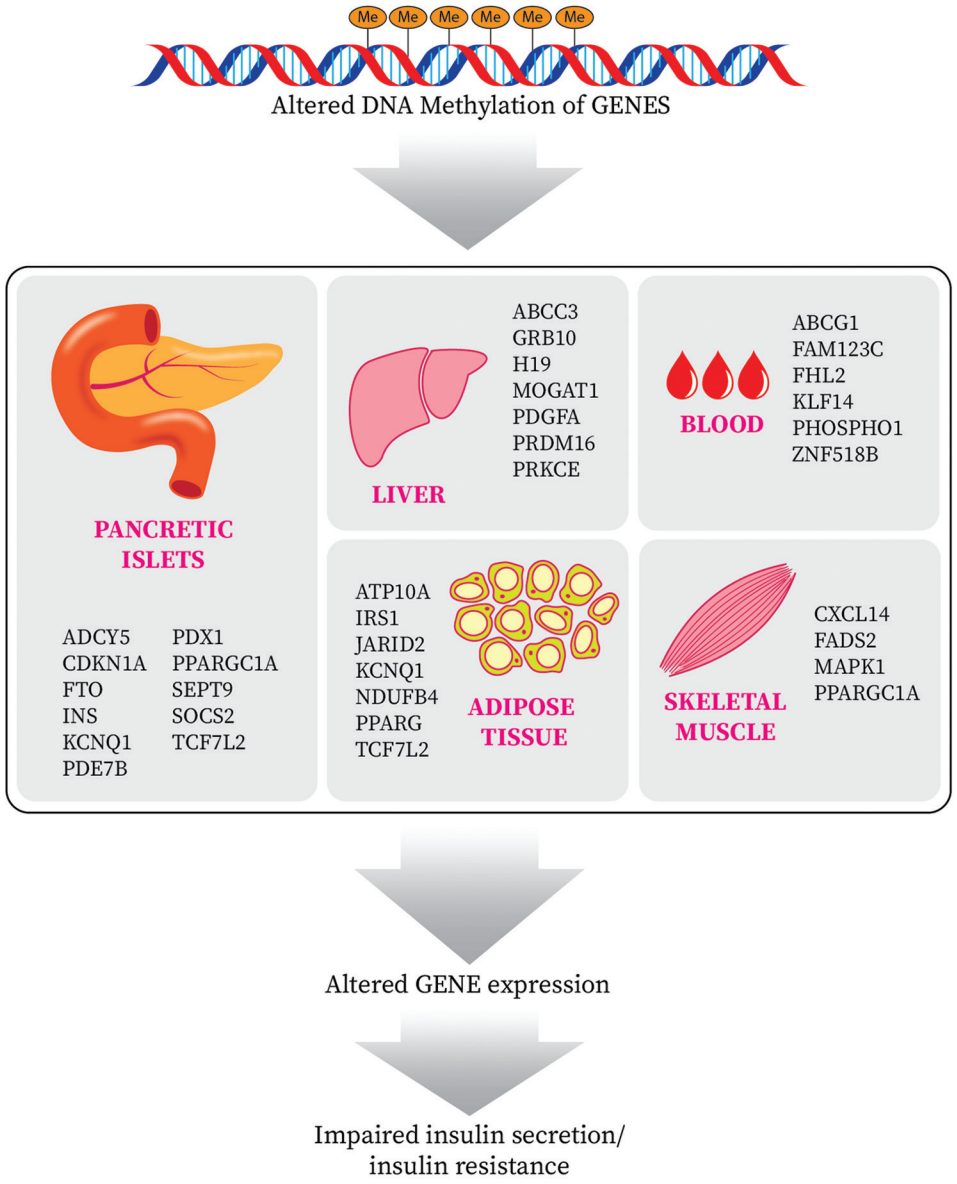

Figure 5: A Schematic representation illustrates altered DNA methylation of genes at different sites in T2D subjects. Some of these genes also exhibit modified gene expression and have been proven to impact traits related to diabetes, such as insulin secretion

Epigenetics and Diabetic Kidney Disease (DKD)

Research on epigenetics and epigenome has contributed to advancing our knowledge regarding the mechanisms underlying diabetic complications. The interplay between T2D and the epigenetics of DKD is burgeoning as a field of study. Several studies have confirmed that epigenetic regulatory mechanisms, including DNA methylation, noncoding RNAs, and histone modifications, critically contribute to the pathogenesis of DKD by providing an additional layer of gene regulation that exacerbates diabetic complications [Figure 6].[39]

The progression of both microvascular and macrovascular complications is attributed to chronic hyperglycemia that induces endothelial dysfunction via multiple pathways. Hypomethylation of CpG islands of the EDN1 promoter leads to its overexpression.,[40] while both hypomethylation and hyperacetylation of histones in the *NF-κB* gene promoter region lead to overexpression of this pro-inflammatory transcription factor in endothelial cells.[41] Moreover, enhanced expression of matrix metallopeptidase-9 (MMP-9) by hyperglycemia-induced H3K9 demethylation causes damage to endothelial cells.[42]

Genome-wide studies have established a correlation between increased DNA methylation and inflammation in DKD.[43] Genome-wide DNA methylation analysis in humans with chronic kidney disease, including DKD, reveals significant differences in DNA methylation at fibrosis-related loci compared to controls, underscoring the importance of epigenetic dysregulation in DKD.[44] Additionally, an epigenome-wide association study utilizing whole blood underscores a strong relationship between DNA methylation alterations and the deterioration of renal

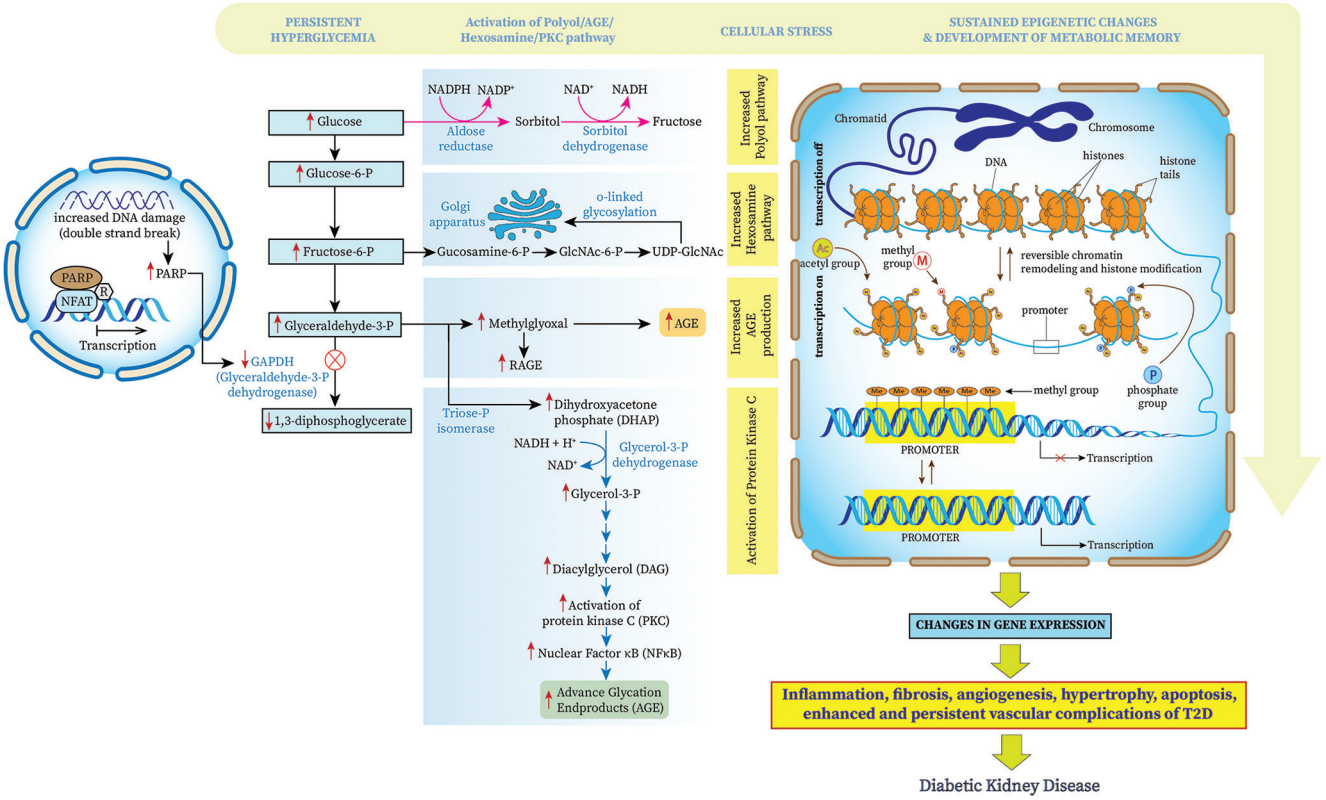

Figure 6: Persistent hyperglycemia in T2D diabetic nephropathy activates various pathways and causes cellular stress. This disrupts epigenetic mechanisms like histone modifications, DNA methylation, and gene regulation by ncRNAs, leading to altered gene expression across tissues and contributing to DN pathogenesis

function in DKD.[45,46] For instance, hypermethylation of the promoter region of Ras protein activator like 1 (RASAL1) has been observed in animal models of DKD.[47]

DNA methylation and altered histone modifications have also been implicated in the TGF-β-dependent activation of genes linked to renal fibrosis, which is critical in the pathogenesis of diabetic nephropathy.[48] Additionally, histone modifications at the promoters of fibrotic genes are associated with DKD.[49] Enhanced TGF-β1 expression drives hypermethylation of RASAL-1, causing activation of Ras-GTP signaling in DKD.[47] Collagen deposition and fibrosis are the outcomes of this mechanism, constituting a critical stage in the development of DKD.[50] Recent findings suggest that reduced methylation of the myoinositol oxygenase (MIOX) gene promotes oxidative stress and fibrosis, further advancing DKD.[51]

Mesangial cell inflammation is exacerbated when histone modifications result in enhanced *TXNIP* gene expression.,[52] while in podocytes of STZ-induced diabetic mice with albuminuria, decreased expression of claudin-1 is observed due to the downregulation of histone deacetylase, SIRT1.[53] HDAC4 upregulation leads to inflammatory changes in diabetic nephropathy by inhibiting the STAT1 pathway.[54]

It has been determined that noncoding RNAs (miRNAs and lncRNAs) are also significant epigenetic actors in the pathogenesis of DKD. For instance, by targeting PTEN, the cluster of microRNA, miR-216/miR-217 promotes TGF-β-dependent activation of Akt kinase, which in turn induces alterations in extracellular matrix (ECM) gene expression and causes hypertrophy in mesangial cells.[55] The presence of lnc-MGC in mesangial cells exposed to high glucose or TGF-β, as well as in the glomeruli of diabetic mice, is triggered by endoplasmic reticulum (ER) stress to facilitate initial occurrences in DKD.[56] AngII upregulates a lncRNA, *Giver,* in rat and human vascular smooth muscle cells (VSMCs), causing VSMC proliferation and oxidative stress.[57]

Interestingly, research indicates that during the preliminary phase of DKD, the overexpression of miR-146a mitigates the activation of proinflammatory genes by downregulating target genes associated with inflammation. Consequently, the synthesis of proinflammatory cytokines, including IL-1β and IL-18, is reduced. However, DKD progresses more rapidly in miR-146a(-/-) mice.[58] Urinary exosomal-derived miRNAs were altered in type 2 DKD. MiR-15a-5p, miR-150-5p, miR-362-3p, and miR-877-3p can potentially emerge as new biomarkers for the early detection of DKD.[59] There are many miRNAs, highlighting the multifaceted role of miRNAs in diverse pathophysiological processes in DKD by targeting various signaling pathways.[60]

Discussion and Conclusion

Emerging research implies that epigenetic processes within chromatin, including histone post-translational modifications (PTMs), DNA methylation (DNAme), and microRNAs (miRNAs), contribute significantly to the advancement of DKD.[39] The enduring effects of epigenetic alterations induced by diabetic stimuli may trigger the phenomenon of metabolic memory.[61] Additionally, specific histone methyltransferases (HMTs) and their associated histone PTMs have been associated with regulating fibrotic and inflammatory genes linked to T2D and DKD.[62] These insights into epigenetic control present opportunities for comprehending and potentially targeting DKD mechanisms. The understanding of additional factors and the regulatory mechanisms controlled by upstream signal transduction pathways in diabetes and DKD is currently limited. However, this field is rapidly evolving, offering the potential to unveil further chromatin factors and epigenetic mechanisms related to DKD. Ongoing advancements such as the Human Epigenome Project and Encyclopedia of DNA Elements will likely aid this progress. Epigenomics, or Epigenome-Wide Association Studies (EWAS), may offer insights into the functional implications of genetic variations in specific gene regions and the crosstalk between genetic and epigenetic processes. Given the reversible nature of epigenetic changes, there is a chance to develop combination therapies involving epigenetic drugs and miRNA inhibitors (antagomirs) to complement current DKD treatments, potentially representing a significant advancement in managing T2D and its complications. The complexities of epigenetic research in DKD are substantial. The cell-specific nature of epigenetic patterns poses challenges in interpreting data from Epigenome-Wide Association Studies (EWAS) using diverse kidney tissues and biopsies. Additionally, the difficulty in obtaining glomerular and tubular biopsies from impacted individuals and matched controls presents a significant hurdle. Given the strong connection between inflammation and most diabetic complications, including DKD, an alternative approach involves assessing easily accessible and noninvasive inflammatory cells like blood monocytes. Despite these obstacles, further exploration of epigenetics is anticipated to uncover essential new biomarkers and therapeutic targets, contributing to improved early detection and treatment of DKD.

In our present study, we evaluated the proportion of DNA methylation in subjects with T2D and those with T2D and DKD. A notable disparity was observed between the groups, ($P < 0.0036$), while comparing means using an unpaired *t*-test [Supplementary Table 2], suggesting that methylation may serve as a valuable biomarker for renal impairment in T2D patients. We believe that studying gene-specific methylation, particularly for genes associated with DKD, would improve our understanding of the mechanisms at play and enhance biomarker efficacy, warranting further investigation through large studies. We also intend to assess the DNA methylation status of the *AGER* and *INS* genes, which are relevant to microvascular complications in diabetes.

In conclusion, this review attempted to bridge a gap between understanding epigenetic modifications in T2D and their implications for the onset and progression of DKD. Future research should focus on how epigenetic signatures in T2D can predict DKD progression. This exploration will investigate the underlying epigenetic signaling to uncover new biomarkers and therapeutic targets, leading to improved management of T2D and its complications.

Acknowledgement

None.

Authors' contribution

SSS and BDB conceptualized, designed, and approved the final version of the manuscript for publication. AM and TD conducted the literature search, performed detailed reviews, and conducted statistical analysis. AM prepared the illustrations for this article. All authors read and approved the manuscript prior to submission for consideration for publication.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Meigs JB, Cupples LA, Wilson PWF. Parental transmission of type 2 diabetes: The Framingham offspring study. Diabetes 2000;49:2201–7.
2. Mahajan A, Taliun D, Thurner M, Robertson NR, Torres JM, Rayner NW, *et al*. Fine-mapping type 2 diabetes loci to single-variant resolution using high-density imputation and islet-specific epigenome maps. Nat Genet 2018;50:1505–13.
3. McCarthy MI. Genomics, type 2 diabetes, and obesity. N Engl J Med 2010;363:2339–50.
4. Darshi M, Van Espen B, Sharma K. Metabolomics in diabetic kidney disease: Unraveling the biochemistry of a silent killer. Am J Nephrol 2016;44:92–103.
5. Barrès R, Osler ME, Yan J, Rune A, Fritz T, Caidahl K, *et al*. Non-CpG methylation of the PGC-1α promoter through DNMT3B controls mitochondrial density. Cell Metab 2009;10:189–98.
6. Ling C, Rönn T. Epigenetic adaptation to regular exercise in humans. Drug Discov Today 2014;19:1015–8.
7. Dong H, Sun Y, Nie L, Cui A, Zhao P, Leung WK, *et al*. Metabolic memory: Mechanisms and diseases. Signal Transduct Target Ther 2024;9:1–29.
8. Ling C, Bacos K, Rönn T. Epigenetics of type 2 diabetes mellitus and weight change - A tool for precision medicine? Nat Rev Endocrinol 2022;18:433–48.
9. Chaffey N. Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K. and Walter, P. Molecular biology of the cell. 4th edn. Ann Bot 2003;91:401.
10. Kouzarides T. Chromatin modifications and their function Cell 2007;128:693–705.
11. Cutter AR, Hayes JJ. A brief review of nucleosome structure. FEBS Lett 2015;589:2914.
12. Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. Cell Res 2011;21:381–95.
13. Becker PB, Workman JL. Nucleosome remodeling and epigenetics. Cold Spring Harb Perspect Biol 2013;5. doi: 10.1101/CSHPERSPECT.A017905.
14. Dong X, Weng Z. The correlation between histone modifications and gene expression. Epigenomics 2013;5:113.
15. Feoktistov AV, Georgieva SG, Soshnikova N V. [Role of the SWI/SNF chromatin remodeling complex in regulation of inflammation gene expression]. Mol Biol 2022;56:244–58.

16. Raab JR, Resnick S, Magnuson T. Genome-wide transcriptional regulation mediated by biochemically distinct SWI/SNF complexes. PLoS Genet 2015;11:e1005748.
17. Saha A, Wittmeyer J, Cairns BR. Chromatin remodelling: The industrial revolution of DNA around histones. Nat Rev Mol Cell Biol 2006;7:437–47.
18. Crusselle-Davis VJ, Archer TK. 2.19 - Chromatin Remodeling, In: McQueen CA, editor. Comprehensive Toxicology (Second Edition), Elsevier; 2010, p. 359-75. Available from: https://www.sciencedirect.com/science/article/pii/B9780080468846002207.
19. Kallin E, Zhang Y, Chromatin Remodeling. In: Lennarz WJ, Lane MD, editors. Encyclopedia of Biological Chemistry. Elsevier; 2004, p. 456-63. Available from: https://www.sciencedirect.com/science/article/pii/B0124437109002532.
20. Moore LD, Le T, Fan G. DNA Methylation and Its Basic Function. Neuropsychopharmacology 2013;38:23–38.
21. Jung M, Pfeifer GP, CpG Islands. Maloy S, Hughes K, editors. Brenner's Encyclopedia of Genetics (Second Edition). Academic Press; 2013. p. 205-7. Available from: https://www.sciencedirect.com/science/article/pii/B9780123749840003491.
22. Ling C, Del Guerra S, Lupi R, Rönn T, Granhall C, Luthman H, *et al*. Epigenetic regulation of PPARGC1A in human type 2 diabetic islets and effect on insulin secretion. Diabetologia 2008;51:615–22.
23. Yang BT, Dayeh TA, Volkov PA, Kirkpatrick CL, Malmgren S, Jing X, *et al*. Increased DNA methylation and decreased expression of PDX-1 in pancreatic islets from patients with type 2 diabetes. Mol Endocrinol 2012;26:1203–12.
24. Yang BT, Dayeh TA, Kirkpatrick CL, Taneera J, Kumar R, Groop L, *et al*. Insulin promoter DNA methylation correlates negatively with insulin gene expression and positively with HbA(1c) levels in human pancreatic islets. Diabetologia 2011;54:360–7.
25. Hall E, Dayeh T, Kirkpatrick CL, Wollheim CB, Dekker Nitert M, Ling C. DNA methylation of the glucagon-like peptide 1 receptor (GLP1R) in human pancreatic islets. BMC Med Genet 2013;14. doi: 10.1186/1471-2350-14-76.
26. Dayeh T, Volkov P, Salö S, Hall E, Nilsson E, Olsson AH, *et al*. Genome-Wide DNA methylation analysis of human pancreatic islets from type 2 diabetic and non-diabetic donors identifies candidate genes that influence insulin secretion. PLoS Genet 2014;10:1004160. doi: 10.1371/JOURNAL.PGEN.1004160.
27. Bysani M, Agren R, Davegårdh C, Volkov P, Rönn T, Unneberg P, *et al*. ATAC-seq reveals alterations in open chromatin in pancreatic islets from subjects with type 2 diabetes. Sci Rep 2019;9:1–12.
28. Bansal A, Balasubramanian S, Dhawan S, Leung A, Chen Z, Natarajan R. Integrative omics analyses reveal epigenetic memory in diabetic renal cells regulating genes associated with kidney dysfunction. Diabetes 2020;69:2490.
29. Daneshpajooh M, Bacos K, Bysani M, Bagge A, Ottosson Laakso E, Vikman P, *et al*. HDAC7 is overexpressed in human diabetic islets and impairs insulin secretion in rat islets and clonal beta cells. Diabetologia 2017;60:116.
30. Nakae J, Biggs WH, Kitamura T, Cavenee WK, Wright CVE, Arden KC. Regulation of insulin action and pancreatic beta-cell function by mutated alleles of the gene encoding forkhead transcription factor Foxo1. Nat Genet 2002;32:245–53.
31. Cao Q, Chen XM, Huang C, Pollock CA. MicroRNA as novel biomarkers and therapeutic targets in diabetic kidney disease: An update. FASEB Bioadv 2019;1:375.
32. Ofori JK, Karagiannopoulos A, Nagao M, Westholm E, Ramadan S, Wendt A, *et al*. Human islet MicroRNA-200c is elevated in type 2 diabetes and targets the transcription factor ETV5 to reduce insulin secretion. Diabetes 2022;71:275–84.
33. Dumortier O, Fabris G, Pisani DF, Casamento V, Gautier N, Hinault C, *et al*. microRNA-375 regulates glucose metabolism-related signaling for insulin secretion. J Endocrinol 2020;244:189–200.
34. Sebastiani G, Po A, Miele E, Ventriglia G, Ceccarelli E, Bugliani M, *et al*. MicroRNA-124a is hyperexpressed in type 2 diabetic human pancreatic islets and negatively regulates insulin secretion. Acta Diabetol 2015;52:523–30.
35. Pullen TJ, Xavier G da S, Kelsey G, Rutter GA. miR-29a and miR-29b contribute to pancreatic β-cell-specific silencing of monocarboxylate transporter 1 (Mct1). Mol Cell Biol 2011;31:3182.
36. Zhong FY, Li J, Wang YM, Chen Y, Song J, Yang Z, *et al*. MicroRNA-506 modulates insulin resistance in human adipocytes by targeting S6K1 and altering the IRS1/PI3K/AKT insulin signaling pathway. J Bioenerg Biomembr 2021;53:679.
37. Dhawan S, Natarajan R. Epigenetics and type 2 diabetes risk. Curr Diab Rep 2019;19:47.
38. Miao F, Wu X, Zhang L, Yuan YC, Riggs AD, Natarajan R. Genome-wide analysis of histone lysine methylation variations caused by diabetic conditions in human monocytes. J Biol Chem 2007;282:13854–63.
39. Lu HC, Dai WN, He LY. Epigenetic histone modifications in the pathogenesis of diabetic kidney disease. Diabetes Metab Syndr Obes 2021;14:329.
40. Mannar V, Boro H, Patel D, Agstam S, Dalvi M, Bundela V. Epigenetics of the pathogenesis and complications of type 2 diabetes mellitus touchREV Endocrinol 2023;19:46-53.
41. Prattichizzo F, Giuliani A, Ceka A, Rippo MR, Bonfigli AR, Testa R, Procopio AD, Olivieri F. Epigenetic mechanisms of endothelial dysfunction in type 2 diabetes. Clin Epigenetics 2015;7:56.
42. Kowluru RA, Shan Y, Mishra M. Dynamic DNA methylation of matrix metalloproteinase-9 in the development of diabetic retinopathy. Lab Invest 2016;96:1040–9.
43. Reddy MA, Natarajan R. Epigenetics in diabetic kidney disease. J Am Soc Nephrol 2011;22:2182–5.
44. Ko YA, Mohtat D, Suzuki M, Park ASD, Izquierdo MC, Han SY, *et al*. Cytosine methylation changes in enhancer regions of core pro-fibrotic genes characterize kidney fibrosis development. Genome Biol 2013;14. doi: 10.1186/GB-2013-14-10-R108.
45. Chu AY, Tin A, Schlosser P, Ko YA, Qiu C, Yao C, *et al*. Epigenome-wide association studies identify DNA methylation associated with kidney function. Nat Commun 2017;8. doi: 10.1038/S41467-017-01297-7.
46. Qiu C, Hanson RL, Fufaa G, Kobes S, Gluck C, Huang J, *et al*. Cytosine methylation predicts renal function decline in American Indians. Kidney Int 2018;93:1417–31.
47. Bechtel W, McGoohan S, Zeisberg EM, Müller GA, Kalbacher H, Salant DJ, *et al*. Methylation determines fibroblast activation and fibrogenesis in the kidney. Nat Med 2010;16:544–50.
48. Kato M, Natarajan R. Diabetic nephropathy—emerging epigenetic mechanisms. Nat Rev Nephrol 2014;10:517–30.
49. Reddy MA, Zhang E, Natarajan R. Epigenetic mechanisms in diabetic complications and metabolic memory. Diabetologia 2015;58:443–55.
50. Zheng W, Guo J, Liu ZS. Effects of metabolic memory on inflammation and fibrosis associated with diabetic kidney disease: An epigenetic perspective. Clin Epigenetics 2021;13:1–16.
51. Sharma I, Dutta RK, Singh NK, Kanwar YS. High glucose–induced hypomethylation promotes binding of Sp-1 to myo-inositol oxygenase: Implication in the pathobiology of diabetic tubulopathy. Am J Pathol 2017;187:724.
52. De Marinis Y, Cai M, Bompada P, Atac D, Kotova O, Johansson ME, *et al*. Epigenetic regulation of the thioredoxin-interacting protein (TXNIP) gene by hyperglycemia in kidney. Kidney Int 2016;89:342–53.
53. Hasegawa K, Wakino S, Simic P, Sakamaki Y, Minakuchi H, Fujimura K, *et al*. Renal tubular Sirt1 attenuates diabetic albuminuria by epigenetically suppressing Claudin-1 overexpression in podocytes. Nat Med 2013;19:1496–504.
54. Wang X, Liu J, Zhen J, Zhang C, Wan Q, Liu G, *et al*. Histone deacetylase 4 selectively contributes to podocyte injury in diabetic nephropathy. Kidney Int 2014;86:712–25.
55. Kato M, Putta S, Wang M, Yuan H, Lanting L, Nair I, *et al*. TGF-β activates Akt kinase through a microRNA-dependent amplifying circuit targeting PTEN. Nat Cell Biol 2009;11:881–9.
56. Kato M, Wang M, Chen Z, Bhatt K, Oh HJ, Lanting L, *et al*. An endoplasmic reticulum stress-regulated lncRNA hosting a microRNA megacluster induces early features of diabetic nephropathy. Nat Commun 2016;7:1–16.
57. Das S, Zhang E, Senapati P, Amaram V, Reddy MA, Stapleton K, *et al*. A novel angiotensin II–induced long noncoding RNA giver regulates oxidative stress, inflammation, and proliferation in vascular smooth muscle cells. Circ Res 2018;123:1298–312.

58. Bhatt K, Lanting LL, Jia Y, Yadav S, Reddy MA, Magilnick N, *et al*. Anti-inflammatory role of MicroRNA-146a in the pathogenesis of diabetic nephropathy. J Am Soc Nephrol 2016;27:2277–88.
59. Xie Y, Jia Y, Cuihua X, Hu F, Xue M, Xue Y. Urinary exosomal MicroRNA profiling in incipient type 2 diabetic kidney disease. J Diabetes Res 2017;2017. doi: 10.1155/2017/6978984.
60. Tang J, Yao D, Yan H, Chen X, Wang L, Zhan H. The role of MicroRNAs in the pathogenesis of diabetic nephropathy. Int J Endocrinol 2019;2019. doi: 10.1155/2019/8719060.
61. El-Osta A, Brasacchio D, Yao D, Pocai A, Jones PL, Roeder RG, *et al*. Transient high glucose causes persistent epigenetic changes and altered gene expression during subsequent normoglycemia. J Exp Med 2008;205:2409–17.
62. Kourtidou C, Tziomalos K. The role of histone modifications in the pathogenesis of diabetic kidney disease. Int J Mol Sci 2023;24. doi: 10.3390/IJMS24066007.

Supplementary Table 1: Significant epigenetics research has been conducted on type 2 diabetes

Authors	Studied on	Site	Year	Subjects	*P*	Conclusion
C. Ling, S. Del Guerra *et al*.	PPARGC1A promoter methylation	Human pancreatic islets	2008	Non-diabetic=48, type 2 diabetic=12	$P<0.04$	The regulation of PPARGC1A expression in human islets is mediated via genetic and epigenetic mechanisms.
Beatrice T. Yang, Tasnim A. Dayeh *et al*.	PDX-1 promoter methylation	Human pancreatic islets	2012	Type 2 diabetic=9, non-diabetic=55	$P=0.0000029$	A negative correlation was observed between PDX-1 DNA methylation and its gene expression in human islets.
B. T. Yang, T. A. Dayeh *et al*.	Insulin promoter methylation	Pancreatic islets	2010	Type 2 diabetic=9, non-diabetic=48	$P<1\times10^{-6}$	In T2DM, insulin promoter DNA methylation is increased and inversely associated with insulin gene expression in pancreatic islets.
Elin Hall, Tasnim Dayeh *et al*.	CpG sites +199 and +205 of glucagon-likepeptide1 receptor (GLP1R) gene	Human pancreatic islets	2013	Type 2 diabetic=10, non-diabetic=55	$P=0.02$	Human pancreatic islets have a negative correlation between DNA methylation of the GLP1R gene and GLP1R expression.
Tasnim Dayeh, Petr Volkov *et al*.	DNA methylation of 479,927 CpG sites	Human pancreatic islets	2014	Type 2 diabetic=15, non-diabetic=34		Impaired insulin and glucagon secretion in human T2D islets is associated with altered DNA methylation.
Mahboubeh Daneshpajooh, Karl Bacos *et al*.	HDAC7 gene expression	Human pancreatic islets	2016	Non-diabetic=85, type 2 diabetic=16	$P<0.004$	HDAC7 expression in human islets is negatively linked with glucose-stimulated insulin secretion (GSIS).
Mahboubeh Daneshpajooh, Karl Bacos *et al*.	HDAC7 gene expression	Clonal beta cells	2016	$n=6$	$P=0.054$	Overexpression of Hdac7 has nominal effects on insulin secretion.
Jones K. Ofori, Alexandros Karagiannopoulos *et al*.	miR-200c expression	Human pancreatic islets	2022	$n=25$ Non-Diabetic and $n=9$ Type 2 diabetic	$P<0.05$	Enhanced miR-200c expression in the islets of individuals with T2D as compared to healthy controls ultimately results in reduced insulin production.

Supplementary Table 2: Paired Sample *t*-test for comparing 'T2D without DKD' and 'T2D with DKD' groups

Paired differences						
Difference between means	Std. error of mean (SEM)	95% confidence interval of the difference		*t*	df	*P* (2 tailed)
		Lower	Upper			
0.4420	±0.1471	0.1491	0.7348	3.004	80	0.0036**