Genetics and Epigenetics of Diabetic Nephropathy

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Key Words

Genetics · Epigenetics · Diabetic nephropathy · End-stage renal disease

Abstract

Background: Diabetic nephropathy (DN) is the most common cause of end-stage renal disease (ESRD) in the USA and worldwide, contributing to significant morbidity and mortality in diabetic patients. A genetic factor for the development of DN is strongly implicated, as only one third of diabetic patients eventually develop kidney disease. Growing evidence also supports an important role of epigenetic modifications in DN. **Summary:** Multiple studies have been performed to identify risk genes and loci associated with DN. So far, only several genes and loci have been identified, none of which showed a strong association with DN. Therefore, a better study design with a larger sample size to identify rare variants and a clinically defined patient population to identify genes and loci associated with progressive DN are still needed. In addition to genetic factors, epigenetic modifications, such as DNA methylation, histone modifications and microRNAs, also play a major role in the pathogenesis of DN through a second layer of gene regulation. Although a major progress has been made in this field, epigenetic studies in

DN are still in the early phase and have been limited mostly due to the heterogeneity of kidney tissue samples with multiple cells. However, rapid development of high-throughput genome-wide techniques will help us to better identify genetic variants and epigenetic changes in DN. **Key Message:** Understanding of genetic and epigenetic changes in DN is needed for the development of new biomarkers and better drug targets against DN. Summarized in this review are important recent findings on genetic and epigenetic studies in the field of DN.

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Introduction

Diabetic nephropathy (DN), a devastating complication of diabetes, develops in 30–40% of patients with both type 1 and 2 diabetes [1]. DN is the leading cause of end-stage kidney disease (ESRD) worldwide and in the USA. DN is clinically defined by the persistent microalbuminuria and gradual decline of kidney function that eventually leads to ESRD. The current treatment strategy of DN, including strict control of blood glucose, blood pressure, and renin-angiotensin system blockade, may slow down but not arrest the progression of DN.

The etiology of DN is multifactorial, including genetic and environmental factors. The role of genetic susceptibility in the development of DN is evidenced by family aggregation [2] and the fact that not all patients with diabetes develop clinically evident DN despite long-term poor glycemic control. Identifying susceptible genetic loci or genes is apparently approach to dissect the mechanisms of onset and progression of DN. Therefore, many efforts have been made to identify genetic associations with DN using a variety of ways, including candidate gene screening, linkage analysis and genome-wide association studies (GWAS). GWAS is an unbiased and hypothesis-free approach to scan for a ‘common variant’, usually hundreds of thousands of single-nucleotide polymorphisms (SNPs) across the whole genome, in order to identify the disproportionately enriched SNPs in diabetic patients with nephrology. Genetic studies that analyzed DN of different kindred have generated a list of associated loci or genes. These associated loci or genes greatly broaden our understanding of the pathogenesis of DN. However, most of the genetic studies in the field of DN are inconsistent with an odds ratio (OR) <2. Likely, these loci or genes are unable to account for a significant proportion of heritability of DN, indicating the existence of factors beyond genetic sequence variations.

Epigenetics has been a rapidly evolving field in the past decade. Epigenetic regulation of gene expression is not related to alterations in primary nucleotide sequence of genes but to alternations in gene expressions through regulatory mechanisms that include DNA methylation, histone modifications (methylation, acetylation, phosphorylation, etc.), and expression of noncoding RNAs such as microRNAs (miRNAs). Methylation is considered to be chemically stable in the post-development stages, while other posttranslational modifications (PTMs) are potentially reversible. Some of these modification processes utilize metabolic intermediates such as reaction substrates or cofactors. For example, methylation transfers a methyl group from S-adenosyl methionine (SAM), a product of essential amino acid methionine metabolism, to DNA or histones. Acetylation, a covalent acetyl group addition to lysine residues of histone, is catalyzed by histone acetyltransferases that use acetyl-CoA as a substrate, and the Sirt family of histone deacetylases (HDACs) uses NAD⁺ as a cofactor. Thus, the metabolic status may affect epigenetic modifications through changing the concentration of the metabolic intermediates. Diabetes is a disorder of several critical metabolic pathways. Therefore, the role of epigenetic modification induced by diabetes in DN is intriguing. In fact, accumulating evidence demon-

Table 1. Genetic variants identified by GWAS

Candidate gene	Ethnicity	Reference
Type I		
FRMD3	EA	3
CARS	EA	3
ZMIZ1	EA	6
ERBB4	Multiple	7
AFF3	Multiple	7
RGMA	Multiple	7
SORBS1	Multiple	8
SP3	Multiple	9
Type II		
ELMO1	Japanese	11
PVT1	Pima Indian	14
MYH9	AA	18
APOL1	AA	18
LIMK2	AA	18
SFI1	AA	18

strates the important role of epigenetic modifications in DN. Likely, the interactive effects of genetics with epigenetics contribute to the development of DN. This review summarizes the current findings on the genetic variants and epigenetic modifications in DN.

Genetics

Type 1 Diabetes

Summarized here are the genetic variants identified from genome-wide screening studies and large-scale meta-analyses (table 1).

FRMD3/CARS: The Genetics of Kidney in diabetes (GoKinD) study [3] conducted a genome-wide SNP screening in patients with type 1 diabetes mellitus (T1DM) of European American (EA) descent with (n = 820) or without (n = 855) kidney diseases to identify risk SNPs for DN. The SNPs that show the strongest association with DN and have successful replication in the Diabetes Control and Complications Trial (DCCT) are rs1888747 (OR = 1.45, p = 5.0 × 10⁻⁷), located in the extended promoter region of *FRMD3* (FERM domain-containing 3), and rs451041 (OR = 1.36, p < 3.1 × 10⁻⁶), located in an intronic region of *CARS* (cysteinyl-tRNA synthetase). *FRMD3* was subsequently demonstrated to be associated with DN in type 2 diabetic Japanese [4] and EA patients [5]. *CARS* association with DN was also replicated in EA patients with type 2 DM (T2DM) [5].

ZMIZ1: In a GWAS, Craig et al. [6] analyzed 555,352 SNPs of pooled DNA from Caucasian patients who had previously participated in the GoKinD study. They first identified 2,870 SNPs showed substantial differences in mean allele frequency, and then selected 22 highly ranked markers in patients with or without ESRD. The strongest association marker with ESRD was located in *ZMIZ1* (MIZ-type containing 1) and *MSC* (musculin). A weaker association with *IRS2*, *TMPO*, *BID*, *KLRA1a*, *ELMO1* and *CNDP1* genes was also observed. Compared to the previous GoKinD study [1], results from the study by Craig et al. [6] showed little overlap in associated SNPs, which is probably due to the differences in experimental design and the ethnicity between these two studies. Craig et al. only analyzed Caucasian individuals, while the previous study included all individuals of multiple ethnicities.

ERBB4/AFF3/RGMA/MCTP2: The Genetics of Nephropathy: An International Effort (GENIE) consortium performed a meta-analysis of GWAS of three existing cohorts: GoKinD, UK-ROI (All Ireland Warren 3 Genetics of Kidneys in Diabetes UK Collection) and FinnDiane (Finnish Diabetic Nephropathy Study) [7]. The study identified risk SNPs associated with primary DN phenotype and established ESRD. A SNP in the intronic region of *ERBB4* ($p = 2.1 \times 10^{-7}$) was the top candidate associated with primary DN. Two SNPs associated with ESRD were also revealed. The strongest genetic association with established ESRD was a SNP residing between *RGMA* (RGM domain family, member A) and *MCTP2* (multiple C2 domains, transmembrane 2; $p = 2.0 \times 10^{-9}$). Another linked SNP was located in *AFF3* (AF4/FMR2 member 3; $p = 1.2 \times 10^{-8}$). *AFF3* expression was upregulated by TGF- β 1 in renal epithelial cells, and knockdown of *AFF3* by siRNA decreased TGF- β 1-induced profibrosis gene expression. These data indicate a role of *AFF3* in renal fibrosis.

SORBS1: Population-attributable risks of SNPs for DN in GENIE database range from 0.5 to 10.5% [7]. Using the latest 1000 Genomes imputation techniques, a multi-stage GWAS was conducted to find out novel gene(s) associated with type 1 DN (T1DN) in patients of European Ancestry [8]. *SORBS1* (sorbin and SH3 domain-containing protein 1) was identified to have a strong association with T1DN in the discovery analysis after comparison to the GokinD database. However, no association was observed after comparison to the UK-ROI and FinnDiane databases. Its mRNA expression was inversely correlated with estimated glomerular filtration rate. The population-attributable risk of *SORBS1* for DN is about 12%.

SP3: It has long been known that gender is a risk factor involved in T1DN [9]. A GWAS analysis of T1DN based on gender identified two nearby SNPs with high linkage disequilibrium on chromosome 2q31.1, located in an intergenic region between *CDCA7* and *SP3*, which are highly correlated with T1DN in women but not in men. This gender-specific association at rs4972593 was replicated in the GENIE database, and no other SNPs, including those previously reported in GENIE [6], were found to be linked to DN in women. The minor A allele of this SNP abolishes several transcription factor binding sites (TFBS). However, expression quantitative trait loci analysis found no significant association between the SNP and the expression level of genes within a 1-Mbp region up- or downstream of the SNP in human HapMap3 lymphoblastoid cell line. *SP3* is highly expressed in the glomeruli of women [10], compared to those of men, while *CDCA7* has not so far been reported to be expressed in glomeruli. *SP3* encodes a transcription factor Sp3, which directly interacts with estrogen receptor- α (ER- α). Target genes of the ER- α /Sp3 pathway include *VEGFA*, *TGFB1*, *COL1A1*, *NOTCH* and *CD2AP*, which are all involved in the pathogenesis of DN. The fact that this SNP is not associated with DN in men may explain why it was not detected in the previous gender-mixed GWAS.

Type 2 Diabetes

ELMO1: The first GWAS for DN revealed *ELMO1* (engulfment and motility protein 1) as a gene conferring susceptibility for type 2 DN (T2DN) in Japanese patients [11]. The genetic association of *ELMO1* with T1DN [12] in EAs and with T2DN [13] in African-Americans (AAs) has been confirmed. *ELMO1* is weakly detectable in podocytes and tubular epithelial cells in normal kidneys and remarkably elevated in diabetic kidneys and in COS cells treated with high glucose. *ELMO1* enhances the expression of extracellular matrix protein genes (*COL1A1*, *MMP2* and *FN1*) in COS cells in a TGF- β -independent manner.

PVT1: A high-density GWAS using a pooled DNA design identified *PVT1* (plasmacytoma variant translocation gene 1) as a candidate susceptibility gene for T2DN in Pima Indians [14]. *PVT1* was upregulated in mesangial cells after high glucose stimulation, and *PVT1* knockdown significantly decreased extracellular matrix (ECM) molecules at both RNA and protein levels, suggesting a role of *PVT1* in ECM accumulation in DN [15].

MYH9/APOL1/LIMK2 and SFI1: The *MYH9* (myosin, heavy chain 9)/*APOL1* (apolipoprotein L1) locus was initially found to be associated to FSGS and non-DN in two

GWAS [16, 17], but not in the DN population. However, a high-density GWAS found evidence of a *MYH9* association with T2DN in AAs, and the association was reproduced in a candidate gene study [18]. A finer mapping of genes susceptible for T2DN in AAs by a GWAS from the same group also revealed the association of *MYH9/APOL1* locus [19]. The *MYH9/APOL1* locus was also linked to T2DN in a family linkage study [20]. The function of *MYH9/APOL1* in DN is rapidly evolving and remains a hot topic of debate. Interestingly, in T2D AAs without DN, *APOL1* nephropathy risk variants are protective, as evidenced by the negative association between *APOL1* nephropathy risk variants and subclinical atherosclerosis and death risk [21]. In the extended study [19], several SNPs in *LIMK2* (LIM domain kinase 2) and *SFI1* [*Sfi1* homolog, spindle assembly-associated (yeast) region] were associated with T2DN in AAs.

Type 1 and 2 Diabetes

Results from genetic association studies for DN are remarkably inconsistent due to the small sample size, limited power, and sparseness of data. A meta-analysis collected 153 genetic mapping studies for established DN of both T1DM and T2DM that were published in April 2010 [22]. Associated genetic variants that occurred more than once were further analyzed. A total of 24 genetic variants in or near 16 genes were finally identified. *ACE* (renin-angiotensin system), *AKR1B1* (polyol pathway), *APOE* and *APOC1* (lipid metabolism), *VEGFA* and *EPO* (angiogenesis), *NOS3* (oxidative stress), *HSPG2* (maintaining glomerular basement membrane electrostatic charge), *GREM1* (cell growth and differentiation), *UNC13B* (apoptosis) and *FRMD3*, *CARS*, *CPVL/CHN2* were associated with DN, while *ELMO3* (Asians), *CCR5* (Asians) and *CNDP1* (type 2 diabetes) were associated with DN only in a subgroup. This large meta-analysis provides some novel biological insight. Pathways including renin-angiotensin, angiogenesis, polyol pathway and oxidative stress are involved in DN, as expected. Of special interest is the involvement of lipid metabolism in DN. Type 2 diabetes causes obvious dyslipidemia, and it has been recently shown that sera in patients with diabetic kidney disease impair cholesterol efflux in podocytes [23]. The exact role of lipid metabolism in DN needs to be further investigated.

An overview of all genetic variants of genes involved in inflammatory cytokines and angiogenesis pathways in DN has recently been published [24]. Eleven genetic variants within or near 9 genes were found to be significantly associated with DN. *VEGFA*, *CCR5*, *CCL2*, *IL-1*, *MMP9*,

EPO, *ADIPOQ* and *IL-8* showed increased risk for DN, and rs1800871 (T) variant in *IL-10* showed protective effect for DN. Enrichment analysis of these DN-associated genes revealed three functional pathways: G protein-coupled receptor signaling pathways (*IL-10*, *VEGFA*, *EPO*, *IL-1* and *IL-8*), receptor binding pathways (*VEGFA*, *EPO*, *IL-1*, *IL-8*, *IL-10*, *ADIPOQ* and *CCL2*), and those related to chronic kidney failure (*IL-6*, *EPO*, *ADIPOQ*, and *CCR2*). The association of *APOE* and *ENOS* with DN is summarized in another meta-analysis [25].

From SNP to Outcome

A list of genetic variants has been generated in the past decade. With the rapid development of next-generation sequencing methods, one can expect novel variants to be identified. How genetic variants function in DN largely remains to be elucidated. Above 85% of disease-associated SNPs are located in noncoding regions of genome [26], including promoters and enhancers. It is deducible that SNPs are able to regulate gene expressions through establishing or abolishing TFBS. An interesting study [27] attempted to establish a mechanistic link between a non-coding SNP and clinical outcome using an integrative approach. The authors selected to investigate the function of a SNP in the promoter of *FRMD3*. Repression in *FRMD3* on the RNA level was found in the kidney of T2DM patients. They then generated a list of genes that coexpressed with *FRMD3* based on the assumption that coexpressed genes may share a 'common regulatory element'. The top pathway enriched by these coexpressed genes was the bone morphogenetic protein (BMP) signaling pathway, a part of the TGF- β superfamily. The rs1888747 risk allele (G) created a TFBS that is believed to be inhibitory. Further analysis revealed a common TFBS module in the promoter shared among *FRMD3* with the risk allele and some BMP pathway members. A testable hypothesis was proposed: SNP rs1888747 in the *FRMD3* promoter region could downregulate *FRMD3* expression, which subsequently abolishes stimulatory effect of *FRMD3* on BMP, leading to a reduction of BMP-mediated renal protection in the kidney of diabetic patients with this risk SNP. This study provides a novel strategy to test how a SNP influences the risk of a certain disease. In addition, most genetic variants associated with DN have a relatively low OR (<2), while the major risk variants have not been identified for DN. The significance of these small-risk variants in the development and progression of DN remains unknown. DN could be caused by the additive or synergic effects of these multiple small-risk variants. However, the ongoing studies with larger

patient populations are needed to further identify major but rare variants for DN. In addition, genetics studies using better defined patient populations, such as patients with progressive DN, will be helpful in identifying genes associated with a more defined disease phenotype.

Epigenetics

Other than qualitative and quantitative control of gene function by genetic variants, epigenetic control of gene regulation provides an extra layer of regulation of genes involved in the pathogenesis of DN. Rapid advances in high-throughput genome-wide profiling and sequencing methods make it feasible to scan epigenetic modification of whole genome, so-called 'epigenome-wide association studies' (EWAS), which is a strong complementarity to GWAS. In this review, we discuss the role of three types of epigenetic modification in DN: DNA methylation, miRNA, and histone modification. The epigenetic modification profile combined with the gene expression profile may provide additional information on the gene regulation in the context of DN.

DNA Methylation

DNA methylation occurs through a covalent addition of a methyl group to 5'-cytosines of CpG dinucleotides, which often cluster at the promoter region, known as CpG islands. This modification within the promoter usually represses gene expression through either directly disrupting the binding of transcription machinery to the promoter or indirectly recruiting corepressors to the promoter via methyl-binding proteins that recognize 5'-methylcytosine. Hyperglycemia has been reported to change DNA methylation [28], indicating a role of aberrant DNA methylation in DN. It has been believed that DNA methylation is very tissue specific. However, recent evidence suggests that DNA methylation patterns vary only slightly among different tissues [29]. Therefore, DNA methylation profiling obtained from surrogate tissues, such as peripheral blood cells and saliva (providing ease of collecting DNA samples) is still informative.

One study utilized the Illumina Infinum Human Methylation27 BeadChip DNA methylation array to investigate the methylation of CpG islands in promoter regions across the whole genome using DNA from peripheral blood cells of T1DM patients with or without kidney disease [30]. The study revealed hypermethylation at 5' of the *UNC13B* gene, in which a genetic variant was identified to be associated with DN [22, 31]. However, this gene,

instead of suppression, was upregulated in cultured rat meningeal cells under a high glucose condition [32]. The discrepancy suggests the complexity of gene regulation, and that hypermethylation of a gene may not necessarily be associated with inhibition of its expression. Another study, using the same array as above [30], identified 187 genes that show differential site-specific DNA methylation in DNA extracted from the saliva of predominantly T2DM patients with or without ESRD [33]. The largest-scale EWAS [34] thus far examined nearly half a million unique methylation sites in blood cells of 255 patients with chronic kidney disease (113 due to T1DM) and 152 control individuals (113 with T1DM without kidney disease). Twenty-three genes had a significant difference in DNA methylation between case and control groups. Six candidate genes identified were known to be involved in kidney disease, including *CUX1*, *ELMO1*, *FKBP5*, *INHBA-AS1*, *PTPRN2* and *PRKAG2*. Among these 6 genes, there was a slight difference in *ELMO1* and *PRKAG2* mRNA levels in peripheral blood cells between patients with chronic kidney disease and controls. Another study analyzed DNA methylation in kidney tubular epithelial cells [35] and showed significant differences of methylation in 1,061 genes in DN patients compared to controls. Differentially methylated loci were mainly located to enhancer regions and TFBS. Of all genes with differential DNA methylation in the proximity, about 40% were correlative regulated. There is very little overlap of genes with differential DNA methylation among the above studies, indicating that aberrant DNA methylation profiles under disease conditions are more tissue-specific than those observed in normal tissues.

Studies from animal models of kidney disease also revealed alteration of DNA methylation. It was reported that DNA hypermethylation was observed in the promoter of *RASAL1*, leading to a decrease in *RASAL1* transcript in primary fibroblasts isolated from fibrotic versus non-fibrotic kidneys [36]. Decrease of *RASAL1* enhanced Ras activity, leading to kidney fibrosis. However, *RASAL1* is not on the list of aberrantly methylated genes identified from human samples.

miRNA

miRNAs are small single-stranded non-coding RNAs containing ~22 nucleotides that modulate the expression of genes by perfect or imperfect complement, leading to mRNA degradation or translation termination. miRNA typically binds to the 3' untranslated region of target mRNA, but it has also been shown to bind to 5' untranslated regions to inhibit translation [37]. One miRNA tar-

gets many genes, due to its imperfect complement binding. In humans, more than 2,500 mature miRNAs are identified and regulate at least 60% of protein-coding genes [38]. miRNAs in intergenic sequences or on the antisense strand of genes are expressed quite independently, while intronic miRNAs are coordinately expressed with their host gene. miRNAs modulate multiple cell processes, and aberrant miRNA expression plays a role in the pathogenesis of various diseases. In kidneys, podocyte-specific deletion of Dicer, a key enzyme for the biogenesis of miRNAs, was shown to cause kidney failure and early death [39]. Many miRNAs involved in DN have been identified [40]. Dysregulated miRNAs are mainly involved in kidney fibrosis through regulation of ECM accumulation, a pathological hallmark of DN. The TGF- β 1 pathway is a master regulator of renal fibrosis. As such, many miRNAs have been shown to regulate the TGF- β 1 pathway or to be regulated by TGF- β 1. miRNAs function as upstream regulators of TGF- β 1 include miR-192, miR-200b/c, miR-21 and miR-1207-5p. Downstream effectors of TGF- β 1 include miR-21 and miR-192. miR-192 is among the best studied miRNA in DN. However, the results on miR-192 expression in diabetic kidney are conflicting. Kato et al. [41] found a significantly higher level of miR-192 in the glomeruli of streptozotocin (STZ)-induced and *db/db* diabetic mice. However, another group found a reduction in miR-192 in the cortex of diabetic *ApoE* knockout mice [42]. In patients with established DN, miR-192 level is lower in the kidney, and a reduced level of miR-192 correlates with tubulointerstitial fibrosis and a decline of estimated glomerular filtration rate [43]. miR-192 was shown to upregulate TGF- β 1 promoter activity in mouse mesangial cells, and inhibition of miR-192 by locked nucleic acids (LNA)-modified antisense miR-192 (LNA-anti-miR-192) decreased TGF- β 1 mRNA in mouse mesangial cells and the mouse kidney cortex [44]. Reciprocally, TGF- β 1 is reported to either decrease [42] or increase [45] miR-192 expression in different cell culture models. miR-192 decreases *Sip1* (also called *ZEB2*), an E-box repressor, by directly binding to its 3' UTR. Decreased *Sip1* relieves the repression of *Col1a2*, causing an increase in expression of *Col1a2*, a key gene associated with the pathogenesis of DN [41]. In STZ-induced diabetic mice, LNA-anti-miR-192 decreased the miR-192 level in the kidney, restored the miRNA-192-mediated inhibition of *ZEB1/2*, decreased the gene expression of collagen, TGF- β 1, and fibronectin, and ameliorated DN in vivo [46]. The discrepancy of the findings on miRNA-192 in DN among different studies could be attributed to different cell types, animal models and disease stages.

Histone Modification

PTMs of histone, mostly in the exposed amino-terminal tails, regulate gene expression by changing the chromatin structure. Important PTMs of histone include methylation, acetylation and phosphorylation. PTMs are mediated by specific enzymes. Lysine acetylation is catalyzed by histone acetyltransferases such as p300 and CBP and lysine deacetylation by HDACs. HDACs are divided into four classes: classes I, II and IV are zinc-dependent enzymes, whereas class III HDACs, sirtuins, are nicotinamide adenine dinucleotide (NAD)-dependent enzymes. Lysine methylation is carried out by lysine methyltransferases (HMTs) and demethylation by lysine demethylases (HDMs). Some PTMs of histones are considered to be transcriptional active marks including H3K9ac, H3K14ac and H4K5ac, and H3K4me2/3, while others are associated with transcriptional repression including H3K9me2/3 and H3K27me3. The role of histone PTMs in DN has been investigated in vitro and in vivo. TGF- β 1 stimulation in rat mesangial cells upregulates the expression of fibrotic genes, such as *Col1a1*, *CTGF*, and *PAI-1*, correlated with deduced association of H3K9me2 and H3K9me3 (repressive mark) and increased association of H3K4me (active mark) in their respective promoters [47]. SET domain-containing 7 (SET7), a H3K4 monomethyltransferase, was found to be upregulated by TGF- β 1 and recruited to the promoter of these genes. High glucose also induced the expression of these genes which were associated with similar changes in histone modification at their promoters. These changes were abolished by a TGF- β 1-specific antibody, indicating that these are TGF- β 1-mediated effects. In another study using rat mesangial cells, TGF- β 1 treatment enhanced mRNA levels of *PAI-1* and *p21* as well as increased the occupancy of H3K9/14ac and CBP/p300 at their promoters [48]. HDAC inhibitor also increased the expression of these genes through increasing binding of H3K9/14ac at their promoters. These in vitro studies clearly demonstrate that histone modification is a key regulator of gene expression in kidney cells. In addition, hyperglycemia also increased mRNA levels of *PAI-1* and *p21* by increasing H3K9/14ac recruitment at their promoter in glomeruli of STZ-induced diabetic mice [48]. Another in vivo study reported that high glucose inhibited DNA methylation and increased H3K9ac at the promoter of the redox-regulating protein p66 (shc) and thereby upregulated p66shc expression in diabetic murine kidney [49]. One report studied upregulated genes implicated in DN and found increased RNA polymerase II recruitment, an increased H3K4me level but decreased H3K27me3 lev-

Fig. 1. Epigenetic regulation of genes in DN. The schema summarizes the epigenetic regulation of gene expression in DN. In the normal kidney, gene expression is regulated at the epigenetic level by PTMs of histone and transcription factor, DNA methylation, and miRNA. In the diabetic kidney, these epigenetic modifications are altered, leading to abnormal gene expression and DN.

els at their respective promoters [50]. One in vivo study used microplate-based chromatin immunoprecipitation method (Matrix ChIP) to screen histone PTMs at promoters of genes encoding MCP-1, receptor for AGEs (RAGE), and PAI-1. The authors found that the increased expression of these genes was associated with higher RNA polymerase II recruitment, active marks and less repressive marks at their promoters in glomeruli of diabetic *db/db* mice compared to nondiabetic *db/m* mice [51]. Losartan, an angiotensin II type 1 receptor blocker, mitigated DN by partially reversing histone PTMs at their promoters.

Another interesting finding from a clinical trial by Miao et al. [52] suggests a potential epigenetic factor in DN. Patients with T1DM who initially received conventional therapy followed by intensive treatment had higher risks of developing diabetic complications, including DN, than those who received intensive treatment at all time, indicative of ‘metabolic memory’. In order to investigate the underlying mechanisms, the authors, using chromatin immunoprecipitation, profiled H3K9Ac, H3K4Me3, and H3K9Me2 in promoters in monocytes and lymphocytes from two groups of T1DM patients treated with either conventional or intensive therapy. Only H3K9Ac in monocytes was significantly different between the two groups. Pathway analysis using genes with hyperacety-

lated promoters showed enrichment in the inflammatory pathway. In addition, a strong association between monocyte H3K9ac and the mean HbA1c level was also observed. This study indicates that enriched H3K9ac at promoters of inflammatory genes could persistently induce inflammation and diabetic complications. The combination of the H3K9ac profile and relevant gene expression level in blood cells might provide a more complete view about the epigenetic mechanism of metabolic memory.

Epigenetics – Adapting to the Environment and Entangling Together

Genetic variants are doomed to be unchangeable. However, cells use epigenetic mechanisms to respond and adapt to the environment. Hyperglycemia puts cells in an environment of ‘extra-nutrient’ and pushes cells to activate three nutrient-sensing pathways: the mammalian target of rapamycin (mTOR), AMP-activated protein kinase (AMPK) and NAD-dependent histone deacetylase (Sirt1) [53]. mTOR is activated by hyperglycemia, and AMPK and Sirt1 expression is decreased by hyperglycemia. These three pathways are deeply involved in DN. mTOR effects histone phosphorylation by its downstream, H3S10-specific kinase. AMPK is able to phosphorylate histone H2B serine 36. Sirt1 deacetylates histones and non-histone proteins, such as DNA methyl-

transferase 1 (DNMT1) [54], to regulate DNA methylation. Histone modifications and DNA methylation could regulate miRNA expression, and miRNAs could in turn regulate histone modification and DNA methylation. For example, miR-29a/b, which is downregulated in diabetes, directly targets *DNMT3A/3B* [55], thereby influencing DNA methylation. miRNA-195, which is upregulated in diabetes, targets *Sirt1* [56], thereby changing histone acetylation. Epigenetic changes form a network to regulate genes involved in the pathogenesis of DN (fig. 1).

PTMs of Transcription Factors

In addition to genetic variants and epigenetic modifications, gene expression regulation may be achieved by direct PTMs of transcription factors and cofactors. It is known that multiple transcription factors are activated not only by protein phosphorylation, but also by protein acetylation, such as NF- κ B [57], Stat3 [58], and p53 [59]. Systems biology analysis of human diabetic kidneys suggests that NF- κ B and Stat3 are key transcription factors involved in DN through activation of downstream proinflammatory gene expression [60]. We have recently shown that acetylation of NF- κ B and Stat3 is increased in diabetic kidneys, leading to their activation [61]. In addition, acetylation of NF- κ B and Stat3 is negatively regulated by *Sirt1*. Knockdown of *Sirt1* in podocytes aggravates inflammation and kidney injury, while inhibition of NF- κ B acetylation by using a specific bromodomain inhibitor attenuates inflammation and kidney injury in diabetic mice.

Concluding Remarks

Rapid developments of high-throughput genome-wide screening techniques have greatly broadened our understanding of genetic and epigenetic changes in disease such as DN. Many genetic studies have been performed in DN, including large studies such as FIND and GoKinD studies. However, although multiple genes and loci have been found to be associated with DN, none of them have a major impact. A better designed study, with a larger sample size and a better defined patient population may help to identify key genes involved in DN. In addition, epigenetic changes including DNA methylation, histone modification, and miRNA regulation also play a major role in the pathogenesis of DN. However, epigenetic study is limited in human kidney disease because of the limited availability of samples and the heterogeneity of kidney tissues with multiple cells. Due to these limitations and the poor differentiation status of cultured kidney cells, recent epigenetic data generated are not consistent among different studies. These technical limitations need to be overcome to generate more solid data on epigenetic changes in DN, so that they can be used as potential biomarkers and drug target for DN.

Disclosure Statement

The authors have no conflicts of interest to declare.

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