

EDITORIAL

How do genetic and environmental factors interact in diabetic kidney disease?



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Diabetic kidney disease (DKD), also known as diabetic nephropathy, is one of the most serious complications of diabetes and results from a complex interplay of metabolic and hemodynamic disturbances in genetically predisposed individuals. Classically, DKD is characterized by persistent proteinuria, elevated arterial blood pressure, and progressive decline in renal function. In recent years, a subset of patients with diabetes and progressive loss of renal function without significant proteinuria either treated or untreated with renin–angiotensin–aldosterone system inhibitors have also been identified. Diabetes accounts for approximately 44% of patients on replacement therapy for end-stage renal disease each year, including dialysis and renal transplantation. Despite the fact that its incidence has been declining in recent years, the current epidemic of obesity and diabetes is anticipated to reverse

this trend and result in a disproportionate number of diabetics with end-stage renal disease, potentially reaching 70% by 2015 [1]. Diabetic individuals with nephropathy have a significantly increased morbidity and premature mortality primarily due to accelerated macrovascular cardiovascular disease, such as coronary heart disease and stroke. In addition to morbidity and mortality associated with DKD, the disease has major societal impact because of the enormous financial burden associated with renal replacement therapy. The increasing number of patients with end-stage renal disease and the associated socioeconomic burden necessitates the identification of cellular and molecular pathways involved in the development and progression of DKD. This should impact adequate classification, prognosis and treatment.

Epidemiological studies investigating the causative factors demonstrate that

“Emerging evidence suggests that the complex interplay of epigenetic factors interacting with genes and environment plays a critical role in susceptibility to diabetes and diabetic kidney disease.”

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DKD is a classic complex trait, the development of which in a given individual reflects contributions by multiple genes, and is modulated by poor metabolic control (hyperglycemic environment). Pathogenesis of DKD is further complicated by the association of diabetes with metabolic syndrome, with hyperlipidemia, obesity and hypertension, which potentially influence the progression of kidney disease. Despite treatments for hyperglycemia, hypertension and hyperlipidemia, and the use of renin–angiotensin–aldosterone system inhibitors for proteinuria, the incidence and prevalence of DKD continues to increase world-wide. Epidemiological data indicate that not all diabetic patients develop kidney disease. Only approximately 5–40% of individuals develop DKD, suggesting that a subgroup of patients is at high risk. Furthermore, the prevalence and incident rate of DKD is high in minority populations, indicating population heterogeneity.

Familial clustering of DKD and differences in the prevalence of DKD among individuals, families and ethnic groups suggests a strong contribution of genetic factors [2]. To identify genes that predispose to DKD, several genetic approaches are being utilized that include linkage scans, candidate gene analysis and genome-wide association studies (GWAS). Genome-wide linkage scans identified several chromosomal regions that are likely to contain DKD susceptibility genes, and association analyses have evaluated positional candidate genes under linkage peaks. These complimentary approaches have demonstrated that polymorphisms in the carnosinase 1 gene on chromosome 18q, the adiponectin gene on 3q, and the *ELMO1* gene on 7p are likely associated with susceptibility to DKD [3]. GWAS performed for DKD have identified novel risk loci including *SLC12A3*, *ELMO1*, 4.1 protein ezrin, radixin, *FRMD3* and *SASH1* gene [4–6]. A recent meta-analysis identified additional genetic variants associated with DKD, that includes when the genetic variants located in angiotensin converting enzyme 1, ApoE and nitric oxide synthase 3 [7].

Although genome-wide searches to identify causal genetic variants influencing DKD led to the identification of few credible associations pointing to cellular pathways playing a prominent role in linking genes and environment, at present, no single gene with a large effect has been identified that confers susceptibility to the development, progression and/or severity of DKD

and related traits. Despite significant advances in controlling the environmental influences (hyperglycemia, high blood pressure and hyperlipidemia), there remains a high risk of kidney disease progression in the diabetic population. In this context, a critical barrier to the progress of gene discovery for DKD is the identification of a missing link that connects genes and the environment. Emerging evidence suggests that the complex interplay of epigenetic factors interacting with genes and environment plays a critical role in susceptibility to diabetes and DKD. Epigenetics is the study of heritable changes in gene expression without changes in the DNA coding sequence and occurs via specific DNA modification including mitotically stable DNA methylation, post-translational histone modification (PTMH) proteins (acetylation, methylation, phosphorylation and ubiquitination), and small noncoding RNAs, such as miRNAs (miR). These diverse molecular mechanisms are closely intertwined to ensure the faithful propagation of an epigenetic state over time.

DNA methylation occurring on the 5' position of the pyrimidine ring of cytosine in the context of the dinucleotide sequence CpG forms one of multiple layers of epigenetic mechanisms modulating gene expression. Emerging reports suggest that DNA hypermethylation is likely to be involved in the pathogenesis of insulin resistance, diabetes and DKD [8–13]. Hypermethylation of *RASAL1* is associated with the perpetuation of fibroblast activation and fibrogenesis in the kidney, suggesting that DNA hypermethylation regulates the cellular reprogramming leading to glomerular and interstitial fibrosis [11]. Methylation profiling in the genomic DNA of Irish patients with Type 1 DKD identified an association between a promoter methylation of *UNC13B* and diabetic neuropathy [12]. Altered methylation in *MYL9*, *MMP10*, *TIMP4* and *MTHFR* was recently reported to be associated in DNA isolated from the saliva of African–American patients with Type 2 DKD [13]. Together, differential DNA methylation and associated dysregulation of gene expression can be causal for DKD.

The genetic basis for interindividual variation in DNA methylation profiles was recently demonstrated. GWAS and genome-wide methylation data were recently integrated to identify haplotype-specific DNA methylation in a T2DM and obesity susceptibility locus [14]. Using this strategy, a recent study analyzing single nucleotide

polymorphism (SNP) gene expression and the DNA methylation pattern in 77 HapMap Yoruba individuals identified SNPs that affect both gene expression and DNA methylation, thus providing evidence for epigenetic mechanisms affecting multiple quantitative trait loci through interacting with genes and environment [15].

PTMH in chromatin is another epigenetic mechanism modulating gene expression, and has been implicated in the pathology of diabetes and its complications. Using a chromatin immunoprecipitation linked to microarray approach, Miao *et al.* recently profiled H3-lysine trimethylation (H3K4me3, H3K27me3, H3K9me2, H3K9me3 and H3K9) and lysine acetylation (H3K9Ac and H4K16Ac) in blood cells of patients with Type 1 diabetes and healthy individuals [16]. There was a marked variation in H3K9Ac levels in the upstream regions of HLA-DRB1 and HLA-DQB1 within the *IDDM1* locus between cases and controls, indicating that the acetylation status of H3K9 may contribute to Type 1 diabetes through regulating the expression of HLA-DRB1 and HLA-DQB1. Since the diabetic milieu is known to modulate the PTMH, variations in PTMH at the promoter/enhancer regions of DKD susceptibility genes may result in dysregulated expression of these genes and contribute to DKD.

Recent studies provided compelling evidence for a pathogenic role of ncRNAs such as miR in DKD [17]. miRs are a family of endogenous short, 21-nucleotide long, noncoding RNAs that recognize target mRNAs through partial complementary elements in the 3'-UTR of the mRNAs and inhibit their expression by translational repression or destabilization. Kato *et al.* first identified miR-192 as a regulator of collagen synthesis in DKD [18]. In cultured mesangial cells and in diabetic kidneys, TGF- β upregulates miR-192 expression, which in turn targets the E-box repressor protein SIP1. TGF- β also downregulates δ EF1. Downregulation of SIP1 and δ EF1 by miR-192 relieves the repression of *Col1a2* gene expression, resulting in increased collagen production and deposition in the mesangium in DKD [18]. Furthermore, miR-192 upregulates the levels of miR-200b/c in mouse mesangial cells, which, in a coordinative manner, upregulates *Col1a2* expression by inhibiting Zeb1 expression through binding to the 3'UTR of Zeb, an E-box repressor of *Col1a2* [19]. In addition to miR-192, TGF- β also upregulates the expression of miR-216a and miR-217 in mesangial cells, which in

turn activate Akt and downstream signaling through inhibition of the phosphatase and tensin homolog, PTEN [19]. Inhibition of miR-192 ameliorates renal fibrosis in DKD, indicating that miR-192 may represent a therapeutic target for DKD [20]. Together, these studies have established a regulatory role of specific miRs in TGF- β -mediated pathology in DKD.

A new layer of gene and environment interaction has been discovered through post-transcriptional miRNA-mediated expression of a large proportion of genes. SNPs in miRNA target sites (miR SNPs) represent a specific class of regulatory polymorphisms in the 3'-UTR that may contribute to heritable variations in gene expression [21]. Genetic alteration in the Zeb1 3'-UTR (M1 or M2) results in a complete loss of response to miR-200b, indicating that miR SNPs located at Zeb1 may prevent the binding of miR-200b and protect individuals from DKD [19]. As the list of miRs and target SNPs keeps growing, the need to confirm the effect of target SNPs on gene expression becomes more pressing, and will eventually help achieve a deeper insight into the molecular pathways involved in DKD.

Recent advances in genomic technology and associated computational models have positioned investigators to initiate large-scale studies of genetic and epigenetic variations associated with DKD. Furthermore, the International Human Epigenome Consortium was recently launched to map 1000 reference epigenomes for human cells. These maps will be equivalent to the human haplotype map and will help improve our ability to conduct epigenome-wide association studies and integrate these with GWAS and next-generation sequencing data. This should help uncover cross-talk between genetics and epigenetics, and significantly enhance our understanding of complex diseases including DKD. Recent developments in identifying genetic variants that regulate the expression of methylases such as histone methyltransferase and DNA methyltransferase may also yield important insights into the complex interplay of epigenetics interacting genes and environment in DKD.

In conclusion, by elucidating the interactions between genes and the environment, epigenetics may play a major role in defining DKD susceptibility. Identification of genetic and epigenetic-mediated dysregulation of genes involved in the development and/or progression of kidney disease in a diabetic environment will open new avenues

for detailed functional studies, and may eventually help facilitate prediction, prevention and the development of improved treatment for this devastating complication of diabetes.

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