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Epigenome-Wide Association Studies of DNA Methylation in Kidney Diseases

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As a parallel study design to a genome-wide association study, an epigenome-wide association study (EWAS) uses a variety of microarray-based or sequence-based analytical techniques to detect differential changes in the distribution of methyl groups on thousands of specific DNA nucleotides across the whole genome. Associations between these epigenetic marks and clinical phenotypes can identify epigenetic variants contributing to human diseases. These associations may ultimately help to explain disease pathogenesis and facilitate development of new diagnostic approaches and treatments. EWAS methods have developed rapidly in the last 10 years, with reduced cost and increased efficacy. For example, EWAS using DNA methylation arrays include progression from Illumina 27k to Illumina 450k and then to Illumina Methylation EPIC v2.0 array (San Diego, CA). The latter includes over 860,000 cytosine-phosphate-guanine (CpG) probes.[1]

DNA methylation was one of the earliest identified epigenetic modifications, described in 1940. It can alter chromatin structure, DNA conformation, DNA stability, and DNA interaction with proteins. DNA methylation is involved in many biologic processes, including cell differentiation, embryonic development, X chromosome inactivation, and tissue specificity without influencing protein encoding sequences.[2] Significant differentially methylated CpG (dmCpG) sites identified in EWAS are associated with diverse complex diseases, including cancer, Alzheimer's disease, and diabetes mellitus. As a method to study epigenetic pathogenesis of complex diseases, EWAS of DNA methylation is cost-effective and provides a high throughput approach to study the association between epigenetic changes and complex human diseases.

In a paper published in this issue of *KI Reports*, Smyth *et al.*[3] conducted a longitudinal EWAS of DNA methylation in peripheral blood mononuclear cells, an easily obtained clinical research sample, from 154 kidney transplant recipients evaluated pretransplant and posttransplant, with an average of 17 years of follow-up. This is the largest EWAS study of kidney to date, using the low cost and high-throughput Illumina Infinium MethylationEPIC platform. The authors identified 5 dmCpG sites that showed consistent differences between peripheral blood mononuclear cells longitudinally collected pretransplant and postkidney transplant.

This study provides a reference database of dmCpGs for future studies to discover novel biomarkers for diagnosing or predicting complications of kidney transplantation. Because the primary renal diagnoses for kidney transplant include more than 7 kinds of kidney diseases, phenotypes of these causative kidney diseases are very variable. Thus only 5 dmCpGs have been identified in kidney recipients pretransplant and posttransplant. In addition, 2 of the 5 dmCpGs sites, including cg23597162 (within JAZF1) and cg17944885 (located between ZNF788P and ZNF625-ZNF20), which have prior associations with chronic kidney disease.[3] The identification of few dmCpGs is a common limitation for discovery studies of novel biomarkers in any human diseases using EWAS.[1]

Besides the above longitudinal EWAS in peripheral blood mononuclear cells from recipients of pretransplant and postkidney transplant, EWAS of DNA methylation has been reported in various kidney diseases in the past decade including many forms of primary and secondary kidney diseases. Urinary albumin-to-creatinine and estimated glomerular filtration rate are important biomarkers to diagnose chronic kidney disease and to evaluate

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kidney function decline. Schlosser *et al.*[4] conducted blood-based EWAS of DNA methylation for detecting associated CpGs with estimated glomerular filtration rate (n = 33,605) and albumin-to-creatinine (n = 15,068). Of these, 69 CpGs were associated with estimated glomerular filtration rate and 7 were associated with albumin-to-creatinine. Associations of kidney function with DNA methylation, such as a dmCpG site within *JAZF1*, encoding the protein named "juxtaposed with another zinc finger protein 1," was validated in kidney tissue.[4]

IgA nephropathy (IgAN) is the most common cause of primary glomerular disease. CD19+ B cells isolated from peripheral blood mononuclear cells from 92 patients with IgAN and 92 healthy subjects who were age and sex matched were subjected to Illumina Infinium MethylationEPIC array for DNA methylation site analysis. Seventy-two dmCpGs were identified to significantly associate with IgAN, which included those present in the genes *GALNT6*, IQSEC1, *CDC16*, and SYS1. The protein products of these genes were found to be involved in the pathway related to tubular atrophy and interstitial fibrosis. The authors concluded that CD19+ B cell gene DNA methylation may play an important role in the pathogenesis of IgAN.[5]

Lupus nephritis is the most common immune-mediated glomerular disease associated with systemic illness. Coit *et al.*[6] used DNA samples from whole blood and performed Illumina Infinium MethylationEPIC array to assess site-specific DNA methylation of over 850,000 methylation sites across the genome. Methylation levels at 2 dmCpG sites correlated with the lupus disease activity index. Demethylation of a CpG site within *GALNT18* is associated with the development of proliferative lupus nephritis.[6]

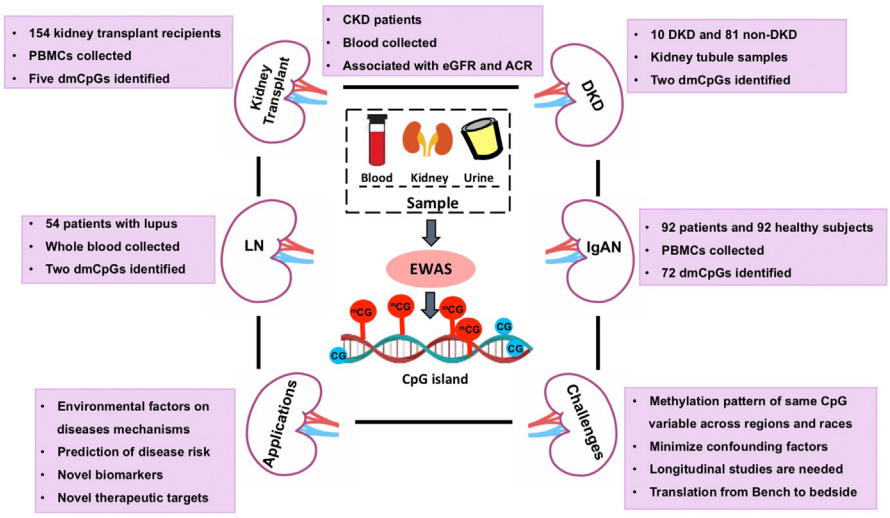

Figure 1. The current status, applications, and challenges of EWAS in human kidney diseases. Blood, kidney tissue, and urine samples can be used to isolate DNA for EWAS in patients with kidney diseases, including kidney transplant, diabetic kidney disease, lupus nephritis, and IgAN. Differentially methylated cytosine-phosphate-guanine sites associated with different kidney diseases are main findings from EWAS. The applications and future challenges of EWAS technology. ACR, albumin-to-creatinine ratio; CKD, chronic kidney disease; DKD, diabetic kidney disease; dmCpG, differential methylated cytosine-phosphate-guanine; eGFR, estimated glomerular filtration rate; IgAN, IgA nephropathy; LN, lupus nephritis; PBMCs, peripheral blood mononuclear cells.

Diabetic kidney disease (DKD) will affect approximately 40% of diabetic patients, who are nearly half a billion people worldwide. DKD is currently the leading cause of end-stage kidney disease. Gluck *et al.*[7] identified 2 CpG sites in human kidney tubules in subjects with DKD (n = 10) and those without DKD (n = 81) using Illumina Infinium HumanMethylation450K BeadChip. One dmCpG (cg 20597486) was associated with renal interstitial fibrosis and the other one (24818418) was related to the estimated glomerular filtration rate decline and the gene expression change of epidermal growth factor.[7]

EWAS is an emerging tool to identify epigenetic variations as biomarkers in complex kidney diseases. The profiles of DNA methylation in many other kidney diseases, including primary, secondary, and hereditary kidney diseases remains unknown. DNA samples can be extracted from blood, urine, and kidney tissue, and used for EWAS of DNA methylation.[6–8] The methylated CpG sites-related genes may be a source of novel biomarkers for early diagnosis, monitoring response of treatment, and predicting outcomes in kidney diseases. They might also serve as presymptomatic biomarkers of hereditary kidney diseases such as Alport syndrome and polycystic kidney disease.

EWAS can be used to elucidate many aspects of kidney diseases. EWAS may identify novel therapeutic targets for common and rare kidney diseases. Because various environmental factors can contribute to the incidence of lupus nephritis and DKD, the EWAS approach may uncover the underlying mechanisms of specific environmental factors that contribute to these diseases. EWAS of DNA methylation has very promising applications in cancer and kidney diseases because of its

ability to recognize epigenetic changes.

EWAS also faces challenges. Because epigenetic modifications are chiefly affected by the environment and genetics, a particular CpG site that is identified in one kidney disease case may be absent in other patients with the same disease. For example, environmental factors such as exposure to ultraviolet rays can trigger flares of lupus nephritis and so epigenetic patterns must account for diverse environmental confounders. Longitudinal studies will likely be required. The current Illumina EPIC 860+ array can identify many dsCpG sites in a specific kidney disease.[1,9] However, next-generation sequencing and third-generation sequencing have improved analysis of EWAS identified dmCpG sites. Nevertheless, it may take long to translate such data into improvements in clinical practice.

In conclusion, EWAS of DNA methylation has been used to identify novel biomarkers and underlying mechanisms in various clinical settings. These include longitudinal studies of kidney transplant and IgAN and cross-sectional studies of lupus nephritis and DKD, using blood and kidney samples. EWAS provides a novel approach to study the role of environmental and epigenetic factors. EWAS can discover novel diagnostic biomarkers, underlying disease mechanisms, and new drug targets. EWAS also faces challenges including the influence of the environment and population diversity, the need for longitudinal samples, and translation of findings to clinical practice (Figure 1).

DISCLOSURE

All the authors declared no competing interests.

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