The practical implications of the findings in Nadkarni *et al.* are that increased TNFR1 and 2 and KIM1 identify patients with early kidney disease, similar to their discriminatory role in biomarking nephropathy in patients with diabetes. If the results are corroborated in other trials, this would serve as a warning sign for practitioners to implement therapies that could delay progression of kidney disease. These high-risk individuals would be ideal for selective enrollment in clinical trials aimed at mitigating APOL1 nephropathy, due to enrichment of events and amplification of the ability to discern the therapeutic end points of decreasing renal outcomes in *APOL1* high-risk genotype individuals. In addition, inclusion of patients with earlier disease, who are potentially more amenable to intervention, could ameliorate the progression of kidney disease–associated complications at earlier time points (Figure 1).

As for all observational studies, adjustment for confounders can attenuate but not eliminate the possibility of residual confounders. Thus, data on urine albumin and/or protein are lacking for 58% of the participants. Therefore, it is not possible to fully exclude baseline kidney disease, even in the subset with highest eGFR.

In the era of precision medicine, we should aim to characterize the differential kidney disease risk and conduct risk stratification profiles in each individual without or with high-risk *APOL1* genotypes to improve clinical care and to mitigate the burden of CKD in individuals at increased genomic or nongenomic risk.

DISCLOSURE

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DNA methylation yields epigenetic clues into the diabetic nephropathy of Pima Indians

Karol Bomsztyk[1], Oleg Denisenko[1] and Yuliang Wang[1,2]

Environmental factors drive epigenetic programming. DNA methylation is the best studied modification transmitting epigenetic information. A study by Qiu *et al.* examined potential epigenetic roots for the decline of renal function in Pima Indians. A genomewide survey of blood leukocytes uncovered differentially methylated DNA sites in regulatory regions of genes associated with chronic kidney disease. This longitudinal study provides the first clues on epigenetic links between environmental factors and a high prevalence of diabetic kidney disease in Pima Indians.

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The classic definition of epigenetics refers to the heritability of cell phenotypes, via either mitosis or meiosis, that is not encoded by the genome. Cell identities are defined by patterns of gene expression, and chromatin structure is the key mediator of epigenetic programming of gene function.[1] More than 40 years ago, DNA methylation emerged as the first epigenetic modification. DNA methylation involves the addition of a methyl group to the cytosine ring of cytosines preceding a guanosine in the DNA sequence (cytosine-phosphate-guanine [CpG] dinucleotides) to form methyl cytosine (5-methylcytosine). Since then, much has been learned about how DNA methylation cooperates with histone modifications to control gene transcription. Generally, methylation of CpG in promoter regions is associated with "gene silencing" (Figure 1). In contrast, DNA methylation in the gene bodies can increase gene expression by

[1]*UW Medicine Lake Union, University of Washington, Seattle, Washington 98109, USA; and* [2]*Paul G. Allen School of Computer Science & Engineering, University of Washington, Washington, 98195, USA*

Correspondence: *Karol Bomsztyk, UW Medicine at Lake Union, Box 358050, University of Washington, Seattle, Washington 98109, USA. E-mail: karolb@u.washington.edu*

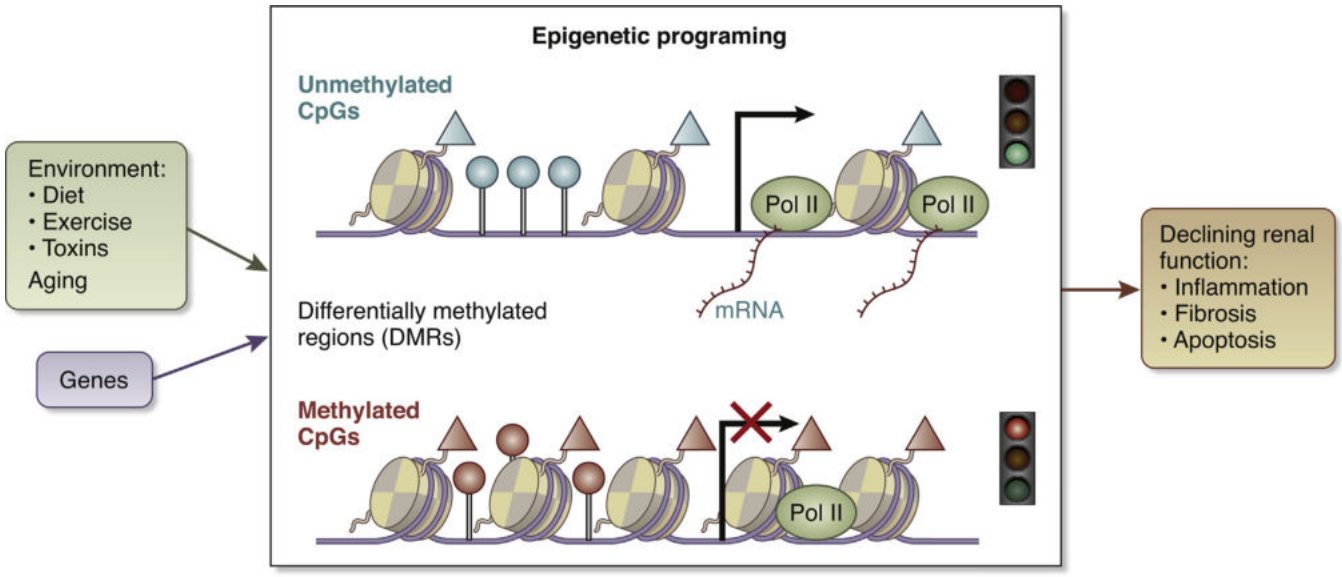

Figure 1 | Environmental factors, epigenetic reprogramming, and declining renal function. Environmental factors, on a genetic background, cause epigenetic reprogramming, in part through DNA methylation (lollipops) and histone modifications (triangles) and altered gene expression leading to decline in renal function. As illustrated here, there is interaction between DNA methylation and histone modification to control RNA polymerase II (Pol II) transcription. CpGs, cytosine-phosphate-guanine dinucleotides; DMRs, differentially methylated regions.

modulating transcription elongation and RNA splicing. The mechanisms of differential transcriptional effects of DNA methylation on promoter versus gene bodies remain largely unexplored.

Epigenetic programing has been linked to environmental factors (such as nutrition, smoking, chemical exposures, drugs, and other stresses), physical activity, and aging. Altered epigenetic processes are major contributors to disease. Epigenetic alterations in cancer have been known for >30 years because the observation that tumor progression is associated with the global loss of DNA methylation. This pioneering discovery generated great interest, and to this day DNA methylation remains by far the most studied epigenetic mark of other diseases, including kidney disease.

The association between chronic kidney disease and DNA methylation was first reported >10 years ago.[2] At about the same time, reports began to appear linking hyperglycemia, metabolic syndrome, and diabetic nephropathy to epigenetic programming. Introduction of high-throughput genomewide platforms propelled the field by allowing the assessment of DNA methylation in large patient cohorts with kidney disease including diabetic kidney disease.[3] The majority of those studies used blood leukocytes, whereas only 1 used kidney samples.[4] DNA methylation has been correlated with the decline in kidney function.[3,5] Differentially methylated regions (hypermethylated and hypomethylated) associated with kidney disease have been identified in promoters, gene bodies, and enhancers. Several of the differentially methylated regions were found at loci that either encode genes previously implicated in the pathogenesis of renal disease or sites containing regulatory elements controlling chronic kidney disease–relevant genes.[4] Although most of these surveys used the same Infinium HumanMethylation450 Beadchip (Illumina, San Diego, CA) array platform, none of the differentially methylated regions have emerged as shared among these kidney studies. It has been suggested that the lack of agreement between these DNA methylation genomewide studies may reflect differences in severity or causes of kidney disease.[3]

Pima Indians of Arizona have an extremely high prevalence of type 2 diabetes and kidney disease attributable to diabetic nephropathy.[6] The genetically related counterparts living in Mexico, whose lifestyles remain native, do not experience these morbidities. The differences in the lifestyles of these genetically related Pima subpopulations provide evidence that environmental factors play an important role in disease origins and suggest involvement of epigenetic programming. This question was addressed in a large longitudinal study of Pima Indians by Qiu *et al.*[7] published in this issue of *Kidney International*. The authors used the Illumina Infinium HumanMethylation450 Beadchip to assess DNA methylation at ~485,000 CpG sites across the genome in peripheral blood leukocytes.

After adjusting for multiple hypotheses testing, no one individual methylation locus showed a significant association with baseline estimated glomerular filtration rate (eGFR). It has been previously demonstrated that in genomewide association studies, a substantial proportion of borderline significance associations represent replicable, possibly genuine, associations, after considering additional data from subsequent studies.[8] The top eGFR-associated differentially methylated sites were localized to regulatory regions such as promoters and enhancers. Further, these regions were

enriched in transcription-binding sites, providing another link between epigenetic programing and aberrant transcription leading to declining renal function in this patient population (Figure 1). After performing a weighted gene coregulatory network analysis on 28,376 CpG loci in promoter regions, comethylation modules that showed significant associations with the eGFR were identified. These eGFR-associated modules were enriched in genes involved in cell cycle, apoptosis, and metabolism. Network-level analysis is an attractive option, especially when individual loci show weak but potentially coordinated associations with the phenotype of interest. Contiguous CpG loci often show significant comethylation, and differentially methylation loci tend to be enriched in these correlated methylation regions.[9] Therefore, testing the associations between *a priori*–defined correlation methylation regions with the phenotype of interest may further boost statistical power when the sample size is small.

The investigators identified 77 sites that correlated with subsequent eGFR decline. The strongest association was found at a site on chromosome 3 near the transcription start site for the *CDGAP* (*ARHGAP31*) gene, which encodes Rho GTPase Activating Protein 31. This gene is expressed in many tissues including the kidney, and mutations of this gene have been associated with renal abnormalities. Interestingly, hypomethylation of this site in visceral adipose tissue is associated with insulin resistance.[7] Further, this protein appears to regulate vascular endothelial growth factor–mediated angiogenesis. Taken together, the authors suggest that *CDGAP* (*ARHGAP31*) could be an epigenetically programmed gene that increases the risk of diabetic nephropathy in these individuals.

Cell specificity is dictated by epigenetic programming. This raises a concern whether methylation of eGFR-associated genes in blood leukocytes is also found at these loci in the kidney. To address this question, the authors took advantage of their previous human chronic kidney disease datasets from another cohort (it did not include Pima Indians) that examined DNA methylation and fibrosis in renal biopsy samples.[4] Among the 77 differentially methylated regions associated with the eGFR found in the current study, 5 regions showed an association of DNA methylation with altered gene expression and renal fibrosis in kidney datasets. The fact that this set of 5 genes did not include the top hits, especially the loci near the *CDGAP* gene found in the current study, may reflect epigenetic programming specific to blood leukocytes and/or the Pima Indian cohort. Confirmatory studies are needed, but the identification of common differentially methylated regions found in the 2 different studies suggest a paradigm that analysis of DNA methylation in blood leukocytes could report renal fibrosis.

Baseline eGFR and urinary albumin-to-creatinine ratio are used to predict renal function decline. The authors found that combining baseline eGFR and albumin-to-creatinine ratio with methylation of 2 CpG sites improved the ability to predict the development of kidney disease and progression to ESRD. However, the whole dataset was used for marker selection (20 top candidates), and split-sample validation was done *after* the selection of markers. It is generally recommended that feature selection and model validation be performed on separate samples from the beginning to avoid overfitting. One of the 2 sites is located in an intron of *FSTL5* (a gene that is preferentially expressed in the nervous system). The function of this site is unclear because it resides within the intron of a gene that does not appear to be expressed in the kidney. Still, it is of interest as a potential biomarker because it was also identified in the Chronic Renal Insufficiency Cohort.[5]

Epigenetics of chronic kidney disease is a fledgling field. This is the largest longitudinal study thus far that examined DNA methylation in a cohort at high risk of the development of kidney disease and end-stage renal disease. This survey provides the first epigenetic clues as to how environmental factors of Pima Indians could be linked to the high prevalence of diabetic kidney disease in this population (Figure 1). Specifically, the results show that DNA methylation of blood leukocytes at certain sites can correlate with the eGFR, predict renal decline, and report renal fibrosis. These observations are of potential clinical importance. Illumina's HumanMethylation450 Beadchip have been used extensively to survey DNA methylation, and in many such studies, sites of interest have been validated with other methods that are generally simple and more quantitative than these beads arrays. This was not done in the current study. Yet, such confirmation would go a long way to bring these finding to clinical settings. Most of these types of studies have been done in blood leukocytes because obtaining kidney samples in most settings is not practical. Other than cancer, there are no reports that looked at DNA methylation in the urine of chronic kidney disease cohorts. There is great interest in the cancer field in liquid biopsy samples in which blood and other body fluids are used to follow cancer progression and treatment. Urine is the logical source to predict and follow kidney disease using epigenetic markers including DNA methylation. Longitudinal DNA methylation analysis of urine samples cannot only establish potential causation as in the current study, but also provides better mechanistic connections. This and other DNA methylation cohort studies (documenting the importance of epigenetic programming) underscore the utility and need to develop epigenetic urinalysis techniques. This task may not be as difficult as it may seem given the fact that hundreds of renal cells are shed in the urine every day and the availability of methods to assay DNA methylation at a single cell level.

DISCLOSURE

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Facing cinacalcet-induced hypocalcemia: sit back and relax?

Pieter Evenepoel[1] and Rukshana Shroff[2]

A *post hoc* analysis of data from the EVOLVE study demonstrates that cinacalcet-induced hypocalcemia is common, mostly asymptomatic, and resolves spontaneously. These findings are reassuring and may warrant therapeutic inertia. However, previous studies in parathyroidectomized patients suggest that calcium repletion may be beneficial and safe from bone and cardiovascular perspectives, respectively, and as such call into question the appropriateness of a "sit back and relax" attitude toward cinacalcet-induced hypocalcemia.

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Dysregulation in mineral and bone metabolism in patients with chronic kidney disease (CKD) may contribute to the develop-mnt of vascular calcification, fractures, and premature cardiovascular morbidity and mortality. This clinical syndrome is currently referred to as CKD mineral and bone disorder (CKD-MBD). A cardinal manifestation of CKD-MBD is the increase in parathyroid hormone (PTH) levels. Secondary hyperparathyroidism (SHPT) starts in the early stages of CKD and becomes almost universal by the time of dialysis start. The armamentarium to tackle SHPT has expanded substantially over the last 2 decades. The introduction of i.v. vitamin D analogs, and especially cinacalcet in 2004, had a major impact on clinical practice and contributed to a drop in the parathyroidectomy rate. Cinacalcet is an allosteric activator of the calcium-sensing receptor, making the receptor more sensitive to extracellular calcium. The calcium-sensing receptor was initially described in the parathyroid glands, where it acts as a controller of free ionized calcium concentration in the blood via the regulation of PTH synthesis and secretion. Subsequent studies also localized the calcium-sensing receptor in other tissues, including the vasculature, gastrointestinal tract, and kidney. In the thick ascending limb, the activation of the calcium-sensing receptor will result in decreased calcium reabsorption and consequent hypercalciuria.

At present, on average 20% of dialysis patients are treated with cinacalcet. Gastrointestinal discomfort and hypocalcemia are the most commonly reported side effects of cinacalcet therapy. Given the lack of data on the epidemiology, natural history, and clinical relevance of cinacalcet-induced hypocalcemia, clinicians are often uncomfortable with this side effect. Therefore, the study by Floege *et al.* (2018)[1] in this issue of *Kidney International* is highly welcomed. The authors performed a *post hoc* analysis of the EVOLVE study to investigate the incidence, predictors, and therapeutic consequences of hypocalcemia. At least 1 episode of hypocalcemia developed among 58.3% of patients randomized to cinacalcet, versus 14.9% treated with placebo. The likelihood of developing hypocalcemia was related to severity of SHPT. Of patients manifesting low calcium, equal proportions manifested mild (defined by total serum calcium [tCa] ≥8 to <8.4 mg/dl), moderate (tCa ≥7.5 to <8.0 mg/dl), and severe (tCa <7.5 mg/dl) hypocalcemia. The hypocalcemia in cinacalcet-treated patients, moreover, was generally asymptomatic and self-limited, and its occurrence was not associated with hard (cardiovascular) outcomes. As such, the study by Floege *et al.*[1] largely confirms data from previous prospective intervention trials and from a recent North American retrospective study of a large dialysis organization database.[2]

The transient nature of most hypocalcemic events, with spontaneous

[1]*KU Leuven, Department of Immunology and Microbiology, Laboratory of Nephrology, Leuven, Belgium; and* [2]*Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK*

Correspondence: *P. Evenepoel, Nephrology, University Hospitals Leuven, Herestraat 49, B-3000 Leuven, Belgium. E-mail: Pieter.Evenepoel@uzleuven.be*