APOL1 and Proteinuria in the AASK
Unraveling the Pathobiology of APOL1

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Nucleic acid variants in the sequence of the gene for *APOL1*, which change the amino acid sequence, strongly associate with nondiabetic kidney diseases only in blacks (1). *APOL1* risk genotype–associated kidney disease phenotypes in blacks include hypertension-associated kidney disease, FSGS, HIV-associated nephropathy (HIVAN), and other diseases, which span standard pathologic classifications, and additional clinical (*e.g.*, eGFR decline) and histopathologic phenotypes. Unlike almost all other common disease-associated polymorphisms, *APOL1* kidney disease–associated variants have large effect sizes. Given this, defining the biology responsible for the genetic association could identify novel, high-effect treatment targets for a major health disparity.

APOL1 is an innate immune effector protein that causes trypanolysis (1). However, two trypanosome subspecies evolved mechanisms to block APOL1's trypanolytic activity, permitting transmission of African sleeping sickness. The APOL1 proteins encoded by the kidney disease–associated variants restore trypanolytic activity against one of the subspecies. Analogous to the link between the hemoglobin mutation that confers protection from malaria but also causes sickle cell disease, a single copy of a variant *APOL1* allele protects the carrier from sleeping sickness, but two copies confer increased risk for kidney diseases. Driven by positive selection in sub-Saharan Africa, because variant APOL1s confer resistance to sleeping sickness, the *APOL1*-associated kidney disease risk genotype is common. From 12% to 15% of black individuals carry the risk genotype (*i.e.*, two *APOL1* kidney disease risk alleles). Fortunately, most individuals with the *APOL1* risk genotype do not develop kidney diseases, suggesting that a second stressor is needed to initiate disease. Genetic association studies have identified the only known environmental trigger: uncontrolled HIV infection. Without antiretroviral therapy, individuals with the *APOL1* risk genotype have a 50% lifetime risk of developing HIVAN.

In contrast, most blacks who are hypertensive with high-risk *APOL1* genotypes do not develop CKD, suggesting that high BP accelerates kidney disease progression but does not initiate kidney disease. The African American Study of Kidney Disease and Hypertension (AASK) cohort has been studied to characterize associations of *APOL1* variants with clinical phenotypes in subjects with CKD and high BP. At entry, the AASK participants had CKD (measured GFR between 20 and 65 ml/min per 1.73 m^2) attributed to hypertension and <2 g proteinuria. Prevalent proteinuria is more common at baseline in the AASK subjects with high-risk *APOL1* genotypes compared with those with low-risk *APOL1* genotypes (48% versus 25%; $P<0.001$) (2). In this issue of the *Clinical Journal of the American Society of Nephrology*, Chen *et al.* (3) asked if incident proteinuria differed in the AASK subjects with and without high-risk *APOL1* genotypes. The study included 480 of the original 1094 AASK participants: 362 with prevalent or unknown proteinuria at study entry and 252 without *APOL1* genotypes were excluded. In aggregate, 53% (n=254) of this study population developed proteinuria during a median follow-up of nearly 7 years. Incident proteinuria was more frequent in individuals with high-risk *APOL1* genotypes (56 of 82 participants) compared with those with the low-risk genotypes (198 of 398 participants; hazard ratio, 1.82; 95% confidence interval [95% CI], 1.35 to 2.46; $P<0.01$). Excluded subjects with prevalent proteinuria were younger, had a lower eGFR, and had a higher frequency of high-risk *APOL1* genotypes, suggesting that the excluded AASK subjects had more advanced or severe CKD than subjects included in this analysis. The effect size of *APOL1* high-risk genotypes on proteinuria may have been underestimated by an analytic design that required that these participants be excluded.

When all AASK participants with *APOL1* genotypes (n=693) were analyzed, those with high-risk genotypes more commonly reached the composite kidney outcome (an eGFR decline >50% from baseline or ESRD) (2). Chen *et al.* (3) also found a faster eGFR decline in subjects without proteinuria at study entry but with high- compared with low-risk *APOL1* genotypes (−1.65; 95% CI, −2.05 to −1.25 versus −1.11; 95% CI, −1.32 to −0.90; P interaction =0.01). Interestingly, the rate of eGFR decline before and after proteinuria onset was independent of *APOL1* genotype risk status. Therefore, the overall faster rate of eGFR decline in these AASK participants with *APOL1* high-risk genotypes reflects the increased frequency of proteinuria in this group compared with participants with low-risk genotypes. Previously, an association between high-risk *APOL1* genotypes and ESRD in an unadjusted analysis was lost after adjustment for proteinuria (4). Somewhat surprisingly, these data, in aggregate,

suggest proteinuria, after it is established, is the dominant risk for adverse kidney disease outcomes, regardless of *APOL1* risk genotype status.

How does the study of the AASK participants by Chen *et al.* (3) inform hypotheses about mechanisms of APOL1-associated kidney diseases? Proteinuria reflects glomerular filtration barrier dysfunction. Podocytes are key components of the glomerular filtration barrier, and APOL1 is abundantly expressed within podocytes of healthy kidney (5). Emerging data describing APOL1 effects on podocyte biology may explain the increased frequency of prevalent and incident proteinuria in blacks with high-risk *APOL1* genotypes. *APOL1* is a human-only gene, but several transgenic animal models have been published. Similar to humans, mice with podocyte-restricted expression of a reference or variant *APOL1* transgene lack markers of cell death or do not have overt kidney disease, even after 300 days of observation (6). Despite the absence of "clinical" kidney disease, mice expressing a variant APOL1 transgene have reduced podocyte density compared with age-matched reference APOL1 transgenic mice. As proposed by the podocyte depletion hypothesis, this reduced podocyte reserve may predispose to overt kidney disease development when a stress is superimposed (7). Similar results were published in zebrafish with podocyte-specific transgenic expression of a variant *APOL1* transgene (8). Podocytes had ultrastructural evidence of injury, but the fish had no overt kidney disease phenotypes. In contrast, mice with inducible reference or variant *APOL1* transgene expression and fruit flies expressing reference and variant *APOL1* transgenes showed variant-dependent podocyte death and filtration barrier dysfunction (9–11). Like the mice with constitutive expression of a variant *APOL1* transgene, the transgenic flies and mice with inducible, variant *APOL1* transgenes have fewer nephrocytes (podocyte-like cells in the fly) or podocytes, respectively. In aggregate, variant APOL1 seems to cause podocyte depletion by either cytotoxicity or alternatively, podocyte detachment (the mice with constitutive variant APOL1 expression had fewer podocytes but no cell death). Interestingly, soluble urokinase plasminogen receptor, a circulating biomarker that predicts kidney disease incidence and progression, interacts with variant but not reference APOL1 to activate a podocyte integrin adhesion molecule and cause podocyte detachment in culture and soluble urokinase plasminogen receptor–dependent proteinuria in mice (12).

Another common feature of these different animal models is the dysregulation of intracellular trafficking of membrane-bound vesicles. Marilyn Farquhar and colleagues noted 60 years ago the abundance of vesicles within the podocyte, a finding confirmed in subsequent studies of normal and diseased podocytes (13). Deletion of a protein regulator of vesicular trafficking resulted in an FSGS-like phenotype in mice, emphasizing the importance of trafficking pathways for podocyte homeostasis (14). Linking APOL1 function with vesicular trafficking, reference but not variant APOL1s interact with a vesicle-associated membrane protein (15). More work is needed to dissect the pathways responsible for podocyte depletion and identify better CKD treatments.

From human data, we know that APOL1 function is not required for normal filtration barrier function, that APOL1 is an innate immune effector protein, and that a "second hit" is required for kidney disease to ensue in subjects with the *APOL1* risk genotype, such as an uncontrolled HIV infection in HIVAN. Integrating these observations with the experimental data described above, we propose that APOL1 is not required for normal kidney function but rather, samples the ambient environment by interacting with vesicles trafficking though the podocyte. When a disease-causing stress is sensed, reference APOL1 detects and contains environmental danger signals carried by vesicles. Variant APOL1 proteins fail to do so, permitting glomerular filtration barrier dysfunction. Of course, APOL1-independent pathways may also lead to proteinuria, and as shown in the study by Chen *et al.* (3), progressive kidney function loss ensues no matter the patient's *APOL1* genotype.

What are the next steps to translate a groundbreaking discovery, the association of *APOL1* variants with non-diabetic kidney diseases, to the clinic? Certainly, better models are needed to define mechanisms of *APOL1*-dependent kidney diseases. Few, if any, studies have analyzed APOL1 function in cells or animals exposed to a stress. Exploiting the strong genetic association between HIV-associated kidney disease and the *APOL1* kidney disease risk variants is a plausible approach to implement. Hypertensive-associated kidney disease most likely represents a number of molecular mechanisms that generate similar clinical findings and kidney histopathology. To more effectively manage and treat this inexorable disease, we need kidney tissue to better characterize the APOL1-dependent and -independent molecular pathways responsible for hypertensive-associated kidney disease in blacks. The Kidney Precision Medicine Project provides an unprecedented opportunity to accomplish this goal (16). This study will use omics tools to produce molecular phenotypes from kidney biopsies and then integrate these data with histopathology phenotypes generated by computational methods and longitudinal clinical data abstracted from electronic health records. Population health is the latest mantra, often chanted uncritically, for innovative health care delivery models, but it will fail unless precision medicine defines the populations.

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