Apolipoprotein L1 and the Genetic Basis for Racial Disparity in Chronic Kidney Disease

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Persons of African ancestry living in the United States carry a disproportionate burden of chronic kidney disease (CKD). Compared with Americans of European ancestry, African Americans have an approximately fourfold greater lifetime risk of ESRD and, on average, require initiation of renal replacement therapy at a younger age. This group also has a unique risk of HIV-associated nephropathy (HIVAN). Unraveling the basis for these racial disparities in CKD offers opportunities to understand its pathogenesis, to identify biomarkers of risk, and to conceive of new treatments or preventive strategies.

Recent progress has been made in identifying genomic factors that explain the excessive chronic kidney disease (CKD) risk in nondiabetic African Americans. In 2008, two groups demonstrated highly significant associations of markers on human chromosome 22q with idiopathic focal segmental glomerulosclerosis (FSGS), HIV-associated nephropathy (HIVAN), and nondiabetic end-stage renal disease (ESRD) in African Americans.1,2 The strongest association was centered on genomic variants within MYH9 encoding a nonmuscle myosin heavy chain expressed in glomerular podocytes. Despite the attractiveness of MYH9 as a candidate to explain the association of 22q with CKD, further studies failed to identify plausible functional variants within this gene. In 2010, two groups reported an even stronger genetic association of FSGS in African Americans with variants in APOL1 encoding apolipoprotein L1.3,4 MYH9 and APOL1 are separated by a mere 14,000 nucleotides and coexist within a broader block of genomic sequence that exhibits features such as linkage disequilibrium, suggesting maintenance through evolutionary pressure. APOL1 variants associated with FSGS were not interrogated in the original genetic mapping studies because these variants were not found in databases available at that time through the international haplotype mapping (HapMap) project. Subsequently, data produced by the 1000 Genomes project annotated APOL1 variants, including a compound missense allele (glycine-342/methionine-384; designated G1) and an in-frame deletion (deletion of asparagine-388 and tyrosine-389; designated G2), which emerged as the critical markers for risk of disease within this interval. Moreover, G1 and G2 were mutually exclusive, never being observed together on the same chromosomal copy. These advances helped demonstrate that coding sequence variants within APOL1 accounted for a large fraction of observed FSGS risk in African Americans. However, demonstrating associations with other renal disease phenotypes was needed, as was clarity about the potential biologic contribution of APOL1 to pathogenesis of renal disease.

In this issue of JASN, five new studies addressing the genetics, clinical importance, and biology of APOL1-associated renal disease in African Americans will help advance our understanding of this problem.5–9 In two of the articles, investigators examined the association of APOL1 variants with HIVAN.5,6 In previous studies, risk of HIVAN in African Americans was associated with the aforementioned MYH9 variants, but in light of the new findings implicating APOL1 coding alleles as the functional variants responsible for FSGS risk, it became important to determine if HIVAN risk was genetically similar or whether MYH9 alleles were still relevant. Kopp et al. report results of a case-control genetic association study examining the importance of APOL1 variants (G1, G2) to HIVAN risk by comparing allele and genotype frequencies in African Americans with biopsy-proven HIV-associated collapsing glomerulopathy compared with HIV-infected African Americans with normal renal function. The investigators demonstrated an astonishing odds ratio (OR) of 29 for HIVAN risk, conferred by two APOL1 risk alleles. They also found that two APOL1 risk alleles confer a similarly impressive OR of 16.9 for idiopathic FSGS, as well as an earlier age of onset and more rapid progression toward ESRD. HIVAN and FSGS risk confounded best to a recessive inheritance model, while cases heterozygous for only one APOL1 risk allele had marginal or no association with kidney disease. Papeta et al. report qualitatively similar findings for recessive genetic association models in a smaller HIVAN case-control study (OR = 10.9), whereas genetic association determined using an additive genetic effect model was also significant but less robust. The study by Pa-
peta et al. also demonstrates that APOL1 variants are not associated with IgA nephropathy.

Together, these studies provide compelling evidence that APOL1 confers genetic risk for HIVAN in African Americans. Further evidence against a significant biologic contribution of MYH9 is provided by the study by Papeta et al., with demonstration of absent nephropathy in the offspring of mice generated by crossing HIV-1 transgenic mice, in a genetic background protective against nephropathy, with Myh9 hemizygous mice. Haploinsufficiency for Myh9 did not promote overt albuminuria orglomerular histopathology, and therefore the plausibility that this gene is the biologic culprit for HIVAN seems less likely. However, Myh9 might contribute to other forms of CKD, as suggested by increased susceptibility to doxorubicin glomerulopathy in podocyte-specific Myh9 knockout mice.

Although case-control association studies have become the foundation for new discoveries in the modern genomic era, such studies have important liabilities that can skew results, including ascertainment bias and mismatched control subjects. To thwart this concern for the recent discoveries regarding APOL1, Friedman and colleagues examined whether variants in this gene associate with two proxies of CKD, microalbuminuria and reduced estimated GFR, in the Dallas Heart Study, a large population-based cohort with a predominance of African American participants. Consistent with the prior case-control association studies, nondiabetic carriers of two APOL1 variants had approximately 3 times higher rates of microalbuminuria and reduced GFR (≤60 ml/min/1.73 m²) than subjects with 0 to 1 variant alleles. In further analyses, rates of microalbuminuria and reduced GFR were not different between nondiabetic African Americans with 0 to 1 APOL1 risk alleles and nondiabetic subjects of European ancestry. The latter observation provides an important perspective on the relative level of CKD risk among genotype-defined groups. Future longitudinal studies using this population could be very useful for determining the predictive value of APOL1 genotype in assessing ESRD risk, which is essential information for exploiting these findings in clinical practice.

Given the population allele frequency of APOL1 variants, the number of African Americans who carry two copies of the risk alleles likely exceeds three million. The public health impact of this large population of potentially at-risk individuals is magnified further by the observed accelerated progression to ESRD. To further quantify the evidence for earlier onset ESRD in APOL1 variant carriers, Kanji et al.⁹ investigated the age at hemodialysis initiation for nondiabetic African Americans with ESRD participating in an observational cohort study, the Accelerated Mortality on Renal Replacement (ArMORR) study. The data indicate that subjects who carried 1 to 2 APOL1 risk alleles exhibited a significantly younger age at initiation of hemodialysis compared with noncarriers. Specifically, carriers of 1 or 2 APOL1 G1 alleles initiated dialysis 6 and >10 yr earlier, respectively, than noncarriers. Findings for the less frequent G2 allele were not conclusive. The effect observed for single allele carriers differs from the other studies that implicated recessive genetic mechanisms in renal disease susceptibility, and this may imply that the rate of progression to ESRD can be independently influenced by APOL1 variants.

The high carrier frequency of APOL1 renal disease risk alleles in African Americans has evolutionary origins by way of natural selection for a trait protective against infection with subspecies of the protozoan parasite Trypanosoma brucei that causes sleeping sickness endemic to sub-Saharan Africa. Apolipoprotein L1 lyses trypanosomes by causing osmotic swelling of parasite lysosomes through a pore-mediated mechanism and renders humans resistant to infection. However, one trypanosome subspecies responsible for African sleeping sickness (T. b. rhodesiense) produces a virulence factor—serum resistance associated-factor (SRA)—that neutralizes APOL1 by binding to its C-terminus. However, both APOL1 risk variants (G1, G2) alter amino acid residues directly within the C-terminal SRA binding site thus preserving lytic activity and conferring T. b. rhodesiense resistance to heterozygous carriers. This heterozygote advantage is reminiscent of malaria resistance conferred by β-hemoglobin mutations in sickle cell disease. In both situations, the selective advantage of parasite resistance bestowed on heterozygous carriers creates disease-prone homozygous carriers in the population. For perspective, the allele frequency of hemoglobin-S among African Americans is 5 to 10%, and this is dwarfed by the combined allele frequency of APOL1 G1 and G2 risk alleles (37% in the Dallas Heart Study⁸), but sickle cell disease in homozygous hemoglobin-S carriers exhibits complete penetrance.

How do APOL1 variants predispose to CKD? Renal lesions associated with APOL1 risk alleles, including FSGS and HIVAN, are characterized by glomerular podocyte dysfunction. Rare Mendelian forms of FSGS and congenital nephrotic syndrome have been associated with mutations in several genes encoding podocyte-expressed proteins. It is likely that predisposition of CKD associated with APOL1 variants is recessive, and it is plausible that human APOL1 may contribute to glomerular structure and/or function, with injury being the result of loss of function.

As a first step in determining the function of APOL1 in the kidney and glomerulus, Madhavan and colleagues provide the evidence for intrarenal APOL1 protein expression. Similarly to many other genes linked to FSGS, these investigators demonstrate that APOL1 is expressed in podocytes. Additionally, they found APOL1 expression in proximal tubular cells, which may be relevant to the tubulointerstitial injury that is prominent in HIVAN. In biopsies from patients with HIVAN or FSGS, podocyte and tubular expression of APOL1 is reduced and de novo expression is observed in renal arterioles, most likely in vascular smooth muscle cells. The latter finding is intriguing, given the association of arteriolar lesions with ESRD in African Americans due to FSGS. Future studies are needed to validate these findings and to determine whether renal APOL1 expression varies by genotype.

The mechanism by which APOL1 variants predispose to
FSGS and HIVAN is not known. Additional experiments are needed to determine if circulating and/or intracellular APOL1 is important for renal function and how variant forms might contribute to kidney dysfunction. A higher incidence of transplanted kidney graft failure occurring in organs procured from donors carrying two APOL1 risk alleles suggests that intrinsic renal expression may be important. The protein consists of specific functional domains, including a pore-forming domain in the N-terminal region, C-terminal SRA interaction domain, as discussed above, and a membrane-addressing domain. In addition to trypanolytic membrane pore-forming activity, APOL1 may contribute to lipid metabolism, vascular function, and autophagy. The latter function is intriguing in view of recent data demonstrating the importance of autophagy and autophagic flux for maintaining podocyte health.

Animal models could also be valuable for determining disease mechanisms, but the options are limited, as only humans and some non-human primates express APOL1 natively, thus precluding the possibility of employing knockout mouse or rat models. Transgenic mice overexpressing wild-type human APOL1 have helped determine the biology of trypanosome resistance, and together with transgenic animals carrying specific APOL1 risk alleles, might provide one approach to elucidate mechanisms responsible for renal injury. Investigating mechanisms of susceptibility in APOL1-associated renal disease should also consider potential triggering mechanisms or whether variant alleles represent intrinsic time bombs. The robust associations of APOL1 with HIVAN reported in this issue of JASN suggest that viral infection or inflammation might provide local or systemic triggers that potentiate glomerular injury in patients carrying two risk-associated variants. A wealth of new information in this issue should stimulate the next wave of discoveries.

DISCLOSURES
None.

REFERENCES


The Aging Kidney Phenotype and Systemically Derived Stem Cells

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The desire to extend lifespan and health has occupied the scientific and lay communities during most of recorded history. This quest includes the search for some magical elixir: Ponce de Leon vainly sought the legendary Fountain of Youth in Florida, and charlatans continue to sell potions that promise longevity. Another tack in legends, novels, and history is the belief that youth could be maintained by taking a young partner, in hopes that living tissues or cells could transfer youth. The downside of this was the fear that having an aged partner can drain youth. These two approaches to aging persevere in modern biology.

Fortunately scientists have taken up this challenge using modern ideas and methods.1 The source of humoral factors or cells that might be involved in either the repair of renal injury or the normal replacement of parenchymal cells is now a topic of considerable research and debate.2–13 In part, the debates may result from the fact that the models chosen to examine the questions have been quite diverse, namely organ development, responses to acute injury, or those related to the more orderly process of cell turnover as a part of normal postnatal aging. Because each of these processes may excite unique and time-dependent responses, it is important to focus experiments and their interpretation quite clearly.

Humoral factors affect tissue repair in both aging and acute kidney injury. A series of parabiotic experiments involving aged and young mice provided evidence that some humoral factors were passed between the young and old mouse that restored skeletal muscle satellite cells, a skeletal muscle progenitor cell, and some of the phenotypic changes of aging in the aged mouse toward normal values.14 Although the humoral substances could have been derived from the young donor and passed to the aging recipient, it remains possible that the young partner was also able to handle some toxic metabolite that accumulated in aging. Candidates for such substances include advanced glycation end products, because they accumulate in aging and result in phenotypic changes resulting from depletion of cellular antioxidant mechanisms.15,16

The concept that soluble factors are important in tissue regeneration is also extended by the observation that diffusible factors derived from macrophages in ischemia-perfusion injury foster regeneration of tubular cells.6 Thus, phenotypic changes in parenchymal cells with aging could be influenced by changes in distant organs or in the behavior of migrating cells.

The source of cells that might be involved in either normal turnover of kidney parenchymal cells or their response to injury has been recently reviewed.7 The experiments from the laboratory of Fogo and colleagues17 in this issue of JASN extend previous studies showing both that the aging phenotype to young mice could be transferred by bone marrow transplantation (BMT) and that at least some of the glomerular changes in aging could be reduced.18 The current study addresses this problem by using male donors and examining recipient kidneys for the Y chromosome.17 Although some previous studies did not include lineage tracing, mesangial cells isolated from the glomeruli of young recipients of marrow transplants from old mice had a similar phenotype to mesangial cells isolated from aging mice.18 More convincing evidence of the transfer of a specific sclerosis-prone phenotype and genotype was provided in earlier studies from the same laboratory, in which the glomerular phenotype depended on the genotype delivered with transplanted bone marrow and the number of cells with that phenotype that repopulated the glomeruli.19 These studies were extended to another form of glomerulosclerosis, diabetic nephropathy, in which the sclerotic lesion, but not the hyperglycemia, was transferred to the recipient by BMT.20 Because mesangial cells isolated from the recipients expressed both an altered phenotype and genotype and glycermia remained normal, this report additionally suggested that not all organs were repopulated by BMT. In summary, repopulation of the glomeruli and transfer of a disease phenotype by BMT have been shown in two different models of glomerulosclerosis, in normal aging, and in a model of IgA nephropathy.21

The current study uses 129SvJ mice and examines a number of phenotypic changes that had not been previously studied,