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The Apolipoprotein L1 (*APOL1*) Gene and Nondiabetic Nephropathy in African Americans

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ABSTRACT

Mapping by admixture linkage disequilibrium (LD) detected strong association between nonmuscle myosin heavy chain 9 gene (*MYH9*) variants on chromosome 22 and nondiabetic nephropathy in African Americans. *MYH9*-related variants were posited to be the probable, but not necessarily the definitive, causal variants as a result of impressive statistical evidence of association, renal expression, and a role in autosomal dominant *MYH9* disorders characterized by progressive glomerulosclerosis (Epstein and Fechtner syndromes). Dense mapping within *MYH9* revealed striking LD patterns and racial variation in risk allele frequencies, suggesting population genetic factors such as selection may be operative in this region. Genovese and colleagues examined large chromosomal regions adjacent to *MYH9* using genome-wide association methods and non-HapMap single nucleotide polymorphisms identified in Yoruba from the 1000 Genomes project. Statistically stronger associations were detected between two independent sequence variants in the Apolipoprotein L1 gene (*APOL1*) and nondiabetic nephropathy in African Americans, with odds ratios of 10.5 in idiopathic FSGS and 7.3 in hypertension-attributed ESRD. These kidney disease risk variants likely rose to high frequency in Africa because they confer resistance to trypanosomal infection and protect from African sleeping sickness. Risk variants in *MYH9* and *APOL1* are in strong LD, and the genetic risk that was previously attributed to *MYH9* may reside, in part or in whole, in *APOL1*, although more complex models of risk cannot be excluded. This association likely explains racial disparities in nondiabetic nephropathy as a result of the high prevalence of risk alleles in individuals of African ancestry.

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Polymorphisms in the nonmuscle myosin heavy chain 9 (*MYH9*) gene exhibit strong association with nondiabetic chronic kidney disease (CKD) in African Americans. The increased risk attributable to this genomic region is among the highest observed in any complex genetic trait.[1–4] The odds ratio (OR) for *MYH9* E1 risk haplotype associations in idiopathic FSGS and HIV-associated collapsing glomerulopathy (also termed HIV-associated nephropathy) ranges from 5 in African Americans to 8 in European Americans with statistical evidence reaching $P < 1.0 \times 10^{-27}$.

Evidence for association on chromosome 22q was detected using admixture mapping (mapping by admixture linkage disequilibrium [LD]) followed by fine mapping.[5] *MYH9*, expressed in the kidney and previously associated with glomerulosclerosis in patients with autosomal dominant *MYH9* disorders,[6] was rapidly identified as potentially responsible in independent reports from two investigative groups (National Institute of Diabetes and Digestive and Kidney Diseases [NIDDK] and National Cancer Institute) and the NIDDK-supported Family Investigation of Nephropathy and Diabetes [FIND].[1,2] Marked association was also demonstrated in African Amer-

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icans with ESRD clinically attributed to hypertension in the absence of a kidney biopsy, thereby linking hypertension-attributed ESRD (HA-ESRD) with FSGS and focal global glomerulosclerosis (FGGS).[3,4] Furthermore, preliminary analysis of the African American Study of Kidney Disease and Hypertension (AASK) indicated that propensity to lose kidney function over time is associated with *MYH9* risk variants.[7]

The consistent evidence of association provided an explanation for the long-observed excess risk for ESRD in individuals of African ancestry relative to European ancestry. Much has transpired in the short time since the *MYH9* association with CKD was first detected. Everyone has been awaiting identification of causative polymorphisms and demonstration that these affect renal function and histology in animal and cell culture models. Dense mapping and sequencing of *MYH9* suggested the strongest associations with CKD are with single nucleotide polymorphisms (SNPs) in the 3′ (centromeric) portion of the gene but have not identified functional variants.[8] The global allele frequency patterns for *MYH9* risk alleles and the extended haplotype homozygosity in West Africans suggest that the region encompassing *MYH9* may be under selective pressure.[9]

Recently Genovese *et al.*[10] observed that genetic variation in the *APOL1* gene, located immediately centromeric of *MYH9*, is also strongly associated with nondiabetic CKD, and they presented evidence that the statistical association is stronger than that of *MYH9*. They suggested these coding variants are in fact causally related to kidney disease and provided an explanation for selection of *APOL1* kidney disease risk polymorphisms as protective from an infectious disease common in Africa.

APOL1 ASSOCIATION WITH NONDIABETIC NEPHROPATHY IN AFRICAN AMERICANS

Apolipoprotein L1, located <20 kb from the 3′ end of *MYH9*, is a member of a gene family located on chromosome 22q. *APOL1* is the only member in this family that produces a secreted protein, a product known to be associated with HDL particles in the circulation.[11]

An association analysis comparing 205 African Americans who had biopsy-proven FSGS with 180 African American control subjects without nephropathy was performed using novel SNPs identified in Yoruba participating in the 1000 Genomes project.[10] These SNPs reveal the strongest evidence of association within a 10-kb region in the last exon of *APOL1*, not in *MYH9*. The most robust signal in this small sample includes a two-SNP haplotype consisting of derived nonsynonymous coding variants rs73885319 (S342G) and rs60910145 (I384M) in perfect LD ($r^2 = 1.0$), termed the "G1" allele. The frequency of the 342G:384M G1 allele was 52% in case patients and 18% in control subjects. After controlling for effects of the G1 allele, a second strong *APOL1* signal was identified, a 6-bp deletion termed "G2" (rs71785313), which removes two amino acid residues. Because of the low likelihood of recombination as a consequence of the close proximity of these alleles and the recent origin of these mutations, G2 seems mutually exclusive from G1; that is, they never appear together on the same chromosome. The frequency of the G2 allele is 23% in case patients and 15% in control subjects. The OR for association with biopsy-proven FSGS, contrasting patients with zero or one G1-G2 risk alleles with those who have two risk alleles (recessive effect), was 10.5 (95% confidence interval [CI] 6.0 to 18.4). Controlling for the effects of both the G1 and G2 alleles by statistical techniques, no additional significant associations were observed, including in *MYH9*. Conversely, after controlling for multiple *MYH9* variants, the *APOL1* signal remained significant. Thus, statistically, the *APOL1* variations are the more plausible and parsimonious explanation for the association. Conversely, alternative analytic approaches in a larger FSGS cohort indicated a modest but independent association with FSGS susceptibility for variants in *MYH9* (C.A.W., G.W.N., J.B.K., unpublished observations), which could be due to rare and as-yet-undiscovered variation in this region of chromosome 22.

These findings were extended to HA-ESRD, including 1030 African American patients with HA-ESRD and 1025 geographically matched control subjects from the southeastern United States.[10] This patient group again reveals the strongest disease association with the G1 tag SNP rs73885319 (S342G; $P = 1.1 \times 10^{-39}$); controlling for rs73885319, G2 indel rs71785313 was also strongly associated ($P = 8.8 \times 10^{-18}$). The OR for the G1-G2 association with HA-ESRD was 7.3 (95% CI 5.6 to 9.5), with a combined G1-G2 $P = 10^{-63}$. This *P* value is 35 orders of magnitude stronger than for *MYH9* in the same study population, with no overlap in OR (*e.g.*, OR 2.38; 95% CI 1.93 to 2.95; $P = 1.22 \times 10^{-15}$ recessive, for the *MYH9* E1 haplotype). As for FSGS, controlling for the G1 and G2 alleles revealed no residual associations in patients with HA-ESRD.

The observed mode of inheritance of the *APOL1* kidney risk variants was fully recessive in the FSGS cohort (no effect for one risk allele and an OR 10.5 for two risk alleles), whereas a mild dominant effect was observed in the larger cohort with HA-ESRD (OR 1.26 for one risk allele and OR 7.3 for two risk alleles). Although a mild dominant effect cannot be fully excluded, it is entirely possible that such an effect could be explained by as-yet-undiscovered variants or by sporadic mutations in patients with the recessive model.

Importantly, these findings do not exclude the possibility that additional rare variants in *APOL1*, *MYH9*, or nearby genes are involved in kidney disease susceptibility. Extended LD exists in this region as a result of selective forces and likely explains previous reports of association within *MYH9*; the *MYH9* E1 haplotype was present in 89% of *APOL1* G1 haplotypes and 76% of G2 haplotypes. The strong LD pattern in this region confounds the identification of causal variants using purely statistical approaches, and functional and genetic approaches will be required to determine definitively the risk variants across the chromosome 22q region.

SELECTION FOR *APOL1* NEPHROPATHY RISK VARIANTS

It has been postulated that harboring nephropathy risk variants in *MYH9* and/or *APOL1* protects from another disease; an effect necessary to explain the high frequencies of nephropathy risk variants in the general African American population.[9] G1 and/or G2 *APOL1* risk variants were present in >30% of African American chromosomes in the report by Genovese *et al.*,[10] approximately half of the 60% frequency for the *MYH9* E1 risk haplotype previously reported in this racial group.[1] These data for *APOL1* result in approximately 10 to 12% of African Americans carrying two alleles and being at risk, compared with approximately 36% of African Americans carrying two copies of the *MYH9* E1 haplotype; the effect of *APOL1* seems to be approximately threefold greater than the previously reported effect of *MYH9* in terms of risk for nephropathy.[12] Hence, approximately 12% of HIV-negative and >50% of HIV-positive African American *APOL1* G1/G2 homozygotes or compound heterozygotes may be susceptible to kidney disease. The predictive value of these strongly associated *APOL1* alleles on nephropathy risk will need to be clarified before consideration of population genetic screening.

Why were these *APOL1* mutations maintained in African populations? The most likely explanation is that in the battle between host and pathogen, the *APOL1* mutations provided selective advantage to carriers, at the cost of increased risk for kidney disease. *Trypanosoma brucei brucei* infects many mammals but is unable to infect humans because human serum contains a complex, trypanosome lytic factor (TLF), comprising apolipoprotein L-1, haptoglobin-related protein, and apoliprotein A-1 that confers innate protection against *Trypanosoma brucei brucei*. ApoL1 leads to formation of anionic pores in the parasite's lysosomal membrane, resulting in lysosomal swelling and death of the organism. In concert with hemoglobin, haptoglobin-related protein acts as a co-factor promoting binding of the trypanolytic ApoL1 on the trypanosome surface and facilitating internalization of the lytic factor. Both *Trypanosoma brucei rhodesiense* and *gambiense* have evolved a mechanism to evade lysis by TLF; the pathogen-encoded serum resistance–associated protein abrogates lytic activity by TLF, leading to infection and sleeping sickness.[11]

Genovese *et al.*[10] demonstrated a trypanolytic effect with serum from carriers of *APOL1* nephropathy risk mutations, compared with absence of trypanosomal killing with serum from individuals lacking *APOL1* nephropathy risk variants. Recombinant ApoL1 proteins with or without these variants showed a similar difference in trypanolytic effect. Both G1 and G2 restore the lytic activity of human serum, providing a likely selective advantage to carriers against sleeping sickness as a result of *Trypanosoma brucei rhodesiense* but not *gambiense*. These findings, together with evidence of recent evolution of *APOL1* occurring in the past 10,000 years, suggest these variants were selected for within Africa because they conferred protection against potentially lethal trypanosomal disease but at a cost of increased susceptibility to glomerular injury.

This story of protection from trypanosomal infection by *ApoL1* variants is remarkably similar to the protection from malaria by *HgbS*.[13] One curious difference between the two genes, however, is that whereas heterozygous carriers of *HgbS* (approximately one in 12 African American births) are surprisingly prevalent among African Americans with renal failure,[14] heterozygous carriers of *APOL1* risk alleles (approximately one in two African American births), unlike the recessive genotype, are at less risk for nondiabetic nephropathy.

POTENTIAL EFFECTS OF *APOL1* VARIANTS ON THE KIDNEY

Northern blot analysis revealed mRNA encoding ApoL1 strongly expresses in the placenta, lung, and liver and more weakly in heart and pancreas.[15] *In situ* hybridization of human vascular tissue also revealed ApoL1 expression in endothelial cells and possibly macrophages.[15] Importantly, mRNA encoding ApoL1 and protein are expressed in cultured human podocytes (J.B.K., unpublished observations). Future studies should delineate whether circulating ApoL1, kidney cell–expressed ApoL1, particularly expressed by the podocyte, or both contribute susceptibility to kidney disease.

Nothing is known about the mechanisms by which ApoL1 variants induce kidney injury, but several possibilities can be imagined. First, ApoL1 variants expressing in the podocyte or another renal cell may cause injury or fail to support a critical cellular function. ApoLs share structural and functional similarities with proteins in the Bcl2 family of proteins, which are involved in apoptosis.[11] Possible toxic renal effects include roles for ApoL1 in triggering programmed cell death with resultant glomerulosclerosis. ApoL1 is also important in autophagy.[16] Mice deficient in a critical autophagy protein are more susceptible to glomerular injury, which may well contribute to human CKD associated with this locus.[17] Second, circulating ApoL1 could be important in the pathogenesis of CKD. Dysfunctional HDL particle formation may result from inheriting G1 and G2 variants, potentially leading to inflammation in the renal vascular endothelium with arteriolar nephrosclerosis. This vascular change frequently accompanies both FSGS and FGGS in African Americans without diabetes. Alternatively, free ApoL1 may be present in the plasma, although this remains to be demonstrated. Thus, ApoL1 may become available for uptake by podocytes after passage across the glomerular filtration barrier. The mechanisms that underlie ApoL1-induced renal injury are of considerable interest. The role of polymorphisms in the adjacent *MYH9* gene and other genes in this region should also be explored, with an eye toward the possibility that *MYH9*–*APOL1* gene interactions exist.

CONCLUSIONS

Susceptibility for several forms of idiopathic glomerulosclerosis, including FSGS,

collapsing glomerulopathy, and FGGS, have been linked to genetic variation in a region of chromosome 22q. The previous designation of "*MYH9*-associated nephropathy" requires revision.[4,12] It now seems that these kidney diseases are more strongly associated with polymorphisms in the *APOL1* gene on chromosome 22, an observation replicated by Tzur *et al.*[18]

With the evidence of *APOL1* association with nondiabetic forms of CKD and the role of selection bringing nephropathy risk variants to substantial frequency in African Americans, it will be necessary to move beyond statistical tests of association to cellular and mouse models for evaluating effects of gene variants. These models should determine the potential role of ApoL1 in the pathogenesis of the podocyte depletion common in FSGS and podocyte proliferation seen in idiopathic and HIV-associated collapsing glomerulopathy. Although *MYH9* may ultimately prove not to be the proximate cause of FSGS and related diseases or may interact with *APOL1* and other nearby gene variants to initiate renal disease, it remains clear the disease process labeled HA-ESRD in many cases represents a primary inherited renal process within the spectrum of glomerulosclerosis. The strong link among FSGS, collapsing glomerulopathy, FGGS, and HA-ESRD requires a search for unifying disease mechanisms that lead to these diverse renal histologic phenotypes. Finally, the *APOL1* gene is found only in humans and certain non-human primates and is lacking in rodents. Although transgenic mouse models may be informative, it may also be fruitful to study nonhuman primates, as well as clinical studies, to define the pathophysiology of this important pathway of nephropathy susceptibility.

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DISCLOSURES

NIH has applied for a patent on the clinical use of *APOL1* and *MYH9* genetic testing for kidney disease susceptibility testing, with co-inventors C.A.W., G.W.N., J.B.K., and M.R.P.

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