A compelling genetic basis for the excess burden of nondiabetic kidney disease in blacks has been provided. APOL1 kidney risk variants account for approximately 70% of the African ancestry disparity in the progression of a rather wide spectrum of etiologies of nondiabetic CKD. The discovery was enabled by combining population genetics and evolutionary medicine approaches, which have been the subject of several recent reviews (1–3). The odds ratios (ORs) for increased risk of progressive kidney injury conferred by these variants (ranging from OR, 7 to OR, >80 depending on underlying etiology) compared with those previously described in other population–based disease risk association studies (typically ranging from OR, 1.1 to OR, 2.0) places the APOL1 kidney disease risk variants among the highest ever reported for a common variant associated with a common disease.

Consistent high–level APOL1 risk variant association has been conspicuously absent for diabetic kidney disease (1), save for some studies in which it was difficult to exclude nondiabetic etiologies among subjects with concomitant diabetes and kidney disease enrolled without pathologic validation of diabetic kidney disease (4,5).

Therefore, the underpinnings of increased risk for diabetic kidney disease in blacks remain an enigmatic health disparity with resolution that presents a challenging research imperative. Genome–wide association studies (GWASs) as well as candidate gene approaches to study diabetic nephropathy more generally have identified several genomic regions of possible interest (6). A relevant area of inquiry has included identification of possible genetic risk loci for albuminuria (7,8). Such loci should be mechanistically revealing to the understanding of CKD progression, because albuminuria reflects damage to glomerular macromolecular ultrafiltration and/or dysfunctional proximal tubule processing of albumin with consequent abnormalities in downstream vasoactive and fibrotic pathways (9) The reported heritability of albuminuria in the general population and in patients with diabetes ranges from 0.2 to 0.46 (8). Albuminuria is the most frequently assessed marker of kidney damage measured in clinical practice, and it has been associated in a meta-analyses of approximately 1.5 million individuals from multiple cohorts with all-cause mortality, cardiovascular mortality, and ESRD (10,11). Boger et al. (7) reported the statistical association of a common cubulin (CUBN) missense variation (rs1801239; I2984V) with both an elevated urine albumin excretion ratio (P<0.001) and albuminuria (P=0.001) in population sample sets including diabetic and nondiabetic subjects. Although the CUBN variant association was statistically significant after appropriate false discovery rate corrections in this meta-analysis comprising 63,153 individuals of European ancestry and 6981 African ancestry individuals, the identified variants only accounted for a fraction of heritability. Tzur et al. (12) reported a unique extended haplotype structure at the CUBN locus and suggested that population ancestry–based haplotype structure could be leveraged to identify causative variants predicting albuminuria–related diabetic kidney disease risks in African ancestry individuals.

In this issue of the Clinical Journal of the American Society of Nephrology, Ma et al. (13) took advantage of next generation exome sequencing at both the CUBN and the megalin (LRP2)–encoding gene loci. CUBN encodes a large 460-kD glycosylated extracellular protein that interacts with other membrane proteins involved in endocytosis. In the proximal tubule, CUBN interacts with megalin, forming a multireceptor complex. Megalin is a 600-kD transmembrane protein initially identified as the autoantigen in Heymann nephritis, the classic historical experimental model for membranous nephropathy. Amnionless is responsible for trafficking of CUBN to the membrane. The multiligand endocytic receptor CUBN interacting with megalin has been identified as essential in the process of kidney proximal tubular uptake of filtered proteins (reviewed in ref. 14). Mendelian recessive loss of function mutations in either CUBN or AMN cause Megaloblastic Anemia 1 (Online Mendelian Inheritance in Man no. 261100) combining proteinuria and B12 deficiency, whereas rare recessive loss of function mutations in LRP2 cause severe multisystem syndromes, which include proteinuria. Reabsorption at these sites is responsible for recovery of essential components but also, clears other biologically active substances from the ultrafiltrate so that more distal kidney tubular segments are not exposed to these substances. The relationship between genes with complete loss of function that results in such high–penetration rare Mendelian inheritance disorders and the association of common variants at these same loci with corresponding common disease phenotypes is never predictable but worthy of exploration.

Beyond APOL1: Genetic Inroads into Understanding Population Disparities in Diabetic Kidney Disease

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Ma et al. (13) used multiple well defined comparative cohorts to focus on associations in black population sets, including patients with diabetic and nondiabetic kidney disease, patients with diabetes without kidney disease, and controls without diabetes and without kidney disease. The study examined association with ESRD in contrast to the prior work, which examined association with albuminuria. By using both a discovery and validation approach as well as multiple sample sets, a number of novel inferences of both clinical and mechanistic importance emerged. The same CUBN variant, rs1801239, encoding an amino acid I2984V substitution, which was previously associated with albuminuria in the mostly European GWAS, was associated with type 2 diabetic ESRD in black cohorts. Furthermore, a newly identified LRP2 missense variant, rs17848169, encoding amino acid change N2632D was associated with protection from type 2 diabetes–associated ESRD. These variants were not significantly associated with either nondiabetic forms of ESRD or type 2 diabetes mellitus without kidney disease by trait discrimination analysis. These findings could potentially point to otherwise elusive functional variants that promote or prevent from diabetic nephropathy.

An examination of the Yoruba population (YRI) 1000 Genomes database and other YRI–containing sample sets shows that the occurrence of the missense risk variant at rs1801239 (the first index at the CUBN locus) appears on a derived low–frequency European CUBN haplotype (12). This European haplotype may represent a region of extended linkage disequilibrium, conceivably reflecting the effect of positive selective pressure under vitamin B12 or other nutritional influences during human evolution. Ma et al. (13) speculate that the I2984V mutation encoded by rs1801239 in CUBN (as well as N2632D) may interfere with the binding of megalin to CUBN essential for albumin reabsorption. However, because the YRI allele frequencies and haplotype structure suggest a possible European origin for this variant in the black admixed population, it would be useful to conduct association studies using a suite of variants in continental African cohorts (in whom this European origin haplotype is not present), wherein the association with albuminuria can be examined for each variant to identify the causative mutation. This might be the most productive way of resolving a nonfunctional tagging variant from the causative variant followed by biologic proof of causation.

Laudably, Ma et al. (13) present data on rare variants in the manuscript. Rare variants may contribute to missing heritability in common diseases according to one of several disease models (15). These include the nearly intractable infinitesimal contribution of thousands of variants across the genome, broad sense heritability taking into account nongenetic modifiers, and the synthetic association of rare phylogenetically related alleles descended from a major causative variant (16). The findings in Ma et al. (13) strongly motivate designing such an approach with the added power of population substructure to achieve a most important goal.

This is further motivated by the findings in a recent GWAS meta–analysis of 20 studies involving 54,450 European ancestry participants without or with diabetes (8). The association of the CUBN locus with albuminuria was replicated using an index intronic single–nucleotide polymorphism (rs10795433) in presumed high linkage disequilibrium with a causative variant (8). In a discovery GWAS, seven additional loci (HS6ST1, CNTN4, KBTBD8, TFAP2B/PKHD1, CHN2, WDR11/FGFR2, and RAB38/CTSC) containing single–nucleotide polymorphisms in association with albuminuria were identified; of these, RAB38/CTSC and HS6ST1 were examined, and the association was validated in a replication analysis comprising nine studies and 1962 European ancestry individuals with diabetes (8). In these investigations, the authors were also able to show increased albumin excretion in a model of streptozotocin-induced diabetes with RAB38 knockout animals (8). Immunohistochemistry studies showed a decrease of CUBN and megalin consistent with the role of RAB38 in regulating CUBN and megalin at the cell surface. RAB38 is a member of the small RabGTPase protein family that regulates intracellular vesicle trafficking between organelles. With the well characterized cohorts available as described in Ma et al. (13), it should be straightforward to determine the extent to which allele frequency differences in variants at the loci described in Teumer et al. (8) also contribute to the black disparity in diabetic kidney disease and leverage differences in haplotype structure at each locus to infer causative variants.

In terms of pathophysiology, both direct glomerular and indirect tubule–mediated pathomechanisms need to be considered. The glomerular and tubular compartments are embryologically and evolutionarily separated; the renal vesicle develops into the glomerulus, whereas the mesonephric mesenchyme and ureteral bud form the kidney tubules (17). In nonchordates, they are not in direct functional communication. The functions of CUBN and megalin have been best characterized in the tubule compartment, although expression in the filtering structures of insects (18) and expression in human glomerular cells have been described (19,20). Albumin transcellular transport across the podocyte, transcytosis, has recently been shown to be induced by angiotensin II (21). Transcytosis was markedly reduced by gentamicin, an inhibitor of megalin-dependent endocytosis. Functional loss of function variants at gene loci that modulate this pathway of proteinuria may be expected to contribute to glomerular injury and disease. A genetic study cannot distinguish effects mediated at the glomerulus from the functional effect of proximal tubule events on glomerular hemodynamics that also govern albumin permeselectivity. Although the focus of APOL1 functionality is thought to be the glomerular podocyte, a role for APOL1 in kidney disease emanating from the proximal tubule has not been totally excluded. In a recent important study, an intertwining of inhibiting tubular reabsorptive activity with the novel antidiabetic sodium glucose cotransporter 2 inhibitor agent (empagliflozin) on glomerular hemodynamics and progression of diabetic nephropathy is proving to be a game changer (22). Therefore, for any genetic variant encoding a protein with either glomerular and/or proximal tubule expression, it is legitimate and mandatory to consider direct glomerular and indirect tubule feedback mechanisms in considering the interpretation of population– or family–based disease gene discovery findings, such as in the study by Ma et al. (13).

Finally, the interpretation and significance of family– or population–based disease gene discovery on the basis of rare alleles, low ORs, or small effect sizes should not be underestimated. The potential therapeutic importance of genetic loci with such attributes has been shown dramatically for HMGCGR (23). A common variant with an allele frequency of 40% changes LDL by a modest 2.8 mg/dl.
The encoded protein is the target of the statins, drugs that have pronounced LDL effects, can reduce myocardial infarction, and are taken by millions of patients (24). So too, the CUBN and LRP2 loci may have potential as drug targets of therapeutic importance, even with modest ORs and risk allele frequencies. The significance of the statistical associations may also increase when the causation is unrelaxed and epistatic and gene by environment interactions are taken into account.

Disclosures
None.

References


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See related article, “Association Analysis of the Cubilin (CUBN) and Megalin (LRP2) Genes with ESRD in African Americans,” on pages 1034–1043.