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The new era of *APOL1*-associated glomerulosclerosis

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The association between apolipoprotein L1 gene (*APOL1*) variants and non-diabetic chronic kidney disease (CKD) has dramatically altered the landscape in nephrology [1, 2]. The pathogenesis of glomerulosclerosis is now better understood, providing the possibility of novel treatments and hope for millions with, and at risk of, CKD worldwide [3, 4]. The article by Tzur *et al.* [5] in the current issue of *Nephrology Dialysis Transplantation* provides important new information in this fast moving field. To put this paper in perspective, it is appropriate to review how *APOL1* has revolutionized our understanding of non-diabetic glomerulosclerosis.

Relative to European Americans (EAs), nephrologists have observed 'different renal responses' to systemic hypertension and Type 2 diabetes mellitus in African Americans (AAs) [6]. This vague phenomenon was widely applied to explain the higher incidence rates of common forms of end-stage renal disease (ESRD) in AAs compared to EAs. The hypothesis that mild–moderate essential hypertension was the proximate cause of 'hypertensive kidney disease' or arteriolar nephrosclerosis in non-diabetic AAs with low-level proteinuria was deeply ingrained in the literature, despite strong evidence to the contrary [3, 7, 8]. Higher rates of non-diabetic nephropathy in AAs, relative to EAs, were also ascribed to differences in socioeconomic status and environment between population groups.

Three important developments led to identification of *APOL1* and variants in the adjacent non-muscle myosin heavy chain 9 gene (*MYH9*), as initiating factors for most cases of 'hypertension-attributed' nephropathy in those with African ancestry, as well as a spectrum of related kidney diseases in AAs, Hispanic Americans (HAs), Europeans and EAs. The first development was recognition of marked familial aggregation of kidney disease. In AA families, clustering of disparate forms of ESRD was often present. This observation suggested the presence of an overarching kidney failure susceptibility gene inherited independently from systemic disorders, such as hypertension, HIV infection, diabetes and systemic lupus erythematosus [9–13]. The second development was a series of molecular genetic advances, particularly admixture mapping (also called mapping by admixture linkage disequilibrium; MALD) [14]. MALD is a powerful genome-wide method useful to detect associated genes in admixed populations, where ancestral populations have markedly different disease frequencies. AAs are an admixed population with ~80% African and 20% European ancestry, while kidney disease is far more common in Africans than Europeans. MALD detected linkage between genetic markers on Chromosome 22q containing the *MYH9* and *APOL1* genes with focal segmental glomerulosclerosis (FSGS) and HIV-associated collapsing glomerulopathy in AAs [15, 16]. The third development was broad genetic screening for *MYH9* and *APOL1* polymorphisms, whose variants were strongly associated with the clearly defined FSGS phenotype, in large numbers of AAs and HAs with common complex forms of ESRD [1, 2, 17]. These sequential developments led to the realization that FSGS, HIV-associated nephropathy, arteriolar nephrosclerosis and hypertension-attributed ESRD were members of a single disease spectrum [18]. This spectrum has expanded to include sickle cell nephropathy [19] and C1q-associated collapsing glomerulopathy [20].

It is now apparent that many patients with African ancestry and ESRD attributed to diabetes or lupus had been misdiagnosed, they actually have *APOL1*-associated FSGS (with coexisting diabetes or lupus) [21, 22]. *APOL1* genotyping has been used to 'genetically dissect' cases with diabetes and ESRD, enriching for those with diabetic nephropathy by removing those with two *APOL1* risk variants likely with FSGS. This allowed for detection of diabetic nephropathy genes [23, 24]. Variation in *APOL1* is strongly associated with the kidney disease that has historically been labeled 'hypertensive' or arteriolar nephrosclerosis, as in the African American Study of Kidney Disease and Hypertension (AASK) [25]. The strongest *APOL1* association was present in AASK participants with higher baseline proteinuria and progressive nephropathy [26]. Aggressive hypertension control and universal use of angiotensin-converting enzyme inhibitors failed to reliably halt nephropathy progression in AASK, strongly supporting nephropathy initiation from mechanisms other than hypertension. It appears that AASK patients predominantly had focal global glomerulosclerosis (FGGS) with interstitial and

vascular changes that did not correlate with blood pressure [27].

It appears that *APOL1* and *MYH9* are independent nephropathy susceptibility loci. Initial reports implicated the *MYH9* gene as associated with non-diabetic forms of ESRD in AAs. This was due predominantly to strong linkage disequilibrium with the two known *APOL1* coding variants (G1 and G2) that were detected using 1000 Genomes data [1]. However, *MYH9* and/or other nearby genes in strong linkage disequilibrium, but not the *APOL1* G1 and G2 variants, contribute to nephropathy risk in Europeans and EAs, but with lower odds ratios [28–30]. The role of *MYH9* became evident since the E1 haplotype in this gene remains nephropathy associated in European-derived populations, whereas the known *APOL1* risk variants are virtually absent.

Why would deleterious kidney disease-associated coding variants in *APOL1* persist in African-derived populations? The situation appears analogous to the protection afforded from malarial infection by possession of one copy of the hemoglobin S gene, two copies produces the disease state sickle cell anemia. One copy of an *APOL1* G1 or G2 nephropathy risk variant provides protection from the deadly parasitic disease African sleeping sickness, an illness transmitted by *Trypanosoma brucei rhodesiense* after the sting of an infected tse–tse fly. Possession of two *APOL1* risk variants, with apparent autosomal recessive inheritance, leads to glomerulosclerosis, renal microvascular disease and interstitial fibrosis by as yet unidentified mechanisms. In contrast to sickle cell, where two copies of hemoglobin S uniformly produce disease, not all those with two *APOL1* risk variants will develop kidney disease.

It could be hypothesized that the variable histological patterns of FSGS, FGGS and collapsing glomerulopathy are caused by different 'second hits' required for initiation of kidney disease [18]. All individuals with two copies of *APOL1* risk variants do not develop nephropathy; ~50% of AAs with untreated HIV infection develop collapsing glomerulopathy (the risk in treated patients remains undefined) and 18% develop FSGS in the absence of HIV infection [31]. Infection with HIV is one such environmental second hit that greatly increases nephropathy risk and leads to the distinctive pattern of collapsing glomerulopathy. Other patients may develop FSGS or FGGS due to different second hits, environmental or genetic in nature. There is evidence from a genome-wide association study that gene–gene interactions between *APOL1* and podocin (*NPHS2*) increase the risk for non-diabetic nephropathy [32]. *NPHS2* is independently associated with FSGS [33].

It is important that researchers translate this *APOL1* genetic breakthrough from the laboratory to the bedside. Potential clinical uses for genetic screening include among African ancestry deceased kidney donors to identify those with fewer than two *APOL1* risk variants. Transplanted kidneys with zero or one *APOL1* risk variants appear to function for far longer durations than those with two risk variants [34]. Strikingly, AA deceased donor kidneys from those with fewer than two *APOL1* risk variants function for equal durations as EA-donated kidneys, suggesting that variation in *APOL1* may completely account for the shorter allograft survival rates seen with AA donor kidneys. The effect of donor *APOL1* genotypes on allograft survival is more impressive than cold ischemia time, panel-reactive antibody titers and human leukocyte antigen matching. Some have called for widespread screening of AA live kidney donors to reduce subsequent nephropathy risk in donors and improve transplant outcomes [35]. We await replication of our observation. If replicated and extended to living donor kidneys, *APOL1* genotyping could alter selection of donor kidneys and lead to improvements in allograft survival.

Genotyping for *APOL1* will also be useful in patients with African ancestry and in population studies where nephropathy and systemic diseases potentially involving the kidneys coexist, such as diabetes mellitus. Subjects with a higher likelihood of non-diabetic nephropathy may be detectable [23, 24]. This would prove useful in clinical trials of diabetic nephropathy, where patients with FSGS may not respond to the study intervention and should be removed or stratified to allow for analysis of a more homogeneous sample. Patients with atypical forms of diabetes and CKD, those with nephropathy after short diabetes durations or lacking diabetic retinopathy, may benefit from *APOL1* genotyping to detect patients more likely to have disease in the FSGS spectrum. This will be especially important as novel treatments develop. Screening for *APOL1* gene variants will be most relevant in population groups with African ancestry; G1 and G2 risk variant frequencies are vanishingly low in other groups.

Dr Skorecki's group, in collaboration with investigators at Johns Hopkins, have recently shown that patients with HIV infection and fewer than two *APOL1* risk variants were more likely to have immune complex (non-FSGS/collapsing glomerulopathy)-mediated kidney disease, while those with two *APOL1* risk variants more often had FSGS variants and progressed more rapidly to ESRD [36]. They also demonstrated that the presence of *APOL1* risk variants was associated with non-diabetic nephropathy in HAs residing in the USA; these individuals had significant African ancestry [2]. In addition, Ethiopians with HIV infection were found to have negligible rates of HIV-associated nephropathy since they generally lacked *APOL1* risk variants [37]. Ethiopians had nearly equal degrees of Middle Eastern and African ancestry, demonstrating a 'within Africa' difference in genetic composition and associated nephropathy susceptibility.

This group has extended the field by replicating the observation that patients with two *APOL1* nephropathy risk variants developed ESRD at younger ages than those with zero or one risk variants [38]. AA dialysis patients with two *APOL1* risk variants had a mean age at ESRD of 48 years, relative to 57 years in those with zero risk variants ($P = 0.0003$) [5]. HA dialysis patients with two *APOL1* risk variants had a mean age at ESRD of 41 years, relative to 53 years in those with zero risk variants ($P = 0.0003$). A new finding was that AA with a single G1 (not G2) risk variant had an intermediate age at ESRD (mean 52 years; false discovery rate $P = 0.045$), similar to the trend in the Kanji *et al.* [38] report.

What might this 'additive effect' of *APOL1* risk variant association with age at ESRD reflect? *APOL1*-associated nephropathy is typically inherited in an autosomal recessive fashion and higher baseline proteinuria and rates of

progressive nephropathy were also inherited in apparent recessive fashion in the AASK [25]. Hence, it should not be surprising that those with two *APOL1* risk variants progress to ESRD more rapidly. However, the significant additive effect where possession of one G1 variant led to earlier age at ESRD appears somewhat surprising and is important to investigate. Individuals with one *APOL1* G1 variant do not appear to be at markedly increased risk for ESRD; although it is possible that the effect may not be purely recessive; [1] albeit, the recessive model fits best.

It is likely that the *MYH9–APOL1* gene region contains additional risk loci that may be difficult to detect due to high degrees of linkage disequilibrium and selective forces applied over the last 5000–10 000 years in Sub-Saharan Africa as well as multiple repetitive and overlapping sequences spanning the *APOL1–APOL6* gene region. If additional nephropathy risk variants exist, individuals with a single *APOL1* G1 variant would appear to be heterozygous, but in fact would be homozygous for G1 plus the additional risk variant (maintaining recessive inheritance). Other possibilities include the presence of *APOL1* second gene interactions and unidentified non-HIV environmental exposures that may preferentially trigger earlier development of nephropathy in those with one *APOL1* risk variant.

In a potentially related observation, *APOL1* nephropathy risk variants impacted plasma high-density lipoprotein (HDL) cholesterol subfraction concentration in an additive fashion [39]. Lower concentrations of medium-sized HDL particles are seen in those with two versus one versus zero *APOL1* risk variants, an effect thought to reflect altered hepatic HDL synthesis. Additive effects of *APOL1* risk variants on HDL concentration may prove to be important in the pathogenesis of nephropathy, as ApoL1 proteins associate with circulating HDL. Novel treatments may arise from removal of free (or HDL bound) *APOL1* G1/G2 variant-derived ApoL1 protein in the circulation, thereby protecting the kidney or renal microvasculature. Alternatively, expression of *APOL1* in kidney cells may lead to cell death from autophagy or apoptosis. There is great hope for identifying additional environmental second hits, perhaps vaccine-preventable non-HIV virus infections that may trigger initiation of nephropathy in genetically susceptible individuals.

A new era of understanding non-diabetic glomerulosclerosis has dawned from breakthroughs coupling modern molecular genetic techniques with the clinical observation of familial aggregation of nephropathy in AAs. The *APOL1* discovery will impact many aspects of medicine. For nephrology, this includes epidemiology and disease pathogenesis, cell biology, interactions between lipid metabolism and the kidney, outcomes of transplantation and opportunities for developing new treatments for a refractory spectrum of kidney disease. Classification of FSGS should be revisited to better reflect genetic associations impacting natural history and prognosis [40]. Identification of *APOL1*-associated nephropathy provides an opportunity to develop effective interventions that may work as prevention (prior to disease onset) and therapy (after disease onset) for up to 40% of ESRD in AAs as well as in HAs and others with African ancestry around the world.

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(See related article by Tzur *et al*. *APOL1* allelic variants are associated with lower age of dialysis initiation and thereby increased dialysis vintage in African and Hispanic Americans with non-diabetic end-stage kidney disease. *Nephrol Dial Transplant* 2012; 27: 1498–1505.)

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