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Full Review

APOL1 nephropathy: from gene to mechanisms of kidney injury

Etty Kruzel-Davila[1,2,*], Walter G. Wasser[2,3,*], Sharon Aviram[1] and Karl Skorecki[1,2]

[1]Rappaport Faculty of Medicine and Research Institute, Technion—Israel Institute of Technology, Rambam Health Care Campus, Haifa 3109601, Israel, [2]Rambam Health Care Campus, Haifa 3109601, Israel and [3]Mayanei HaYeshua Medical Center, Bnei Brak 51544, Israel

Correspondence and offprint requests to: Walter G. Wasser; E-mail: lcdialysis@aol.com
[*]These authors contributed equally to this manuscript.

ABSTRACT

The contribution of African ancestry to the risk of focal segmental glomerulosclerosis and chronic kidney disease has been partially explained by the recently described chromosome 22q variants in the gene *apolipoprotein L1* (*APOL1*). The *APOL1* variants appear at a high allele frequency in populations of West African ancestry as a result of apparent adaptive selection of the heterozygous state. Heterozygosity protects from infection with *Trypanosoma brucei rhodesiense*. This review will describe the role of the approaches in population genetics for the description of *APOL1*-associated nephropathies and draw inferences as to the biologic mechanisms from genetic epidemiology findings to date. Modifier loci can influence *APOL1* risk for the development of kidney disease. 'Second hits', both viral and non-viral, may explain the discrepancy between the remarkably high odds ratios and the low lifetime risks of kidney disease in two allele carriers of *APOL1* risk variants. Therapeutic strategies for *APOL1*-associated nephropathies will require the prevention and treatment of these 'second hits' and the development of drugs to protect the *APOL1* downstream renal injury pathways.

Keywords: admixture, African American, FSGS, HIV, hypertension-attributed nephropathy

INTRODUCTION

Disparities in kidney disease between individuals in the USA of African ancestry and European ancestry have been clearly delineated. The United States Renal Data System Report demonstrates a consistent, yearly increase in the incidence of end-stage kidney disease (ESKD) among individuals of African ancestry that is 3.5- to 5-fold that of individuals of European ancestry [1]. The estimated lifetime risk for individuals of African ancestry with respect to the development of ESKD approaches 8%, whereas the risk for individuals of European ancestry is 2–3% [2]. The likelihood of developing ESKD in African-American patients infected with human immunodeficiency virus (HIV) is at least 50 times greater than that among European Americans [3]. HIV-infected individuals are also more prone to progress to ESKD [4]. Hypertension-attributed nephropathy accounts for 36% of the annual cases of ESKD in African Americans [1] and represents a prominent cause of kidney disease in this population. Kidney biopsies performed in these individuals demonstrate a pattern of segmental and global sclerosis, arteriosclerosis and chronic tubulointerstitial fibrosis [5].

Four years ago, two independent groups described two common variants located on chromosome 22 that encode the APOL1 protein. This discovery provided an explanation for much of the increase in the rates of ESKD in patients of African ancestry in the USA, which had been a previously enigmatic aspect of health disparities research. This observation, a culmination of decades of investigation, initially focussed on income disparities, overt poverty and racial segregation, which remain highly consequential [6]. The important role of genetic factors was suggested by the painstaking epidemiologic observations of Freedman *et al.* [7], who observed that many patients of African ancestry with ESKD had close relatives who had also been treated with dialysis.

Studies of mapping by admixture linkage disequilibrium (MALD) have been conducted to account for the genetic contributions of African and European ancestries to African-American individuals with kidney disease. This research, performed in the laboratories of Kopp [8], Kao [9] and their associates, as well as in our laboratory [10], led to the

identification in mid-2008 of the 22q13 chromosomal region and its association with non-diabetic chronic kidney disease (CKD) and ESKD. The initial supposition was that the disease-causing variants were located in the *MYH9* gene [8, 9]. Two years later, Pollak and colleagues [11], as well as our laboratory [12], described the association of kidney disease with two coding variants in the *apolipoprotein L1* (*APOL1*) gene by re-sequencing a larger region and by *in silico* data-mining of the 1000 Genomes Project. These studies demonstrated that the *MYH9* gene was in strong linkage disequilibrium with even more highly associated G1 and G2 variants in the *APOL1* gene: The G1 haplotype is composed of two missense variants in nearly absolute linkage disequilibrium, Ser342Gly and Ile384Met, and the G2 allele has an in-frame deletion of two amino acids, del.N388/Y389. The association of kidney disease with the MYH9 haplotype disappeared after controlling for the *APOL1* risk variants [11].

PRINCIPLES OF POPULATION GENETICS AND EVOLUTIONARY MEDICINE

The research leading from the initial hypothesis of the existence of a genetic locus in the early 1990s to the discovery of the locus explains a crucial principle of evolutionary medicine and a major population disparity in cases of kidney disease [11, 12].

The principle of evolutionary medicine is exemplified by the relationship between circulating APOL1 and its ability to lyse *T. brucei*. This subspecies of the parasite, *Trypanosoma brucei rhodesiense* emerged hundreds of years ago in Africa and exhibited resistance to trypanolysis, mediated by APOL1. Pays [13], Raper [14] and colleagues have demonstrated that *T. b. rhodesiense* evades the trypanolytic effect of most forms of circulating APOL1 by the generation of a serum resistance-associated protein (SRA) that confers resistance to trypanolysis by binding to the C terminus of APOL1. However, even one parental allele of the *APOL1* G1 or G2 variant overcomes the resistance of *T. b. rhodesiense* and restores trypanolytic activity [11]. Experiments by Genovese *et al.* demonstrated the lysis of *T. b. rhodesiense in vitro* by *APOL1* G1 and G2 variants. A recent landmark study examined the trypanolytic activity of *APOL1* risk variants *in vivo* [15]. This study reported the extended median survival of mice infected with SRA-expressing trypanosomes after hydrodynamic gene delivery of *APOL1* risk variants compared with wild-type *APOL1*. G2-expressing mice exhibited a prolonged and increased survival compared with G1-expressing mice [15]. The rise to high frequency of the risk alleles in West Africans is due to the apparent adaptive advantage provided by even a single parental G1 or G2 allele against the pathogenicity of *T. b. rhodesiense*. Unfortunately, two copies of *APOL1* risk alleles (i.e. G1/G1, G1/G2 or G2/G2) greatly increase the risk of CKD later in life. Therefore, in areas where sleeping sickness is endemic, mutant *APOL1* homozygotes possess an advantage and therefore became more common in the population. In non-endemic areas such as the USA, these two risk alleles of *APOL1* are mainly associated with CKD (Figure 1). It is this evolutionary force that was conducive to a high odds ratio for the 'common variant–common disease'.

Whereas numerous statistical associations with human phenotypes can be described in common diseases, the typical association or odds ratio (OR) is generally low and is generally well below an OR of 1.5, signifying <50% increased incidence of the disease per inherited variant [16]. The example of *APOL1* association ORs is one of the highest ever described for a common disease association and ranges from 7 to 29 for various aetiologies of non-diabetic ESKD. This association appears to be an exception to the rule because it represents a high-frequency allele with a high odds ratio as a result of powerful selection pressure [17]. Further studies from parental populations in West Africa have documented the highest frequencies of the two risk variants of *APOL1* among the Nigerian Igbo and Yoruba tribes, who live in geographic areas with an overwhelmingly high prevalence of non-diabetic CKD and hypertension [18, 19].

Interestingly, contemporary maps illustrate a discrepancy between areas of modern East Africa that are endemic for *T. b. rhodesiense* and areas of West Africa that exhibit high population frequencies of the G1 *APOL1* risk variant (Figure 2) [20]. In contrast, the G2 allele exhibits widespread distribution. The geographic distribution of the *Trypanosoma* subspecies in Africa may have changed over thousands of years and may have previously been more closely aligned with the *APOL1* allele frequency. However, Thomson *et al.* [15] recently described the *in vivo* trypanolytic activity for *APOL1* risk variants and reported that the G2 variant demonstrated a more pronounced trypanolytic activity than the G1 variant. The combination of the discrepancy between the high G1 allele frequency in West Africa, which does not match the predominance of *T. b. rhodesiense* in East Africa, and the reduced trypanolytic activity of the G1 variant has led the authors to hypothesize that the G2 variant evolved in response to the expression of SRA in *T. b. rhodesiense*, whereas the G1 variant might have evolved in response to complex selection pressures that extended beyond African trypanosomes [15]. The authors searched for other candidate pathogens that may match the high frequency of the *APOL1* G1 risk allele in West Africa and analyzed cases of malaria and controls. However, they did not find a correlation between malaria and the frequencies of *APOL1* risk alleles. It is possible that the G1 variant may have served as an innate immunity protein that provided protection against *Trypanosoma brucei gambiense* Type 2 [15] or other infections, such as Leishmania [21].

The absence of HIV nephropathy in East Africa is an example of a disease population disparity explained by the lack of *APOL1* variants. We reported many years ago that HIV-infected Ethiopian Jews living in Israel exhibited an absence of HIV-associated nephropathy (HIVAN) and a low prevalence of CKD in general [22]. We also found this to be true among Ethiopians living in Ethiopia [23]. Notably, we found that both E1 and S1 African ancestry risk associated haplotypes of the adjacent *MYH9* gene, first suspected to be the responsible locus, exhibit an allele frequency of 0.35 in Ethiopia, which is seemingly at odds with the absence of HIVAN in this region [24]. However, we observed the virtual absence of the *APOL1*

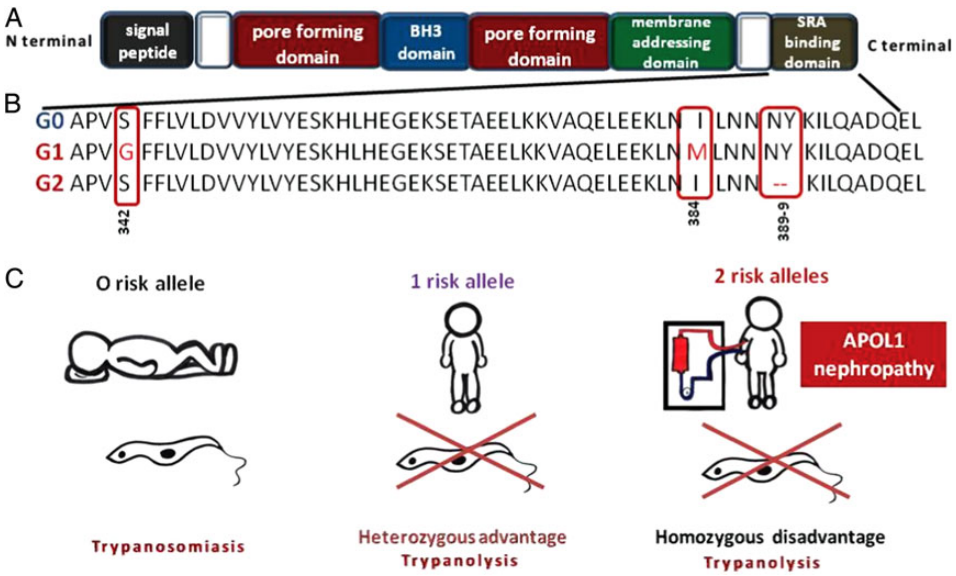

FIGURE 1: (**A**) The structure of *APOL1* with annotated domains. (**B**) G1 (S342G and I384M) and G2 (deletion of N388 and Y389) mutations are located at the C terminus. The close proximity of the G1 and G2 alleles results in a low likelihood of recombination between the two alleles. G2 is mutually exclusive from G1, and these two alleles never appear together on the same chromosome. Therefore, the two risk alleles include the following combinations: G1/G1, G1/G2 and G2/G2. (**C**) Illustration of the different consequences of *APOL1* carriers: individuals with two *APOL1* wild-type alleles (G0) are prone to *T. b. rhodesiense* infection. The depiction on the left represents an individual with zero risk alleles, suffering from African sleeping sickness. Individuals with one *APOL1* risk allele (G1 or G2), represented by the depiction in the middle, are protected from *T. b. rhodesiense* infection and are likely at a lower risk for the development of *APOL1*-associated nephropathy. Individuals with two *APOL1* risk alleles, represented by the depiction in the right are protected from *T. b. rhodesiense* infection but are at an increased risk for the development of progressive adult CKD.

G1 and G2 risk alleles in Ethiopia [23]. This combination of the reported *APOL1* mutations and the absence of HIVAN are consistent with *APOL1* being the gene actually relevant for the risk of HIVAN.

Mode of inheritance and phylogeny

The close proximity of the G1 and G2 alleles results in a low likelihood of recombination between the two alleles; hence, the G1 and G2 alleles appear to be mutually exclusive. These two alleles are in complete negative linkage disequilibrium and never appear together on the same chromosome. Therefore, an individual can carry no more than two copies of G1, two copies of G2 or one copy of each (Figure 1). Almost all studies have demonstrated equivalent CKD association of individuals carrying two copies of G1, two copies of G2 or one copy of each [11, 25, 26]. However, a trend for a higher risk for CKD [27, 28] and albuminuria [27] in G1 homozygotes has also been observed. The cohorts of non-diabetic CKD patients exhibited a highly significant OR under a two *APOL1* risk allele, recessive mode of inheritance (recessive), as follows: a 7- to 10-fold increased risk of hypertension-associated disease, a 10- to 17-fold increase in focal and segmental glomerulosclerosis (FSGS) and a 29-fold increase in HIV nephropathy [11, 12, 25]. Some researchers have reported a small effect of a single risk variant (dominant) in studies that demonstrated a younger age of onset for the start of haemodialysis in individuals with one G1 risk allele [29, 30]. A recent report suggested an additive mode of inheritance in patients with HIV similar to that observed in a combined cohort of FSGS and HIVAN [26, 31]. This additive effect may be an additional mode of injury or may reflect more than one mechanism of kidney disease, which is consistent with the pleotropic forms of *APOL1*-associated kidney disease beyond FSGS.

The 'two risk allele' mode of inheritance should be most consistent with a 'loss-of-function' mechanism of podocyte injury. However, this raises a striking question because many mammalian species lack *APOL1* [32]; thus, *APOL1* is dispensable with respect to the integrity of kidney function. Among non-human primates, chimpanzees lack *APOL1* in their genome. Even in humans, a recent report by Johnstone *et al.* [33] demonstrated the absence of *APOL1* in an individual with normal kidney function from a village in India. Thomson *et al.* analyzed the sequence of the *APOL1* gene from humans, gorillas and Old World monkeys, including baboons, and reported that the G1 and G2 alleles of humans share sequence similarities with the baboon's trypanolytic *APOL1* [15]. There are several possible interpretations to reconcile recessive inheritance with the apparent dispensability of *APOL1* for mammalian (and human) kidney integrity. One possibility is a dose-dependent 'gain-of-injury' required for G1 and G2 risk alleles in which two doses of the allele product are necessary to cross the podocyte injury threshold. Second, the risk variant for non-diabetic CKD in Africans may not be *APOL1*, but could instead be another gene that is in strong linkage disequilibrium with *MYH9* and *APOL1*. A third possibility is that even a single, non-risk (G0) *APOL1* 'protects' from a human-specific 'second hit'. In the absence of such a 'second hit', *APOL1* is dispensable. Recent published studies that demonstrate a differential cellular gain-of-injury or toxicity effect of the G1 and G2 alleles compared with G0 tend to favour the first alternative [15, 34, 35].

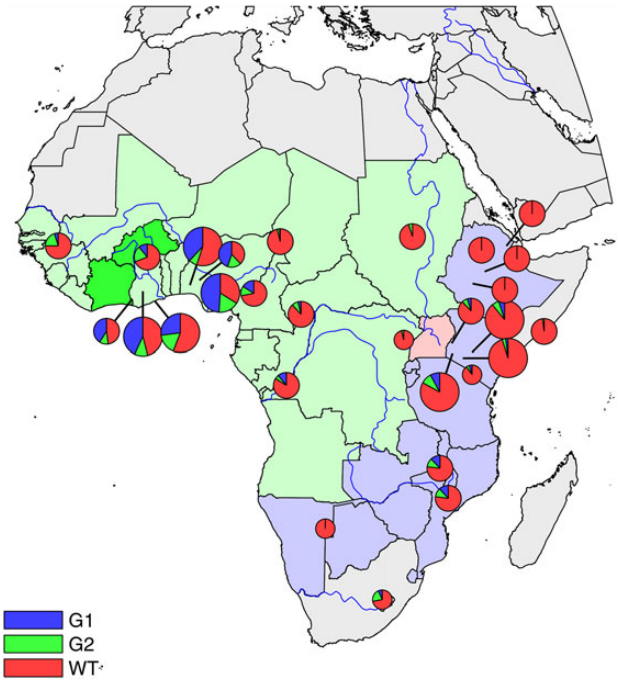

FIGURE 2: Distribution of the G1 and G2 *APOL1* variants across Africa. The allele frequencies of the G1 and G2 variants are indicated as blue and green wedges, respectively. The circle size reflects the number of individuals who were genotyped: small, <10 individuals/20 chromosomes; medium, 10–100 individuals/20–200 chromosomes and large, >100 individuals/200 chromosomes. Countries are shaded according to the presence of subspecies of *T. brucei* that cause African sleeping sickness. Darker green, *gambiense* Types 1 and 2; light green, *gambiense* Type 1; pink, both rhodesiense and *gambiense* Type 1; purple, rhodesiense. Obtained from [15] with permission.

FULL REVIEW

EPIDEMIOLOGIC STUDIES OF *APOL1* NEPHROPATHY

Strong associations of the *APOL1* risk alleles have been described in multiple case–control studies of kidney disease in patients with ESKD, FSGS, HIVAN and hypertension-attributed nephropathy and have been extended to population studies [11, 12, 18, 25, 26, 28, 36–39] (Figure 3).

Population-based studies. Population-based studies were required to corroborate the results obtained from the case–control studies in view of multiple biases described with this study model. The case–control studies of *APOL1*-associated kidney disease were biased due to the potential overestimation of risk because they were performed in patients with advanced disease. In addition, these studies may contain a selection bias in that the control and case populations may not be similar, as well as a survival bias, which is particularly important in studies of groups of patients with high mortality rates, such as individuals who receive renal replacement therapy.

One such population study is the Dallas Heart Study. This cohort study confirmed the *APOL1* risk association and reported that non-diabetic African Americans with two *APOL1* risk alleles exhibited a 3-fold increase in the risk of microalbuminuria and a 4-fold increase in CKD (eGFR < 60 mL/min per 1.73 m^2) (OR 3.9) [28]. These associations were relatively more modest (i.e. the ORs were lower) than those observed in the original case–control studies, likely because the patients exhibited less severe disease in the population studies [40].

Longitudinal studies reveal progression factors in individuals with *APOL1*-associated CKD. A population study of patients with a 25-year follow-up in the Atherosclerosis Risk in Communities Study (ARIC study) reported that individuals with two risk alleles of *APOL1* demonstrated a 1.49-fold increased risk for the development of CKD compared with those with one or zero risk alleles. Among those who had CKD, those with two risk alleles exhibited a 2.2-fold increased risk of progression to ESKD compared with participants with zero or one risk allele over a median follow-up period of 19.7 years [37]. These more modest associations are similar to the results obtained in the Dallas Heart Study but have the advantage of >25 years of follow-up data.

Similarly, the African American Study of Kidney Disease and Hypertension (AASK) and the Chronic Renal Insufficiency Cohort (CRIC) study, both longitudinal CKD cohorts, have confirmed the strong association of *APOL1* risk variants with progressive kidney disease [36, 38]. In the AASK study, 58% of the patients with two risk variants exhibited a doubling of serum creatinine or ESKD, irrespective of the medication that was administered or the level of blood pressure control that was achieved. In contrast, only 36.6% of the patients in the *APOL1* low-risk group exhibited this characteristic (hazard ratio in the high-risk group: 1.88; $P < 0.001$). In the CRIC study, African Americans with two *APOL1* risk alleles exhibited a more rapid decline in eGFR and higher composite renal outcomes than African Americans with zero or one risk allele or European American patients, regardless of diabetes status [38].

Extended spectrum of *APOL1* disease

Systemic lupus erythematosus. Larsen *et al.* [41] described the association between two *APOL1* risk variants and collapsing glomerulopathy. It is unclear whether the finding of collapsing nephropathy in cases of lupus represents a distinct lupus nephritis variant or a coincidental association between these two diseases in a patient population prone to developing both diseases. Freedman et al. [42] have also recently demonstrated the strong impact of the presence of two *APOL1* risk variants on the development of ESKD in patients with lupus and a more rapid progression of kidney disease with a shortened time to the start of dialysis.

Membranous nephropathy. Larsen *et al.* described the association of two risk variants of *APOL1* and collapsing glomerulopathy observed in patients with membranous nephropathy without lupus or HIV. It seems possible that *APOL1* may increase the susceptibility for collapsing glomerulopathy in the presence of various underlying glomerular diseases [43].

Diabetes mellitus. The original case–control studies, as well as the Dallas Heart Study [11, 12, 28], demonstrated a lack of

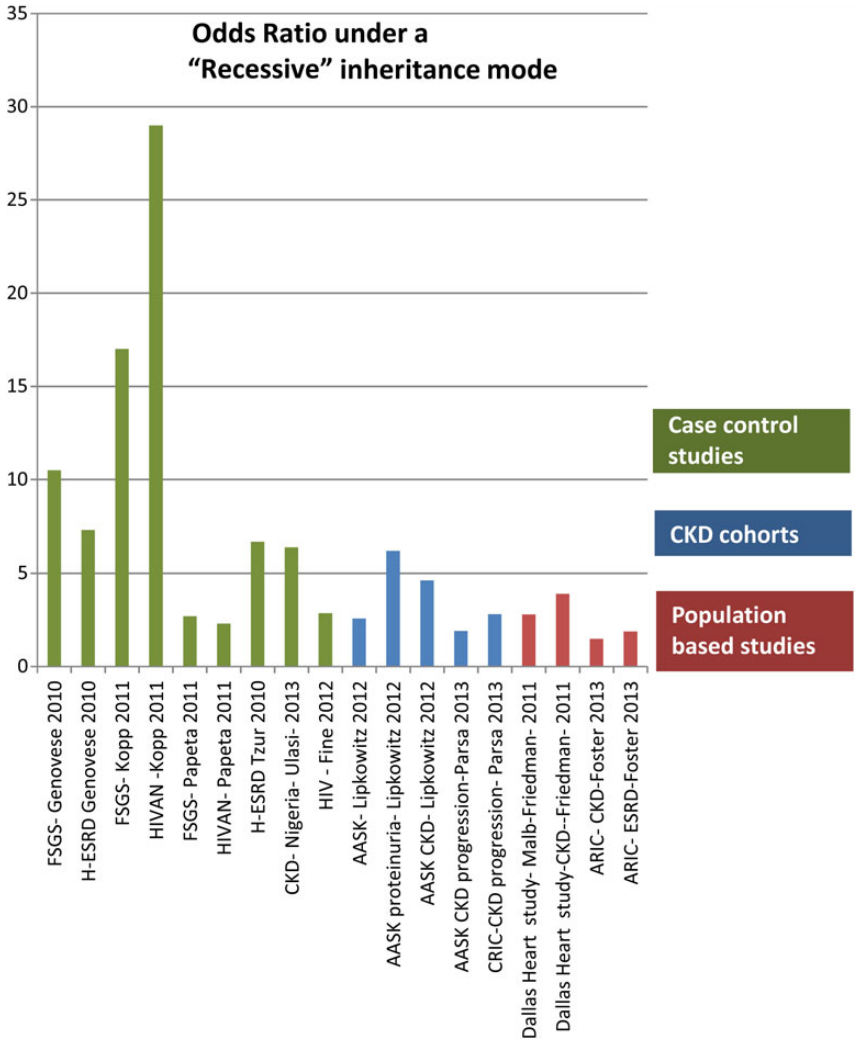

FIGURE 3: The OR for kidney disease in individuals with two *APOL1* risk alleles (G1/G1, G1/G2 and G2/G2) among different studies. OR, odds ratio; FSGS, focal segmental glomerulosclerosis; H, hypertension; HIVAN, HIV-associated nephropathy; CKD, chronic kidney disease; Malb, microalbuminuria [11, 12, 18, 25, 26, 28, 36, 37, 38, 39].

association between kidney disease in diabetic patients and the *APOL1* risk variants. However, the ARIC study reported an association of two risk variants of *APOL1* in diabetic patients with CKD [37]. In a recent publication, Parsa *et al.* analyzed *APOL1* loci in the CRIC study and reported a strong association between kidney disease in diabetic patients and its progression [38]. APOL1 is involved in autophagy [44], a lysosomal degradation process that removes damaged organelles and protein aggregates to maintain intracellular homeostasis in times of stress. This finding led our laboratory to postulate distinct mechanisms in relation to diabetic nephropathy on the one hand versus *APOL1*-associated nephropathy on the other hand. In particular, the autophagy pathway is essential for podocyte integrity and is predicted to be suppressed in cases of diabetic kidney disease [45, 46]. Hence, we propose that any possible association of *APOL1* risk alleles with nephropathy in diabetic subjects may not represent diabetic nephropathy *per se*, but rather *APOL1*-associated nephropathies (e.g. focal segmental glomerulosclerosis) in diabetics. Clearly, this major question will require additional investigation.

Cardiovascular disease. Ito *et al.* [27] analyzed the Jackson Heart Study (JHS) and the Women's Health Initiative study and reported an association between *APOL1* risk alleles and cardiovascular outcomes, including myocardial infarction, stroke and endovascular intervention. The authors demonstrated that individuals with two *APOL1* risk alleles exhibit a 2-fold increased risk for cardiovascular disease events compared with individuals with no risk alleles [27], irrespective of clinical kidney disease. Because subclinical kidney disease is a known risk factor for cardiovascular disease, especially in individuals of African ancestry [47], it is difficult to attribute the increased cardiovascular risk to *APOL1* risk alleles in light of common subclinical kidney disease in this population. Two recent studies that analyzed the Diabetic Heart Study cohort [48] and the Systolic Blood Intervention Trial (SPRINT) study [49] exhibited conflicting results to Ito *et al.*, as they demonstrated a low prevalence of cardiovascular events [49] and lower all-cause mortality [48] in individuals with two *APOL1* risk alleles. Paradoxical findings of both the JHS [27] and Diabetic Heart Study [48] were the

lower levels of coronary [27] and carotid [48] artery calcified atherosclerotic plaque. Further studies are needed to explore the perplexing interaction between *APOL1* risk variants and cardiovascular risk.

CLUES TO THE BIOLOGICAL MECHANISMS FROM POPULATION GENETICS AND EPIDEMIOLOGY

Circulating *APOL1* versus kidney-expressed *APOL1*

APOL1, a 43 kDa protein that belongs to the apolipoprotein family, is the only member of this family that produces a secreted protein, which is bound to circulating HDL particles [13, 44, 50–52]. APOL1 is expressed in various organs, including the kidney [53]. As a BH3-only protein, APOL1 is involved in the autophagy pathway [44]. One of the fundamental questions with regard to *APOL1*-associated kidney disease is whether the risk lies in the circulating APOL1 or the APOL1 that is expressed in the kidney.

Madhavan *et al.* [54] described the expression of APOL1 in sections of normal human kidneys and in kidney biopsies from patients with FSGS and HIVAN. In normal kidneys, APOL1 was detected in the podocytes, proximal tubules and medium-sized arterial and arteriolar endothelial cells. In kidney biopsies in patients with HIVAN and FSGS, expression in the podocytes and tubules was diminished, but *de novo* expression of APOL1 was observed in the vascular smooth muscle cells of the arterial medial wall. Immunohistochemistry studies revealed a more pronounced APOL1 signal in the proximal tubules than in the glomerular compartment [54]. The expression of APOL1 was not correlated with the risk genotypes. These pathological findings do not clarify whether the endogenous expression of APOL1 is changed or whether the extracellular uptake is altered.

Bruggeman *et al.* [31] did not observe an association between the levels of APOL1 in the plasma, *APOL1* genotypes, HDL levels, clinical kidney disease and several cytokines in a cohort of HIV-infected patients with CKD. Their study concluded that there is no association between the *APOL1* genotypes and inflammation in this population, and their results seem to support the idea that APOL1 expressed in the kidney is the major deleterious factor. However, careful analysis questions these conclusions because there was a markedly lower two risk allele frequency of *APOL1* compared with previous studies of HIV-infected patients with CKD [25]. In addition, no correlation was observed between proteinuria and *APOL1* risk variants in the majority of participants with well-controlled HIV infections. All of these factors may raise questions as to whether the individuals who received treatment for HIV represented cases of *APOL1*-associated nephropathy. Therefore, from their study, it is difficult to draw conclusions with regard to the *APOL1* genotype, APOL1 plasma levels and inflammatory mediators in *APOL1*-associated nephropathy. Additionally, the study did not exclude a functional difference in the activity of circulating APOL1 (as opposed to the plasma level of APOL1), which may be attributable to the risk variants.

Kidney transplantation. Clues as to the biologic action of circulating versus endogenous APOL1 may be gleaned from two published studies of kidney transplantation [55, 56]. Reeves-Daniel *et al.* [55] described the relationship between the *APOL1* two risk allele status of the donor kidney and transplant outcomes and suggested that the risk for kidney allograft outcomes is associated with the two risk allele status of the donor kidney. Kidneys from deceased African-American donors that harboured two *APOL1* risk variants failed more rapidly after kidney transplantation than kidneys from donors with zero or one risk variant [55]. Lee *et al.* [56] reported that the *APOL1* two risk allele status of the kidney transplant recipient does not impact the kidney transplant outcome. Taken together, these two studies suggest that the *APOL1* risk allele association is mediated by the gene product isoform that is endogenously expressed within the kidney, not the circulating APOL1. There are some caveats to this study because the 'disease' in this case was kidney 'transplant loss', not FSGS or one of the 'classic' *APOL1*-associated nephropathies. Another caveat is that these studies did not genotype the donor and recipient *APOL1* alleles simultaneously. Nonetheless, the Reeves-Daniel report was sufficient to cause experts in the field to suggest that African-American kidney donors should be screened for *APOL1* [57].

***In vitro* and *in vivo* studies.** Freedman *et al.* have demonstrated APOL1 uptake in podocytes, which explained the apparent discrepancy between the abundance of APOL1 protein in podocytes that was observed in cryosections of kidneys versus the lesser expression of *APOL1* mRNA in podocytes. However, *in vitro* studies in immortalized podocyte cell lines did not exhibit reduced viability, change in podocyte cytoskeletal structure or alterations in the expression of proliferation and differentiation markers in podocytes after APOL1 uptake [58]. Nichols *et al.* [35] demonstrated increased cytotoxicity in HEK293 cells transfected with *APOL1* risk variants, which was similar to *APOL1* transcripts lacking a signal peptide. Another piece of evidence against the role of circulating APOL1 was demonstrated recently in an *in vivo* model using hydrodynamic gene delivery of *APOL1* variants to mice. Deletion of the APOL1 signal peptide did not significantly reduce liver necrosis, which was induced by *APOL1* variants [15]. In conclusion, all of the above-mentioned studies provide indirect evidence suggesting that locally produced or endogenous APOL1 might be the causative factor, opposed to circulating APOL1. However, a role for circulating or paracrine APOL1 has not been definitively excluded.

Second hits

The *APOL1* risk variants have relatively low penetrance compared with Mendelian diseases. Only a subset of individuals who carry two *APOL1* risk alleles develops kidney disease. The lifetime risk for kidney disease in individuals with HIV infection, in the absence of antiviral therapy, is estimated to possibly exceed 50%, whereas the lifetime risk for FSGS is 4% [25]. A fundamental question remains: what are the contributing factors that either trigger progressive CKD or,

conversely, protect individuals with two *APOL1* risk alleles from CKD.

Modifier loci and interactions between genes

Comparisons of risk for kidney disease among Hispanics and African Americans two *APOL1* risk allele carriers demonstrated that Hispanic Americans exhibited a younger age for starting dialysis [30]. This finding led us to further relate the percent of African ancestry outside of the *APOL1* gene to the effect of the *APOL1* risk alleles. A higher percentage of non-African genetic background across the rest of the genome was correlated with increased CKD risk in individuals with two risk alleles (S. Tzur, K. Skorecki, unpublished data), suggesting differences in the population frequency of non-*APOL1* genomic loci that modulate kidney function. Such modifier loci have yet to be identified to account for *APOL1*-associated kidney disease among different populations. Modifier loci may explain the differences in kidney pathologies between FSGS, HIVAN and hypertensive-attributed nephropathy.

Freedman and colleagues have recently reported several interactions between certain genes and *APOL1* [59, 60]. The most prominent effect was observed in *podocin* (*NPHS2*), as well as other genes that include *serologically defined colon cancer 8* (*SDCCAG8*) and a genomic locus near the *bone morphogenetic protein 4* gene (*BMP4*) [60]. The effects of the interaction of these genes and the degree to which these genes increase or decrease the OR of the *APOL1* risk allele have recently been quantified [60].

A family study of first-degree relatives of African-American patients with non-diabetic ESKD in the Natural History of *APOL1*-Associated Nephropathy Study reported that relatives of African Americans with non-diabetic ESKD are enriched for *APOL1* risk variants. After adjustment, two *APOL1* risk variants only weakly predicted overt proteinuria and an eGFR < 60 mL/min per 1.73 m^2 [61]. Other environmental 'second hits', most prominently viruses, are therefore necessary to explain the gaps between lifetime risks in individuals with the same genetic background.

Inflammatory responses

The expression of APOL1 in human embryonic umbilical vein endothelial cells can be induced by lipopolysaccharide [54] and by circulating inflammatory cytokines, including interferon gamma (IFNγ) and tumour necrosis factor alpha (TNFα) [53, 62], which supports the role of APOL1 in innate immunity. Preliminary results from our laboratory (S. Aviram, K. Skorecki, unpublished data) have demonstrated that IFNγ markedly increases the expression of *APOL1* mRNA and protein from near absence at baseline to very high levels in human podocytes. In a recent publication, Nichols *et al.* [35] reported a cohort of patients who developed collapsing FSGS while receiving therapeutic interferon. Not surprisingly, these patients carried two *APOL1* risk alleles. The authors characterized *APOL1* induction by interferon directly or through an interferon-independent, INF regulatory factor 3-dependent pathway [35]. We hypothesize that IFNγ, or other antiviral pathways that may be induced by a second hit, would increase the exposure and vulnerability of podocytes to APOL1, thereby enhancing the deleterious downstream effects of APOL1.

A major question with regard to the role of APOL1 as a modifier of HIVAN is whether APOL1 is mechanistically involved with other mediators of innate immunity to affect the viral clearance of HIV. Taylor *et al.* [63] has recently suggested that APOL1 might contribute to the degradation of HIV. APOL1 targets the HIV Gag protein for degradation by the endolysosomal pathway, thus enhancing endocytosis and lysosomal biogenesis via the promotion of the nuclear translocation of the transcription factor EB (TFEB) and the expression of TFEB-targeted genes. APOL1 depletes the viral accessory protein Vif, which counteracts the host restriction factor APOBEC3G, via lysosomal degradation and secretion of Vif into microvesicles. A major question is whether *APOL1* risk variants differ in their antiviral activity in light of the increased risk for CKD in untreated patients with HIV.

Nephrotropic viral infection

A leading candidate for a second hit that can explain the genetic epidemiologic observations is human nephrotropic viral infection. The prototype for a viral second hit is HIV. The lifetime risk of kidney disease in patients with two *APOL1* risk alleles has been shown to rise from <10 to >50% in the presence of untreated or undertreated HIV infection [25]. Successful anti-retroviral therapy, which reduces the yearly decline in the eGFR, is thought to be one of the most important pieces of evidence indicating that HIV represents an important, pathogenic factor [64].

Fine *et al.* [39] described the utility of *APOL1* risk variants for the prediction of histology in non-HIVAN forms of HIV kidney disease, which are increasingly more common and comprised >71% of a recent series of patients. Patients with two risk variants were more likely to exhibit focal segmental sclerosis (76%) and hypertensive kidney disease (10%) [39]. Of those with no risk variants, histology revealed immune complex diseases (47%) and diabetic nephropathy (28%) [39].

In addition to HIV, a potential human viral nephrotropic second hit is Parvovirus B19. Parvovirus B19 was detected >10 years ago in kidney tissues from patients with collapsing FSGS [65, 66]. *In situ* hybridization studies confirmed the presence of Parvovirus B19, and the viral genome can be detected in kidney biopsies from patients with collapsing FSGS. Most of these subjects were African American, but their *APOL1* status was not determined.

Evidence was sought for non-HIV viral risk modulation in *APOL1* kidney disease. Divers *et al.* examined the association of BK and JC polyomaviruses with the potential for renal or uroepithelial tropism, as well as Human Herpes Virus-6 and Cytomegalovirus (CMV) lymphotropic viruses. These viruses could serve as modifiable environmental factors that interact with *APOL1* to initiate kidney disease [67]. The patient cohort consisted of related and unrelated relatives of African Americans with non-diabetic ESKD. The incidences of HHV6 and CMV were rare. BK viruria was observed in ∼10% of patients and was unrelated to the *APOL1* allele risk frequency or kidney disease. Surprisingly, the authors observed that the patients with two *APOL1* risk variants who had JC viruria were

less likely to develop kidney disease than those who lacked JC viruria. The authors have suggested possible mechanisms for this perplexing negative association. One possible explanation is protection against other nephrotoxic viruses by the modification of cellular immunity. A second explanation is that JC polyomavirus may impact gene transcription in the autophagy and apoptosis pathways that involve *APOL1*. A third explanation could be related to the role of APOL1 as an innate immunity protein that might exert an impact on the ability of the immune system to clear the JC virus. More patient samples are required to clarify the role of these viral interactions with *APOL1* risk variants and for the identification of non-HIV viral 'second hit' triggers of CKD in individuals with *APOL1* risk alleles.

MECHANISMS OF KIDNEY INJURY

In vivo studies

Direct evidence for the pathogenic role of *APOL1* risk variants in the increased risk for CKD is still lacking. However, Thomson *et al.* [15] demonstrated a gain-of-injury effect of *APOL1* risk variants on the liver. The authors engineered *APOL1* variants and expressed them in mice by hydrodynamic gene delivery. Increased hepatic necrosis was observed in mice that expressed the *APOL1* risk variants, with a more pronounced effect observed with the G1 variant. The removal of the signal peptide did not reduce hepatic necrosis significantly, whereas the deletion of the amphipathic helix at the C terminus significantly decreased liver injury, suggesting that cellular toxicity was not related to the secretion of APOL1. Kidney injury was not observed in this model, possibly due to the lower levels of gene expression in the kidney or to insufficient time for the identification of kidney injury. One of the most remarkable differences between the hepatic toxicity of the different *APOL1* variants was the absence of liver injury with the baboon *APOL1* gene. Baboon *APOL1* conferred complete resistance to *T. b. rhodesiense* infection, driven by its inability to bind SRA. The G2 and G1 variants demonstrated reduced binding affinity to SRA (G1 exhibited greater affinity to SRA and less *in vivo* trypanolytic activity) at the physiological pH range found within the endolysosome of the trypanosome. This result suggests that the inability to bind SRA or SRA homologues does not lead to increased liver injury. The absence of liver injury in the case of baboon *APOL1* may be related to the fact that baboon *APOL1* possesses a disrupted BH3 domain [15] and necessitates further studies to explore the role of the *APOL1* BH3 domain in cellular injury.

In vitro studies

Recent observations by Lan *et al.* [34] have demonstrated the detrimental effects of the *APOL1* risk variants in human podocytes. *APOL1* risk variants caused podocyte swelling and reduced cellular viability at lower concentrations compared with wild-type *APOL1*. *APOL1* risk variants increased the permeability of lysosomal membranes, which manifested as diffuse lysosomal Lucifer Yellow and cathepsin L leakage, and might cause the disruption of F-actin. Chloroquine, a known blocker of endolysosomal acidification, and DIDS, a blocker of chloride channels, attenuated the podocyte injury that was induced by *APOL1* risk variants. The authors also reported a role for a secreted factor that is induced by APOL1 (not necessarily secreted APOL1); the addition of conditioned media from podocytes that were transfected with *APOL1* to non-transfected podocytes resulted in decreased cell viability. In parallel, changing the medium of the transfected podocytes every 12 h attenuated injury and increased podocyte viability. Finally, the study also demonstrated that the addition of several adverse host factors, such as H_2O_2, hypoxia, TNFα, puromycin and HIV, resulted in additive podocyte injury, which also fits with the 'second hit' theory. Consistent with human clinical and epidemiologic observations, the deleterious 'second hit' effect was most pronounced in HIV-infected podocytes. This finding represents the first demonstration of a differential effect of *APOL1* risk variants on kidney cells. *In vitro* evidence has suggested that lysosomal disruption mediated by increased chloride permeability, similar to the lysosomal disruption in *Trypanosoma*, may represent one of the deleterious pathways induced by the *APOL1* risk variants.

CONCLUSION

APOL1-associated nephropathy can be summarized with the following paradigm: the genetic risk of African ancestry (which includes *APOL1* susceptibility and perhaps other loci), along with modifier loci, such as podocin or others, and a modifiable environmental second hit leads to progression of various forms of kidney disease, including FSGS and progressive CKD of other aetiologies [68]. This formulation has major public health implications, especially in parts of the world where high-allele frequencies of *APOL1* are present, such as certain regions of West Africa. *APOL1* confers markedly increased susceptibility to kidney disease in tens of millions of people. The prevention and treatment of *APOL1*-associated nephropathy will involve the identification, prevention and treatment of the second hit and/or the restoration of protection of the unsecured podocytes. The World Health Organization recently adopted a similar paradigm, shifting its focus to non-communicable chronic diseases with genetic susceptibility and targeting interventions to prevent the environmental triggers of disease in genetically susceptible individuals [69].

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest. The manuscript has been reviewed and approved by the authors and is not under consideration for publication elsewhere.

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