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A focus on the association of Apol1 with kidney disease in children

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Abstract

Individuals of African origin have an increased risk of developing various progressive chronic kidney diseases (CKD). This risk has been attributed to genetic variants (G1, G2) in apolipoprotein-L1 (*APOLI*) gene. In the pediatric population, especially in children affected by sickle cell disease (SCD), by human immunodeficiency virus (HIV), or with various glomerular diseases, *APOLI* risk variants have been associated with the development of hypertension, albuminuria, and more rapid decline of kidney function. The present review focuses on existing *APOLI*-related epidemiological data in children with CKD. It also includes data from studies addressing racial disparities in CKD, the *APOLI*-related innate immunity, and the relationship between *APOLI* and CKD and pathogenic pathways mediating *APOLI*-related kidney injury.

Keywords APOL1 · Chronic kidney disease · Children · Genetics · Sickle cell disease · HIV-associated nephropathy

Introduction

Basis for racial disparities in CKD

Many regions around the world, particularly low- and middle-income countries (LMICs), are experiencing an increased prevalence of chronic non-communicable diseases, including chronic kidney disease [1]. In the USA, since 1984, the Secretary of Health's Task Force Report on "Black and Minority Health" has highlighted a dramatic health disparity

in blacks, Hispanics, Asian/Pacific Islanders, and Native Americans [2]. This disparity is more pronounced with regard to kidney diseases. Compared with Americans of European ancestry, African Americans have an approximately fourfold greater lifetime risk of kidney failure and, on average, require initiation of kidney replacement therapy at a younger age [3].

To explain this disparity, many theoretical models have been proposed showing the interactions and impact of socio-economic status (SES) (education, employment, and income) on social determinants of health (neighborhood and environment, psychosocial factors, and health care access) [4]. Among self-identified African-Americans, low income (< \$8000/year) was strongly associated with prevalent kidney dysfunction defined by estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73 m² [5]. It has been postulated that a lower SES leads to a lack of affordable healthy foods and poor access to health care, resulting in late diagnosis and treatment of diseases such as diabetes mellitus (DM) and hypertension (HTN), two major causes of chronic kidney diseases (CKD) [6, 7]. Regarding children, it is now generally accepted that there are specific risk factors for the development and progression of CKD in African American children [4]. Some are biological, such as premature birth or low birth weight or the presence of genetic predisposition (especially the presence of the high-risk apolipoprotein L1 (*APOLI*) genotype), and others are social, such as the lack of access to timely health care or adequate treatment [4, 5].

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The present review focuses on *APOL1* as a genetic risk factor of CKD with particular attention on existing epidemiological data in children. It also includes data from studies addressing the *APOL1*-related innate immunity, and the relationship of *APOL1* and CKD and pathogenic pathways mediating *APOL1*-related kidney injury.

Genetic predisposition to CKD among those of African descent

Since the report of dramatic health disparity in American populations between the 1980s and 2008, genetic risk of kidney disease in association with complex kidney phenotypes was largely underestimated [8]. Interestingly, it has been observed that individuals of African origin carry an excessive burden of CKD and kidney failure compared with individuals of other ancestries, even after adjusting for socioeconomic status, lifestyle, and clinical factors including diet and hypertension, suggesting genetic factors as a key contributor to this racial disparity [9]. These observations led to the analysis of ancestry informative population variation data to help explain this ancestry-driven health disparity [10].

In 2008, two research groups [11, 12] provided the first indication that genetic variants on chromosome 22 contribute to the pathogenesis of CKD [13]. They demonstrated highly significant associations of markers on human chromosome 22q with idiopathic focal segmental glomerulosclerosis (FSGS), HIV-associated nephropathy (HIVAN), and nondiabetic kidney failure in African Americans [3]. Using mapping by admixture linkage disequilibrium (MALD) to quantify the ancestry percentage of each genetic locus, both groups identified a locus on chromosome 22q12 associated with nondiabetic kidney disease in those of African descent [13, 14]. This 1.2-Mb locus contained 35 genes, including non-muscle myosin heavy chain 9 (*MYH9*) [13]. In the kidney, *MYH9* encodes an essential protein required for the proper functioning of podocyte cytoskeleton, being involved in cell motility and cell–cell adhesion [11]. Previously associated with glomerulosclerosis in patients with autosomal-dominant MATINS syndrome (macrothrombocytopenia, granulocyte inclusions with or without nephritis, and sensorineural hearing loss, MIM no. 155100) and given its podocyte expression, *MYH9* was rapidly identified as a particularly strong genetic candidate to explain the association of this locus with CKD [13, 14]. However, despite the initial enthusiasm, no plausible causal variants with a clear predicted functional effect have been identified within this gene [3, 10]. However, the extended linkage disequilibrium and haplotype length in the *MYH9* genetic locus suggested the possibility of effective causal variants in the neighboring genes [9].

In 2010, two research groups [15, 16] re-examined the chromosome 22 locus for causal variants outside of *MYH9*,

using data from the International HapMap and 1000 Genomes Projects, consisting of DNA sequences data of hundreds of individuals, including Africans and Europeans. Data from these studies revealed new information on available coding variants within the *APOL1* gene that were in strong linkage disequilibrium with *MYH9* risk haplotypes and exhibited even stronger associations with FSGS, hypertension-attributed kidney failure, and nondiabetic kidney failure [9]. *APOL1* is a 14.5-kb gene with seven exons, located less than 14-kb upstream of *MYH9*, and encoding apolipoprotein L1 (APOL1), a 398 amino acid protein. Two main coding variants in *APOL1* associated with kidney disease have been identified in the last exon (exon 7): the G1 allele, with two SNPs [rs73885319 (A → G) and rs60910145 (C → T)] which result in 2 amino acid substitutions: serine for glycine at position 342 [p.Ser342Gly] and isoleucine for methionine at position 384 [p.Ile384Met], respectively. These two nonsynonymous coding variants are in almost complete linkage disequilibrium since they are always present together within an allele; the G2 allele, located at 12 base pairs away from G1 (rs60910145) site, is a 6 base pair deletion (rs71785313), resulting in a two-amino acid deletion at the C-terminus, asparagine and tyrosine amino acid at positions 388 and 389 (p.N388_Y389del) [15, 16]. The two coding variants in *APOL1* are in complete negative linkage disequilibrium since they never occur on the same allele. Based on odds ratios (OR) and *p* values, *APOL1* risk variants were more strongly associated with kidney failure than potential *MYH9* risk variants [15, 17]. African Americans homozygous or compound heterozygous for *APOL1* risk alleles had increased risk of HIVAN (OR = 29), FSGS (OR = 17), and CKD stage 5 (OR = 7) [10]. Given the high frequency of these kidney risk variants (KRVs) in African Americans and Yoruba (Nigeria) and their absence in individuals of European ancestry and Asians, it has been postulated that positive selection increased the frequency of these *APOL1* KRVs in Africa [16].

How does *APOL1* confer an innate immunity?

Prior to its known role in kidney disease, *APOL1* was initially known as a protector against certain trypanosomes in humans and higher primates [10]. In addition, harboring of *APOL1* KRVs enhanced protective ability against human African trypanosomiasis (HAT), the deadliest form of African sleeping sickness, which was endemic in Africa many years ago and still affects millions of Africans today [10, 15].

APOL1 is part of a six-member family of *APOL* genes (*APOL1*, *APOL2*, *APOL3*, *APOL4*, *APOL5*, and *APOL6*) located on chromosome 22 that arose through gene duplication [18, 19]. Although several *APOL* genes are widespread in the animal kingdom, *APOL1* is present only in a few species in the primate lineage, namely humans, gorillas, and baboons

[18]. APOL1, a protein product of *APOL1* gene, is also expressed widely in tissues, particularly in the lung, placenta, pancreas, liver, and kidney. In the kidney, APOL1 was found to be expressed in extraglomerular vascular endothelial cells, podocytes, and proximal tubular cells [13, 19]. APOL1 circulates in blood at high levels as part of the high-density lipoprotein (HDL) complex that also contains apolipoprotein A1 (APOA1) and haptoglobin-related protein (HPR). This complex, named trypanosome lytic factor (TLF), confers innate protection against subspecies of *Trypanosoma brucei* [18, 19]. Two different serum complexes have been identified as trypanosome lytic factors. TLF1 is the densest fraction of HDL particles (known as fraction 3 or HDL3) and TLF2 is a distinct complex that is poor in lipids but that contains natural IgMs as a major component. Both complexes also contain the primate-specific proteins HPR and APOL1. HPR is involved in targeting TLF1 to the parasite and APOL1 is the trypanolytic toxin of both complexes [19, 20].

As illustrated in Fig. 2, APOL1 has five functional and structural domains: a secretory domain, pore-forming domain, B cell lymphoma 2 homology domain 3, membrane-addressing domain, leucine zipper domain, and serum resistance-associated (SRA) interacting domain, listed from the N-terminal to C-terminal respectively [10]. The secretory domain allows expression of serum protein. HPR enables internalization of TLF1 into the parasite, as the TLF1-bound HPR-hemoglobin complex is an alternative ligand for the *T. brucei* surface receptor for haptoglobin-hemoglobin, which is known as TbHpHbR [20]. While TLF1 uptake occurs by receptor-mediated endocytosis, how TLF2 enters trypanosomes is still unclear. TLF2 can be internalized by trypanosomes in the absence of TbHpHbR. After TLF1 and/or TLF2 uptake, APOL1 seems to be released from the carrier complexes and delivered to the lysosome where it causes osmotic swelling. On the other hand, the kinesin TbKIFC1 allows APOL1 delivery to the mitochondrion, leading to rapid membrane depolarization and TbEndoG nuclease release, resulting in nuclear DNA fragmentation and heterochromatinization [20, 21]. The pore-forming domain of APOL1 is responsible for trypanosome lysis, presumably following ionic-pore formation in endosomal membranes after anchoring via a contiguous membrane-addressing domain [20].

There are three main species of *Trypanosoma*: *Trypanosoma brucei brucei* infects many mammals but is unable to infect humans due to the innate protection conferred by TLFs; *Trypanosoma brucei rhodesiense* (*T.b. rhodesiense*) which occurs in East and Central Africa, causing acute HAT; *Trypanosoma brucei gambiense* (*T.b. gambiense*), widely distributed in West and Central Africa, causes chronic HAT, which is the most common [22]. Both *T.b. rhodesiense* and *T.b. gambiense* have evolved a mechanism to evade TLF lysis. *T.b. rhodesiense* evades lysis by expressing SRA proteins that bind to the C-terminus domain of APOL1, in the process

neutralizing its lytic activity leading to infection and sleeping sickness [10, 20]. In turn, *T.b. gambiense* resists TLFs via a multifactorial defense mechanism. A hydrophobic β -sheet of the *T. b. gambiense*-specific glycoprotein (TgsGP) prevents APOL1 toxicity and induces stiffening of membranes upon interaction with lipids. Two additional features contribute to resistance to TLFs: reduction of sensitivity to APOL1 requiring cysteine protease activity, and TbHpHbR inactivation due to an L210S substitution [20]. The *APOL1* risk variants are located near the C-terminus in the SRA domain (Fig. 2), the binding site for SRA protein, a trypanosomal virulence factor [19, 23]. It has been demonstrated that both *APOL1* risk variants G1 and G2 restore the lytic activity of human serum, and this provides the selective advantage to carriers against sleeping sickness caused by only *T.b. rhodesiense* but not *gambiense* [14, 15]. By restoring the trypanolytic activity of APOL1, these variants confer an adaptive advantage in the endemic regions of Africa, explaining the distribution of G1 and G2 risk variants in Africa [10, 21].

The understanding of distribution of G1 and G2 variants worldwide is driven by the origins, the selection story, and spreading of these variants. It is estimated that *APOL1* risk variants G1 and G2 emerged about 10 thousand years ago in the trypanosome endemic region across sub-Saharan Africa, originally in West Africa, where they have been subjected to intense positive selection [18, 19]. Humans who carried these *APOL1* risk variants had a survival advantage against potentially lethal trypanosomal disease, but at a cost of increased susceptibility to glomerular injury. This protective advantage led to rapid spread of these alleles in Africa, and was then brought to American continents from along the Atlantic coast of Africa, which was the source of the Atlantic slave trade [15, 18, 19].

Modifying factors: is carrying two *APOL1* risk variants sufficient for the development of kidney disease?

APOL1 risk variants confer an increased risk of non-diabetic CKD in African Americans [24]. However, it has been observed that only a minority of individuals who are homozygous or compound heterozygous for *APOL1* risk alleles (less than 15%) are predicted to develop kidney dysfunction and failure, reflecting the incomplete penetrance of *APOL1*-associated disease [19, 25]. This suggests that the presence of two *APOL1* risk variants is necessary, but not sufficient for the development of kidney diseases. Environmental and genetic interactions may contribute as modifier factors or a “second hit” to induce kidney disease [8, 25].

Various environmental factors have been reported, including HIV infection and non-HIV viral infections (such as John Cunningham (JC) polyomavirus), and factors of immune response and inflammation [24]. HIV is the strongest

environmental risk factor for *APOL1* kidney disease. In an individual with HIV infection, the high-risk genotype (HRG) increases the risk of developing HIV-associated nephropathy (HIVAN) by 29–89-fold [26, 27]. The JC polyoma virus has been proposed to mitigate the genetic risk posed by *APOL1* variants [10, 28]. Infection/colonization of the kidneys and urinary tract with JC polyoma virus has a paradoxical protective effect on risk for CKD in the presence of *APOL1* risk variants. The JC virus in the kidney was postulated to either protect against other nephropathic viruses or alter cellular function as protection against other sources of glomerular injury [10]. Moreover, other studies have extended this protective association with JC polyoma virus to non-*APOL1*-associated etiologies of nephropathy [24, 29].

Additional environmental factors, including some markers of inflammation, have been reported. In a case series of interferon (IFN)-induced collapsing nephropathy, all genotyped patients had the *APOL1* HRG [19, 30]. In vitro studies showed that multiple types of IFN could stimulate *APOL1* expression by up to 200-fold [30]. Lupus, HIV, and other FSGS induced by viral infections are known to be associated with high IFN state [19, 31]. Other inflammatory factors, including toll-like receptor (TLR) agonists and tumor necrosis factor (TNF)-alpha, have been shown to induce overexpression of *APOL1* in podocytes and endothelial cells leading to kidney disease [19, 30]. Furthermore, circulating soluble urokinase receptor (suPAR) has been shown to interact with *APOL1* to activate $\alpha v \beta$ integrin and induce podocyte injury [24, 32].

Several groups have studied the role of potential *APOL1* interacting genes in non-diabetic CKD. Some candidate genes, including podocin (*NPHS2*), serologically defined colon cancer antigen 8 (*SDCCAG8*), ecto-NOX disulfide-thiol exchanger 1 (*ENOX*), bone morphogenetic protein 4 (*BMP4*), a null variant in the apolipoprotein L3 (*APOL3*), and glutathione s-transferase mu 1 (*GSTM1*) genes have been explored [24]. However, no genetic modifiers have been shown to have a major impact on *APOL1* HRG penetrance [19, 24].

More recently, Langefeld et al. [33] performed a large genome-wide association study (GWAS) and meta-analysis to identify novel loci for non-diabetic CKD stage 5 in African Americans and to detect new genetic modifiers of *APOL1*-associated nephropathy. No significant genome-wide associations with kidney failure beyond *APOL1* were detected, and no single nucleotide polymorphism (SNP) showed significant genome-wide evidence of interaction with *APOL1* risk variants. Thus, it was concluded that *APOL1*-environment interactions may be of greater importance in triggering nephropathy in African Americans than *APOL1* interactions with other SNPs.

***APOL1* and biological mechanisms of kidney injury**

As previously mentioned, *APOL1* is expressed in various tissues and cell types. However, to date, the manifestations of kidney disease related to *APOL1* risk variants have been limited to the podocytes and possibly microvascular endothelial cells [9]. Although the precise mechanisms by which *APOL1* G1 and G2 variants induce injury in podocytes and other kidney cells, compared with the reference allele G0, remain unclear [24], various pathways of kidney cell damage by *APOL1* are being proposed and much of the data come from studies in cultured cells [9, 34] (Fig. 1). The first proposed mechanism consists of the overexpression of *APOL1* in podocytes associated with cellular injury and glomerular damage. Studies have reported that activation of TLR3 and cytokines such as IFN γ and TNF upregulates *APOL1* expression, regardless of the genotype, resulting in cell injury [30, 34, 35]. However, it has been demonstrated that overexpression of the *APOL1* risk variants is more injurious to cells than overexpression of the wild-type *APOL1* protein [30]. In addition, Poly(I:C), which is a double-stranded RNA TLR3 agonist, increased *APOL1* expression by upregulating IFNs. As in the second mechanism, increased expression of *APOL1* risk variants has been shown to induce inflammasome activation. Once activated, inflammasome processes pro-IL-1b and pro-IL-18 into active forms and induces cell death pathways [9, 30]. The third mechanism postulates that overexpression of G1 and G2 in cultured human podocyte activates protein kinase R (PKR), leading to reduced cell protein synthesis resulting in cell stress or death [36]. Furthermore, the activation of PKR also activates inflammasomes and results in activation of caspase-1 and generation of active IL-1b [30, 36]. In addition, using cell models, increased expression of risk variants has been shown to induce a loss of cellular potassium (K^+) with subsequent induction of stress-activated protein kinase (SAPK) pathways [24, 38]. It has been suggested that *APOL1* acts as K^+ -permeable cation-selective pore in the plasma membrane, and the risk variants G1 and G2 are associated with greater flux and cellular K^+ depletion [9, 24]. Another mechanism postulates that G1 and G2 overexpression impairs mitochondrial function. Indeed, Ma et al. [37] observed that *APOL1* G0 overexpression increased oxygen consumption rate and preserved respiratory capacity, whereas G1 and G2 overexpression decreased oxygen consumption rate and reduced respiratory capacity. This mitochondrial dysfunction could lead to plasma membrane Na^+/K^+ ATPase inhibition, resulting in intracellular potassium depletion. Thus, the authors suggested that mitochondrial dysfunction is present prior to intracellular K^+ depletion in cells overexpressing G1 and G2 alleles. In a sixth proposed mechanism, using cell and animal models, investigators demonstrated increased lysosomal permeability owing to expression of *APOL1* variants

Fig. 1 Postulated pathways of cell injury in APOL1-associated kidney disease. Eight mechanisms proposed to date to explain how APOL1 risk variants induce kidney cell damage. From the first pathway to the eighth, these are the following: overexpression of APOL1 risk variants [30, 35], inflammasome activation [30], PKR activation [36], mitochondrial defect

[37], K⁺/Na⁺ channel activation [38], autophagic flux defect [39, 40], cytotoxicity due to the tripartite complex [32], and upregulation of miR193a resulting in altered actin cytoskeleton [41]; APOL1 Vs ↑: increased expression of APOL1 risk variants

[39]. Furthermore, they reported that expression of *APOL1* variants disrupts endolysosomal trafficking, leading to autophagic flux defects followed by autophagic cell death [34, 39, 40]. In a recent study, Hayek et al. [32] identified high-affinity interactions between suPAR, APOL1, and $\alpha\text{v}\beta 3$ integrin, whereby APOL1 protein variants G1 and G2 exhibited higher affinity for suPAR-activated $\alpha\text{v}\beta 3$ integrin than for APOL1 G0. APOL1 G1 or G2 augmented $\alpha\text{v}\beta 3$ integrin activation and caused proteinuria in mice in a suPAR-dependent manner. They concluded that the synergy of circulating factor suPAR and APOL1 G1 or G2 on $\alpha\text{v}\beta 3$ integrin activation is a mechanism for CKD [24, 32]. More recently, Kuma et al., using a transduced human podocyte cell model, found that overt expression of APOL1 G0 in podocytes downregulated the expression of miR193a. The expression of APOL1 G1 and G2 was associated with upregulation of miR193a resulting in a disorganized actin cytoskeleton [41].

As noticed above, all these studies used cell and animal models to explain the mechanism whereby *APOL1* risk variant expression induces damage in kidney cells, but to what extent these mechanisms are involved in humans is still

unknown. Further confirmatory studies in models much closer to human conditions are needed in order to promote therapeutic approaches to thwart these kidney cell damage pathways.

APOL1 and susceptibility to CKD

The *APOL1* risk alleles have been observed only in individuals of African descent. While the presence of at least one allele (G1 or G2) is sufficient to confer protection against *Trypanosoma rhodesiense* (innate immunity occurring in dominant fashion), the transmission of kidney disease risk is consistent with recessive inheritance. As shown in Fig. 2, for the development of CKD, the presence of two alleles in homozygous (G1/G1 or G2/G2) or compound heterozygous (G1/G2) state is necessary and is defined as a HRG [7, 19]. Carrying one risk allele causes at most only a slight increase in kidney disease risk, whereas HRG increases this risk dramatically [19]. In this way, *APOL1* is similar to sickle cell trait: while patients who carry heterozygous and homozygous mutations in the hemoglobin gene are protected against malaria,

Fig. 2 Sequence variants in apolipoprotein L1 confer an innate immunity to trypanosomal infection and increase susceptibility to CKD in African populations. **a** Schematic illustration of position of *APOL* operon on human chromosome 22q12.3; the *MYH9* gene is also located in this region. The two genes are separated by 14,000 nucleotides. **b** Schematic diagram displaying the genomic organization of members of *APOL* gene family with *APOL1*, *APOL2*, *APOL3*, and *APOL4* gene cluster resulting from tandem gene duplication, whereas *APOL5* and *APOL6* are more distantly related. *APOL1* is in the opposite orientation to the other three genes. **c** The structure of *APOL1* (398 amino acid proteins) with multiple protein domains. **d** G1 (S342G and I384M) and

G2 (deletion of N388 and Y389) sequence variants are located at the C-terminus of *APOL1*. The close proximity of the G1 and G2 alleles results in a low likelihood of recombination between the two alleles. G1 and G2 are reciprocally exclusive and never appear together on the same chromosome. **e** Illustration of the different clinical consequences in *APOL1* carriers: individuals with two *APOL1* wild-type (WT) alleles (G0) are prone to *Trypanosoma brucei rhodesiense* infection. The presence of one *APOL1* risk variant, G1 or G2, expands trypanosomal resistance including *T. b. rhodesiense*. The presence of two *APOL1* risk variants continues to protect against *T. b. rhodesiense*, but increases the risk for *APOL1*-associated CKD

only those with the homozygous mutation develop sickle cell anemia [7, 19]. Nevertheless, a mild dominant influence (OR 1.2 for one risk allele compared with 7.3 for two risk alleles) has been observed in a larger cohort of hypertension-associated CKD. This raised the hypothesis of sporadic mutations which might occur in patients carrying one risk variant or additional rare variants in *APOL1*, *MYH9*, or other neighboring genes [10, 14].

Initially, studies which investigated the relationship between *APOL1* and kidney disease were mostly case-control studies of extreme phenotypes, especially CKD stage 5 and FSGS [8, 15]. Population-based longitudinal studies have been conducted subsequently and showed an association with progressive disease over the years leading to kidney failure [7, 8]. As described in Table 1, various studies have reported the odds of different kidney diseases in patients of African

Table 1 Odds of various kidney diseases in individuals of African ancestry carrying *APOL1* HRG

Population	Disease	OR (95%CI)	Year	Author
African Americans	Hypertensive ESRD	7.3 (5.60–9.50)	2010	Genovese [15]
	FSGS	10.5 (6.0–18.4)		
African Americans	Non-diabetic ESRD	4.86 (2.35–10.06)	2010	Tzur [16]
African Americans	HIVAN	29.2 (13.10–68.50)	2011	Kopp [26]
	FSGS	16.9 (11.00–26.50)		
South African blacks	HIVAN	89.10 (17.68–911.72)	2015	Kasembeli [27]
African Americans	SRNS	4.17 (2.23–7.64)	2018	Adeyemo [42]

ESRD, end-stage renal disease; FSGS, focal segmental glomerulosclerosis; HIVAN, human immunodeficiency virus associated nephropathy; SRNS, steroid-resistant nephrotic syndrome

ancestry carrying *APOL1* HRG [15, 16, 26, 27, 42]. An association between the HRG and increased risk of sickle cell nephropathy and increased risk of severe lupus nephritis has also been reported [43]. Furthermore, the presence of *APOL1* HRG has been found to accelerate progression of CKD to kidney failure in patients with FSGS and hypertensive nephrosclerosis despite immunosuppressive therapy and blood pressure control, respectively [7, 44]. Regarding diabetic nephropathy (DN), the relationship with *APOL1* remains particularly poorly defined. The *APOL1* HRG appears to be associated with progression of DN but not with the incidence of DN [45].

***APOL1* and the susceptibility to kidney disease in children and young adults**

As shown in several aforementioned studies, most existing data on *APOL1* and susceptibility to CKD are focused on adults. There has been a paucity of studies examining pediatric *APOL1*-associated CKD. Yet, there is a strong need for more prospective studies to determine the role of *APOL1* in the pediatric population. In response to this need, the collaboration between the Chronic Kidney Disease in Children (CKiD) Study and the Nephrotic Syndrome Study Network (NEPTUNE) allowed investigation of the *APOL1* status of children with glomerular diseases [46]. Similar to adults, children with glomerular disease who carried *APOL1* HRG had lower eGFR at study entry and experienced a steeper decline in kidney function. It has been demonstrated that children with *APOL1* HRG have a 2–4-fold faster rate of glomerular filtration decline than children carrying a low-risk genotype [46]. Few other studies have investigated the relationship between *APOL1* and CKD in children, including two prevalent diseases shown to be strongly associated with *APOL1*-mediated CKD: HIV infection and sickle cell disease (SCD) [8, 47].

HIVAN

The strongest association between *APOL1* and CKD in adults has been found for HIV-associated nephropathy (HIVAN), with an OR of 29 in the USA [26] and 89 in South Africa [27]. In a case-control study of 451 perinatal HIV infection (PHIV) participants in the Pediatric HIV/AIDS Cohort Study, Purswani et al. found that 27% of CKD cases in the adolescent cohort carried 2 risk alleles. Moreover, there were 3.5-fold increased odds of CKD in those with the *APOL1* HRG with unadjusted incidence of 1.2 CKD cases/100 person-years [47].

More recently, in the Democratic Republic of Congo, we assessed the association between *APOL1* HRG and early-stage CKD in 813 children (401 HIV-infected and 412 from the general population) [48]. From 412 healthy children recruited in the general population, albuminuria (defined as ACR ≥ 30 mg/g) was present in 40 (9.7%). We observed a weak association of HRG and albuminuria in the healthy population, which was not retained in a multivariate analysis. On the other hand, we found a strong association between *APOL1* HRG and albuminuria in HIV-infected children. From 401 HIV-infected children treated with antiretroviral drugs with non-kidney toxicity following the guidelines for integrated HIV management in DRC (the DRC National AIDS Control Program), albuminuria was detected in 72 (18.0%). Almost 78% of HIV-infected children who carried *APOL1* HRG presented with albuminuria, compared with 14.3% of those carrying LRG (unadjusted OR 21.60, 95%CI 7.25–76.62; $p < 0.001$). Multivariate logistic regression analysis showed that *APOL1* HRG (adjusted OR 32.56, 95% CI 9.94–106.58; $p < 0.001$) and a high viral load (defined by a copy number > 1000 /ml) (adjusted OR 10.34, 95%CI 5.29–20.23; $p < 0.001$) were the only independent risk factors associated with elevated albuminuria. Furthermore, HIV-infected children who carried the HRG had a significantly higher blood pressure, and the proportion of those with elevated blood pressure (defined as above 95th percentile) was almost double that observed in those without HRG.

Sickle cell nephropathy

APOL1 risk alleles may also be involved in the development of sickle cell nephropathy. Two recent studies reported the association of *APOL1* HRG with albuminuria and a lower eGFR. Kormann et al. [49] assessed the role of *APOL1* variants in SCD patients living in Europe. From 152 SCD young adult patients of African ancestry recruited in France, investigators showed that *APOL1* HRG was associated in an age-dependent manner with lower eGFR, ($p = 0.008$), proteinuria ($p = 0.009$), and albuminuria ($p < 0.001$). In a cohort of 413 SCD young adults from Cameroon, *APOL1* HRG was significantly associated with albuminuria ($p = 0.01$) and borderline with eGFR ($p = 0.07$) [50].

Nephrotic syndrome

Idiopathic nephrotic syndrome (INS) is the most frequent glomerular disease of childhood. Epidemiologic studies have established that there is significant ethnic disparity in the prevalence and clinical course of steroid-sensitive nephrotic syndrome (SSNS) [42], suggesting the interaction between environmental and genetic factors as major contributors. In view of the role of *APOL1* variants in a variety of CKD in populations of African ancestry, and considering the previous report on the role of the gene encoding major histocompatibility complex-class II-DQ-alpha 1 (HLA-DQA1) in children of South Asian and European ancestry with SSNS, Adeyemo et al. [42] tested the hypothesis that HLA-DQA1 and *APOL1* risk variants are associated with SSNS or steroid-resistant nephrotic syndrome (SRNS) in a sample of 115 African American children. They showed that HLA-DQA1 variants were significantly associated with SSNS in African American children (OR 4.8) but not SRNS. On the other hand, *APOL1* risk variants were not associated with SSNS but showed significant associations with SRNS (OR 4.17).

Furthermore, recently, Watanabe et al. reviewed the specific genes and loci discovered in childhood NS, including *APOL1*-associated NS in black patients [51]. The authors reported that in the pediatric nephrology clinic, they found 60–70% of African American children with NS harbor an *APOL1* HRG. However, they noticed that African-origin children with NS are not routinely screened for the *APOL1* HRG. The authors believe this would surely change in patients with NS if *APOL1*-targeted therapies are developed or modifiable factors are discovered that affect the progression of kidney disease in these children [51]. As with other varieties of pediatric CKD, there is still a paucity of studies examining pediatric *APOL1*-mediated NS. Yet, there is a need for more studies focusing on genetic risk factors of NS to explain disparities in disease incidence and response to therapy. The results may potentially lead to the identification of new therapeutic targets for nephrotic syndrome.

Cardiovascular morbidity

Some reports have shown that almost 14% of African Americans who carried *APOL1* HRG had an increased potential risk of developing CKD and possibly cardiovascular diseases [22]. Within the CKiD Study, Woroniecki et al. [52] reported increased odds of cardiovascular morbidities among African American children with FSGS and carrying *APOL1* HRG. The investigators observed a higher prevalence of uncontrolled hypertension, left ventricular hypertrophy (LVH), elevated C-reactive protein levels, and obesity in children with FSGS carrying *APOL1* HRG than in those bearing 0 or 1 risk allele [4, 52]. On the other hand, in children with hypertensive disease or FSGS, a higher proportion of *APOL1* risk alleles has been observed among those with a family history of kidney failure, emphasizing the important role of family history screening irrespective of genetic testing [8]. However, while the incidence of cardiovascular morbidities and kidney disease is generally higher among *APOL1* HRG compared with that among LRG carriers [52], it is still not clear whether this increased cardiovascular risk occurs due to the underlying CKD or is present independently [8, 52]. More studies are required to elucidate this issue.

APOL1 risk variants prevalence studies: data in children

Data on the incidence of *APOL1* alleles in Africa have shown highly variable frequencies of the risk alleles. Western Africa region has been reported to be the epicenter of *APOL1* variants, with the highest frequencies occurring in Ghana and Nigeria (G1, > 40%; G2, 6–24%); but these risk variants are nearly absent in Eastern African countries, such as Ethiopia [18, 22]. Furthermore, a scarcity of *APOL1*-related genotypic data was observed in Central Africa [22]. In addition, data showed that the G1 allele is highly concentrated in Western Africa, whereas the G2 allele is more widely distributed across the continent [18]. High prevalence of *APOL1* risk variants has also been reported in African Americans living in the USA, one of the destination countries of the Atlantic slave trade. It has been shown that the frequency of G1 and G2 alleles in African Americans is 20–22% and 13–15%, respectively, and approximately 10–15% have two *APOL1* risk alleles (HRG) [18, 19].

Moreover, most studies that have reported prevalence of *APOL1* risk alleles and examined the relationship between *APOL1* and kidney disease were focused on adults. A few recent papers have reported the prevalence of G1, G2, and *APOL1* HRG in the general pediatric population and in children with kidney disease (Tables 2 and 3). As shown in Table 2, in perinatal HIV-infected African American children, Purswani et al. reported that 13% carried *APOL1* HRG and

Table 2 Studies on prevalence of *APOL1* HRG in pediatric population

Population	Country	Author	Year	Participant (<i>n</i>)	<i>APOL1</i> HRG (%)
Perinatal HIV infection AA children	USA	Purswani [47]	2016	302	13
Young adults of African descent with sickle cell	France	Kormann [49]	2017	152	7
HIV-infected children	DRC	Ekulu [48]	2019	401	6
General pediatric population	DRC	Ekulu [48]	2019	412	7

HRG, high-risk genotype; *AA*, African American; *USA*, United States of America; *HIV*, human immunodeficiency virus; *DRC*, Democratic Republic of Congo

43% had one risk allele (G0/G1, G0/G2) [47]. Kormann et al. reported 7% of *APOL1* HRG in a cohort of young adults of African descent with SCD living in France [49]. More recently, in Central Africa, we reported the prevalence of *APOL1* KRVs in 813 children. Of 412 children from the general population, the frequency of the G1 and G2 alleles was 12.4% and 10.4%, respectively, and 7% had *APOL1* HRG. On the other hand, of the 401 HIV-positive children, there were 13.5% and 9.6% of G1 and G2 alleles, respectively, and 5.7% had *APOL1* HRG [48]. In addition, as shown in Table 3, the prevalence of *APOL1* HRG (9–46%) has been reported in pediatric populations presenting with some features of kidney disease [42, 46–49, 52].

***APOL1* implications in kidney transplantation in children and young adults**

Data from the CKiD study showed that African American children reached the start of kidney replacement therapy 3.2 years earlier than non-African American children [46]. Recently, Guan et al. [53] evaluated associations between race and transplant outcomes in children diagnosed with FSGS. The authors observed that graft survival was significantly shorter in black children than that in the non-black group ($p < 0.005$). African-origin children presented with 1.3 increased risk of graft failure, and 1.7 and 1.5 increased odds

of acute rejection and delayed graft function, respectively. Interestingly, some retrospective studies have reported shorter kidney allograft survival from deceased donors with *APOL1* HRG [54, 55]. These studies suggest that the worse survival of kidney grafts from African American donors is due to the presence of HRG. They observed that the recipient *APOL1* genotype status had no effect on the outcomes of kidney allografts, suggesting little role for circulating *APOL1* protein in shorter kidney allograft survival.

Moreover, these observations raise an important ethical concern related to the implementation of routine *APOL1* testing in clinical care, particularly among potential African American kidney donors. It has been suggested that determining *APOL1* genotype among routine kidney donor panel tests in recent African ancestry could better predict outcomes after kidney transplantation [56]. Implications of these observations have a particular resonance in children and young adults, whose parents may wish to donate regardless of the risk related to their *APOL1* status, given that living kidney donation is known to be better for the child. This issue is all the more delicate to address as the number of recipients continues to increase while the number of donors decreases [8]. While there is evidence of the strong association between *APOL1* HRG and worsened allograft outcomes, the causality of *APOL1* HRG has not been established yet [7]. Some authors propose that routine *APOL1* variant testing should be performed in routine clinical care and recommend HRG

Table 3 Prevalence of *APOL1* HRG in children and young adults with features of kidney disease

Population with kidney disease	Country	Author	Year	Participant (<i>n</i>)	<i>APOL1</i> HRG (%)
AA children with FSGS	USA	Woroniecki [52]	2016	140	24
Perinatal HIV infection AA children with CKD	USA	Purswani [47]	2016	23	30
AA children with glomerular disease	USA	Ng DK [46]	2017	104	46
Young adults of African descent with sickle cell with U-ACR ≥ 30 mg/g	France	Kormann [37]	2017	111	9
AA children with SRNS	USA	Adeyemo [42]	2018	50	40
AA children with SSNS	USA	Adeyemo [42]	2018	65	17
HIV-infected children with U-ACR ≥ 30 mg/g	DRC	Ekulu [48]	2019	72	25
General pediatric population with U-ACR ≥ 30 mg/g	DRC	Ekulu [48]	2019	40	12.5

HRG, high-risk genotype; *AA*, African American; *FSGS*, focal segmental glomerulosclerosis; *USA*, United States of America; *HIV*, human immunodeficiency virus; *CKD*, chronic kidney disease; *U-ACR*, urine albumin-to-creatinine ratio; *SRNS*, steroid-resistant nephrotic syndrome; *SSNS*, steroid-sensitive nephrotic syndrome; *DRC*, Democratic Republic of Congo

African carriers not to donate without strong evidence of causality of *APOL1* HRG, which would decrease the number of living donations among African American individuals [7, 8]. Thus, limiting screening to potential donors with a family history has been suggested before considering the extension of *APOL1* genotyping in all donor candidates, until we have further data [8]. Consequently, a consortium, *APOL1* Long-Term Kidney Transplantation Outcomes-Network (APOLLO), is conducting a prospective study on the implication of both donor and recipient *APOL1* genotypes on kidney allograft outcomes. Results from APOLLO will support developing policies for matching donors with recipients to prolong allograft survival [56].

Conclusions

Besides more extensive data in adults, a few recent papers have reported the prevalence of *APOL1* risk variants G1 and G2 in the general pediatric population, in children with kidney diseases, and in those at risk, especially in SCD and HIV-infected children. These data provide further evidence that carrying *APOL1* HRG increases the risk for kidney disease in children of African origin. However, it has been observed that only a minority of *APOL1* HRG carriers are predicted to develop CKD, suggesting the presence of two *APOL1* risk variants is necessary, but not sufficient for the development of nephropathies. A better understanding of trigger factors predisposing to *APOL1* nephropathy is of great clinical importance. Moreover, questions remain about the implementation of *APOL1* testing in routine clinical practice, and particularly among potential kidney donors. Plausible mechanisms whereby *APOL1* risk variants promote podocyte injury have been proposed. The relevance of these cellular pathways needs to be confirmed in humans with *APOL1*-related nephropathies. This might allow the targeting of pathways that are more critical for organ damage in order to develop appropriate therapeutic agents.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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