



Published in final edited form as:

Trends Mol Med. 2018 August ; 24(8): 682–695. doi:10.1016/j.molmed.2018.05.008.

APOL1: The balance imposed by infection, selection, and kidney disease

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Abstract

Chronic kidney disease (CKD) affects millions of people and constitutes a major health and financial burden worldwide. People of African descent are at an increased risk of developing kidney disease, which is mostly explained by two variants in the Apolipoprotein L1 (APOL1) gene that are found only in people of West African origin. It is hypothesized that these variants were genetically selected due to the protection they afford against African sleeping sickness, caused by the parasite *Trypanosoma brucei*. Targeting mutant APOL1 could have substantial therapeutic potential for treating kidney disease. In this review, we will describe the intriguing interplay between microbiology, genetics, and kidney disease as revealed in APOL1-associated kidney disease, and discuss APOL1-induced cytotoxicity and its therapeutic implications.

Keywords

Apolipoprotein 1; chronic kidney disease; genetics

APOL1 in chronic kidney disease- a game changer

Chronic kidney disease (CKD) and **end stage renal disease (ESRD)** affect millions of people of African ancestry throughout the world. African-Americans, while only 13% of the general population in the US, constitute 32% of dialysis patients [1, 2]. Much of this increased risk is attributed to two risk variants (named G1 and G2) that were recently identified in the Apolipoprotein L1 (*APOLI*) gene [3]. About 40% of all African-American dialysis patients carry these variants [4] and it is estimated that these variants explain about 70% of the excess ESRD risk in this population.

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Competing interests: The Susztak lab receives research support from Biogen, Boehringer Ingelheim, Celgene, GSK, Merck, Regeneron and ONO Pharma for work not related to this manuscript.

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Variants in APOL1 are thought to have arisen as a result of positive genetic selection, as they confer resistance against *Trypanosoma brucei rhodesiense*, a parasite that causes **African sleeping sickness** [5]. In sub-Saharan Africa, sleeping sickness threatens millions of people. During the most recent epidemic the prevalence reached 50% in some regions. While having one risk variant (G1 or G2) imparts this crucial resistance against sleeping sickness, having two risk alleles significantly increases the risk of developing kidney disease [6]. It is estimated that 20% of people with two risk alleles will eventually develop kidney disease [7]. Genetic variants increase CKD risk with an odds ratio of 2–89, depending on other environmental factors such as HIV infection. Understanding the mechanism by which these risk variants cause kidney disease will be important for future therapeutic targeting.

A tale of two mutations: The interplay between the human Apolipoprotein L1 and the Trypanosome

The first studies that analyzed the potential contribution of genetic factors to CKD/ESRD development in the African-American population used admixture linkage disequilibrium (MALD) methods. These studies identified a genomic region on chromosome 22 that showed an increase in African ancestry marks compared to healthy controls [8, 9]. The initial analysis proposed that the risk variant is located on the intronic region of *MYH9*. In 2010, using denser genotyping data from the 1,000 genome project, two groups reported that the association can be explained by coding variants in the Apolipoprotein L1 gene [10, 11]. After controlling for the *APOL1* variants, the *MYH9* locus was no longer associated with kidney disease [10]. G1 represents two missense mutations that are in near-complete linkage disequilibrium, and G2 results in deletion of two amino acids. Both variants are in the C-terminal domain on the gene. The G1 and G2 alleles are common, with 50% of African-Americans carrying one or two risk alleles and 10–15% carrying two risk alleles (G1/G1, G2/G2 or G1/G2) [12, 13]. Having two of the risk alleles significantly and reproducibly increased the risk for a wide range of kidney diseases including focal segmental glomerulosclerosis (FSGS), lupus nephritis, sickle cell disease, hypertensive renal disease, and HIV associated nephropathy (HIVAN) [14–20].

APOL1 is a recently evolved gene. It is present only in humans and a few primates [21, 22]. It circulates as part of the high density lipoprotein (HDL) complex and imparts resistance to infection with *Trypanosoma Brucei* through lysosomal swelling and lysis of the parasite [23–25]. Several Trypanosome subspecies (*T b rhodesiense* and *T b gambiense*), developed mechanisms to avoid APOL1-mediated lysis and cause African sleeping sickness in humans, a potentially lethal disease [26, 27]. *T. b. rhodesiense* escapes from APOL1-induced toxicity by expressing a protein called serum resistance-associated protein (SRA), that binds to and neutralizes APOL1 [28]. It is hypothesized that genetic variants in *APOL1* emerged as a result of positive genetic selection [29]. The selective sweep originated in West Africa and mostly involved the G1 allele, although evidence of a selective sweep has also been shown for the G2 allele [3]. Long-range haplotype tests based on extended haplotype homozygosity suggest that the selective sweep occurred within the last 10,000 years [30]. Recently, studies have added important insight into the evolutionary genetics of APOL1 risk variants. G1's and G2's protective properties against *T b rhodesiense* and *T b gambiense* were

demonstrated in an epidemiological study in West and East Africa [31]. G2 was shown to protect against *T b rhodesiense* in East Africa, thus explaining the high allele frequency and its association to this region. In a study in West Africa, G1 did not protect against *T b gambiense* but was rather associated with a latent asymptomatic infection. On the other hand, a different study in Central Africa failed to identify a protective effect of G2 against *T b rhodesiense* [32]. These results suggest that the relationship between the evolution of APOL1 risk variants and the *trypanosome* might actually be more complex than previously imagined.

APOL1 genetic variants: Proving causality

The properties of the G1 and G2 *APOL1* genetic variants are highly unusual. The allele frequencies of the G1 and G2 *APOL1* risk variants are high [13], and the odds of disease development with either allele are also unusually high for a complex trait variant [7]. In addition, while most complex trait variants are non-coding, G1 and G2 are in the coding region of the gene, resulting in alterations in the translated protein. Animal models are currently the most effective tool to establish causality between genetic variants and disease. Such models can aid the understanding of disease mechanisms and drug development. However, *APOL1* is a recently evolved gene that exists only in the genome of humans and some primates, making the construction of animal models challenging. To avoid this problem, several groups have targeted the entire *APOL1* locus when designing animal models. While the genetic knock-in of the human locus should be a powerful method, it is not necessarily expected to lead to phenotype development as only 20% of people with two risk alleles develop kidney disease [33]. Furthermore, neither the regulation of *APOL1* nor the disease causing cell type is well understood [34, 35]. To this end, our lab has generated a conditionally inducible G0, G1 or G2 *APOL1* mouse model, where the transgene can be induced by doxycycline administration [36]. By mating this mouse model with cell-type specific inducer mouse strains, cell-type specific *APOL1* expression can be achieved. In our mouse model, expression of G1 or G2 *APOL1* variants in **podocytes** induced albuminuria. Leakage of serum albumin into the urine is one of the key phenotypic manifestations seen in patients with high-risk *APOL1* genotypes. The albuminuria was most likely the result of structural damage to the glomerular filter—including podocyte foot process effacement and solidified glomerulosclerosis—observed in the animal model, as well as in patients with *APOL1*-associated kidney disease [36]. As a result of the severe functional and structural damage, *APOL1* transgenic animals developed azotemia and died of renal failure. (Figure 1) Molecular studies, using RNA sequencing of podocyte-specific *APOL1* transgenic animals, indicated similarities in the mouse model to the transcriptomes of human FSGS patients with *APOL1* high risk genotype, further supporting that the mouse model recapitulates the human disease [37].

A previous study described a mouse model with podocyte-specific constitutive expression of G0 or G2 *APOL1* that did not develop glomerulosclerosis or proteinuria. In this model, *APOL1* expression was significantly lower in the G2 mice compared to G0 mice. The constitutive expression of *APOL1* potentially caused a selective pressure and decreased toxicity. Yet, this mouse model developed podocyte loss over time and a phenotype similar

to preeclampsia, suggesting that even under constitutive and lower expression of APOL1, podocyte loss can be demonstrated, supporting a causal role [33].

Using the conditionally inducible transgenic approach, expression of APOL1 in kidney **tubule cells** did not result in phenotype development in our study, indicating cell type specific toxicity [36]. Furthermore, studies using mice with hydrodynamic gene transfer-mediated expression of APOL1 in the circulation also failed to induce renal phenotype development [30]. Animal model studies are consistent with human observational studies, indicating that kidney-specific expression of risk variant APOL1 is critical for phenotype development. Studies from the transplant literature indicate that the kidney genotype, rather than the recipient genotype, correlates with inferior outcome after kidney transplantation. High-risk allele donor allografts are associated with decreased graft survival, independent of the recipient genotype [38–42] (see Clinician’s Corner, Box 1).

In humans, the majority of APOL1 circulates in the plasma [23]. Most APOL1 is secreted by the liver, as evidenced by mass spectrometry of liver transplant patients [43]. On the other hand, transplanting low or high risk variant APOL1 livers had no effect on liver graft survival [44]. There is also no correlation between circulating APOL1 concentrations and renal phenotype (GFR or proteinuria) in a large population-based cohort [45]. On the other hand, our group found a significant negative correlation between glomerular APOL1 transcript level and kidney function in patients [36]. The clinical observations are consistent with *in vitro* and animal model results. Deletion of the *APOL1* signal peptide did not abolish toxicity in human embryonic kidney (HEK) cell line 293T transfected with risk allele *APOL1*, indicating that the endogenous (i.e. cell autonomous) and not the secreted (circulating) APOL1 is important for disease development [46]. A recent study suggested that APOL1 may form a complex with soluble urokinase-type plasminogen activator receptor (suPAR) and integrins on the podocyte plasma membrane [47]. This complex was identified using surface plasma resonance (SPR) studies, and confirmed *in vitro* in transfected HEK cells and in human cultured podocytes using immunoprecipitation and integrin activation studies, respectively. Furthermore, expression of risk variant *APOL1* in mice using gene delivery technique induced proteinuria as well as integrin activation; no proteinuria was observed following the expression of *APOL1* in suPAR-deficient mice. However, the origin of the APOL1, either exogenous or endogenous that is secreted from the podocyte and re-internalized through the suPAR-integrin complex, was not determined. Taken together, these data suggest that G1 and G2 variants are pathogenic mutations and that cell autonomous expression of *APOL1* in podocytes plays a key role in kidney disease development.

The second hit: environmental hits are necessary for disease development

While the risk of chronic kidney disease development for people with G1 and G2 APOL1 variants is significant, close to 80% of people with two risk alleles will still not develop disease [7]. Characterizing the role of environmental factors as second hits is crucial to the understanding of APOL1 associated kidney disease. Such studies indicate that the high-risk *APOL1* genotype confers an odds ratio of 30–90 for HIV associated nephropathy (HIVAN). The lifetime risk for HIVAN in untreated HIV-infected individuals carrying two risk alleles

is 50% [7, 19, 20, 48, 49]. Indeed, 60–70% of patients who present with classic HIVAN (collapsing FSGS pathology) have the high-risk APOL1 genotype. Similarly, a subset of patients with the high-risk APOL1 genotypes who received interferon injections for other disease conditions developed proteinuria and histological lesions such as FSGS [50]. These studies strongly support that viral infections, most likely via an interferon-mediated mechanism, present an important second hit for kidney disease development [51, 52].

In cultured human podocytes and endothelial cells, interferon is one of the strongest inducers of APOL1 RNA and protein expression, as APOL1 has multiple STAT-binding sites on its regulatory regions [36, 53]. Thus, a relatively simple model has been proposed, in which environmental factors induce kidney disease development by increasing risk variant APOL1 expression in podocytes. (Figure 2) Multiple observations support this hypothesis. In animal models and cell culture systems, *APOL1*-induced toxicity not only depends on risk variant *APOL1*, but it is also highly dose dependent [30, 54–56]. Increasing APOL1 mRNA or protein expression is associated with higher toxicity [36]. Furthermore, transcriptomic studies of a large collection of human kidney glomerular samples indicated that APOL1 transcript expression is higher in patients with kidney disease and the amount linearly correlates with kidney function [36]. Taken together, this data suggests a possible mechanism in which environmental triggers, such as viral infections, induce interferon expression, which then increases APOL1 concentration above a certain critical threshold that leads to podocyte injury and progressive CKD. (Figure 2)

Several other factors have been proposed as second hits [57]. For example, it was published that serum suPAR is a good predictive biomarker for kidney disease development among people with the high-risk *APOL1* genotype [47]. suPAR can potentially bind to podocyte integrins and APOL1, triggering the disease. Further studies are needed to identify factors that serve as second hits and the mechanism by which these factors cause disease.

Gained or lost? APOL1 recessive mode of inheritance and gain of function mutation: are they truly contradictory?

Studies indicate that APOL1 variants bind Trypanosomal SRA less efficiently than wild type APOL1 [18]. The variant therefore represents a gain-of-function variation, whereby the *Trypanosome* is unable to escape the lytic activity of APOL1. However, most studies have been unable to detect a statistically significant increased risk for kidney disease in patients carrying only one risk variant [11, 15]. The recessive mode of inheritance usually suggests a loss of function mechanism. Whether the G1 and G2 variants induce kidney disease by loss or gain of function has been a matter of extensive discussion [58].

Studies indicate that knock-down of the APOL gene cluster in zebrafish results in renal phenotype development, thus supporting a loss-of-function mechanism which is consistent with the mode of inheritance [59]. However, the *APOL1* gene is not conserved in most species and it is unclear whether the phenotype is APOL1 related [21]. Moreover, an individual with homozygous loss of function *APOL1* has been identified in India [60]. This individual presented with repeated episodes of Trypanosomiasis, but no obvious renal

abnormalities were detected. These observations indicate that *APOL1* is a non-essential gene and therefore the renal phenotype is unlikely to be caused by a loss-of-function.

There are several possible explanations for the need of two risk alleles for phenotype development. One possibility is that APOL1 is present in the body as a multimer [61]. The multimerization model proposes that APOL1 toxicity is antagonized by an unknown factor that interacts with wild-type APOL1, and that this antagonism is attenuated by binding to G1 and G2. When one risk allele APOL1 is present, there is sufficient APOL1 multimer, that carry at least one wild-type APOL1, thus the toxicity-blocking binding of the factor is retained. When two risk alleles are present, multimers lacking wild-type APOL1 predominate, resulting in toxicity. The ability of APOL1 to multimerize *in vitro* has been reported, yet there is no formal proof of this model [61]. Another alternative could be that the effect of a single risk allele is small and larger studies are needed to identify a significant association. A recent study indicates that in Sub-Saharan Africa, where both APOL1 risk variants and HIV incidence is much higher than in previously studied African American populations, even heterozygous individuals have a significantly increased risk of developing CKD [49]. In addition, because the *APOL1* risk variant associated toxicity is dose dependent, it is possible that having two risk alleles increases the likelihood of reaching the critical threshold to induce kidney disease. (Figure 2)

APOL1-mediated Trypanosome lysis and renal injury: Similar mechanisms?

APOL1 is a 398 amino acid protein. Five functional domains have been defined in the protein: the S domain is a secretory signal, the MAD (membrane-addressing domain) is a pH sensor and regulator of cell death, the BH3 domain is associated with programmed cell death, the PFD (pore forming domain) and SRA (serum resistance-associated) binding domains confer resistance to *Trypanosoma brucei* [62–64]. The G1 and G2 variants alter the SRA binding domain of the protein, therefore specific attention has been paid to this domain. The structure of SRA was recently determined through crystallography and the APOL1-binding site was identified [65]. This finding highlighted flaws in the current SRA-APOL1 interaction model, and may help identify SRA-like candidates for APOL1 binding in human cells. The C-terminal coiled coil domain of APOL1, which directly interacts with SRA, has also been recently characterized through nuclear magnetic resonance (NMR) and molecular dynamics simulations [66]. The model predicts a helical hairpin, comprised of two antiparallel helices, that has a more compact and stable fold in G0 than G1 or G2, affecting SRA binding, as well as potentially human protein targets.

APOL1 circulates in the human blood as part of the high density lipoprotein complex. A model based on studies of APOL1 in planar lipid bilayers measuring pH-dependent ion conductance was recently described. The model stipulates that APOL1 is endocytosed by the parasite and inserts into the vesicular membrane when exposed to the low lysosomal pH [67]. Later, upon APOL1 recycling to the plasma membrane and exposure to neutral pH, it creates cation channels that transport sodium, with secondary chloride and potassium fluxes, thus inducing osmotic swelling and death of the parasite [68]. This model suggests that SRA prevents APOL1 from inserting into endocytic membranes, so when recycled to the plasma membrane, it is released from the parasite without creating lethal channels. The resemblance

of the APOL1 pore-forming domain to the bacterial pore forming colicinA is the structural basis of this hypothesis.

Multiple publications suggest that APOL1 forms cation channels in membranes; either sodium or potassium [69–71]. Yet, other studies indicate that APOL1 functions as an anion channel that is activated in low pH, transporting chloride across lysosomal membranes, thus inducing lysosomal swelling and cell death [54, 72–74]. A recent study offers an inclusive model that reconciles APOL1 anion and cation channel activity, by its pH-switchable permeability properties [75]. The study used purified recombinant APOL1 in a vesicle-based approach. They found that APOL1 confers chloride selective permeability when allowed to insert into vesicles and tested at low pH, and calcium dependent potassium permeability when inserted at low pH but tested at neutral pH. This model indicates that APOL1 is taken up by endocytosis and the endosomal acidification triggers APOL1 membrane insertion, creating anion channels that affect the lysosomal pH gradient. When APOL1 is targeted to other membranes, such as the plasma membrane, it is exposed to neutral pH and its channel activity is switched from anion to cation permeability [75].

A limitation of these studies is that they were all based on an artificial lipid bilayer or liposome systems, acknowledging that in intact cells, ion conductance, especially secondary ion currents, is likely to be more complex. Apart from the lysosomal and plasma membrane, a recent work indicated that APOL1 can also insert into the mitochondrial membrane, inducing the release of factors that further enhance trypanolysis [73]. Understanding the mechanism by which APOL1 creates channels to mediate trypanolysis may help to understand its pathogenic mechanism in kidney disease.

Indeed, studies by the Pollak group indicate the role of potassium efflux in mammalian cytotoxicity as well [76, 77]. Exogenous expression of APOL1 using APOL1 cRNA injections in *Xenopus* oocytes increased ion permeability and induced morphological toxicity [78]. The currents were mainly potassium and calcium fluxes with a secondary chloride flux and were influenced by extracellular pH. Elevated extracellular potassium concentration diminished APOL1-associated morphological toxicity. Other reports, including a recent paper by the same group, also found an abnormal potassium efflux in HEK cells transfected with risk variant APOL1, which preceded APOL1-induced cell death [76, 79]. Increasing extracellular potassium, again abrogated these cellular effects, including cytotoxicity. According to this model, APOL1 pore activity plays an important role in initiating a cascade of events leading both to trypanosome death and to human kidney cytotoxicity. The mechanisms by which the channels are created, the membranes involved and the precise intracellular events that follow will require further investigations.

Endocytosis: APOL1 intracellular voyage

APOL1 undergoes a complex endo-lysosomal vesicle trafficking in the *Trypanosome*. Several lines of evidence suggest that APOL1 risk variants interfere with normal trafficking and these alterations are important for toxicity [54, 70]. Indeed, SRA and APOL1 colocalize in the endosome of *Trypanosome brucei*, as SRA traffics to the flagellar pocket of the parasite [27].

Unbiased screen studies in yeast, using the risk allele *APOL1*, indicated that APOL1 risk variant-associated toxicity was increased in mutant strains with defective endosomal trafficking [80]. The interaction screen indicated a role for VPS36. VPS36 protein is a subunit of endosomal sorting complex required for transport II (ESCRT-II). This protein plays a complex role in the sorting of ubiquitinated membrane proteins during endocytosis. In *Drosophila*, variant *APOL1* expression lead to disturbances in the endocytic function and acidification of the nephrocyte; the *Drosophila* podocyte [80, 81].

Structural homology searches to identify proteins similar to the APOL1-interacting domain of trypanosomal SRA, identified VAMP8 as a potential binding partner of APOL1 [82]. **VAMP8** is a **SNARE protein** involved in autophagy through the direct control of autophagosome and lysosome fusion [83]. Experimental data suggest that G1 and G2 variants could alter VAMP8 binding of APOL1, thus affecting vesicular trafficking and membrane fusion [82].

In human cultured podocytes, we showed that APOL1 is associated with intracellular vesicles, mainly the late endosome, also called multivesicular body compartment [36]. Transfection of HEK cell line with *APOL1* risk variants resulted in a massive increase in the late endosome compartment. On the molecular level, we showed poor acidification of the endocytic vesicle in risk variant *APOL1*, which was associated with a defect in fusion of endocytic vesicles with lysosomes. This fusion step is mediated by the STX17/VAMP8 interaction [36]. Since APOL1 can bind to VAMP8, this model could potentially define the molecular defect induced by risk variant APOL1. We did not identify a defect in the lysosomal compartment in our studies using human podocytes and transfected HEK cells [36]. However, another study did suggest an increase in lysosomal permeability in human podocytes transduced with risk variant APOL1 lentivirus [54]. In summary, several unbiased and targeted experiments indicate that APOL1 might play a role in the fusion of endocytic vesicles with the lysosome.

A dynamic interplay exists between endocytosis and autophagy [84, 85], and several lines of evidence suggest that risk variant APOL1 interferes with autophagy. BH3 proteins, such as APOL1, play critical roles in regulating autophagy; specifically autophagosome formation [86, 87]. Earlier research suggested that wild type APOL1 protein expression induced the formation of autophagic vacuoles and activated translocation of LC3II (a marker of autophagosomes) to the vacuoles. APOL1 expression in autophagy deficient mouse embryonic fibroblast cells did not induce cell death, indicating the role of APOL1-induced autophagy in cell death [86, 87]. Binding of APOL1 to PI3P, a derivative of membrane phospholipids that plays a role in autophagosome formation, was thought to mediate this effect. Deletion or replacement of APOL1 BH3 domain has been shown to reduce APOL1-associated toxicity in transfected HEK cells[46].

Studies testing the effect of risk variants on autophagy have shown seemingly conflicting results. *APOL1* risk variant was associated with an increase in LC3II abundance (a marker of autophagosomes) in HEK and hepatoma cell lines and in primary human hepatocytes [55, 88]. Our group examined autophagy in risk variant *APOL1* transfected HEK and HeLa cells. We found that while there was an increase in LC3II at baseline, treatment with chloroquine,

a lysosomal blocker, was associated with lower LC3II protein expression indicating a block in autophagy flux secondary to a defective fusion of autophagosomes with lysosomes. The block in autophagy flux was confirmed by other methods such as electron microscopy analysis and immunofluorescence staining of autophagic vesicles in human podocytes and transfected HEK cells [36]. Other studies were mostly based on simply quantifying LC3II protein expression without analyzing the flux and therefore were unable to detect significant differences in autophagy flux when G0 and G1, G2 variants were compared [89]. In summary, model organisms and cell biology data indicate that risk variant *APOL1* induced toxicity is mediated by a defect in late endosome-lysosome fusion (a known VAMP8-mediated process) and a downstream defect in autophagy flux.

Risk allele *APOL1* cytotoxicity: How do podocytes die?

Most investigators agree that exogenous expression of *APOL1* in podocytes, transfected HEK293 cells and transfected hepatoma cells is associated with increased cell death [54–56]. Expression of the exogenous reference allele, G0 also seems to induce some toxicity in most *in vitro* systems. One study reported no significant differences in cytotoxicity between G0 and G1, G2 variants in transfected HEK cells [89], while most other groups identified a dose dependent toxicity induced by *APOL1* in which G1 and G2 variants are more toxic than G0.

Transgenic expression of G1 or G2 *APOL1* in podocytes, resulted in podocyte loss and dedifferentiation in mice [36]. In a *Drosophila* transgenic model system, G1 was more toxic to the fly nephrocyte than G0, manifested as nephrocyte hypertrophy and ultimately the death of the organism [81]. By contrast, in zebrafish, exogenous *APOL1* expression caused only endothelial and podocyte damage, more pronounced in G1 and G2 compared to G0, with no significant kidney-related phenotype [90]. In summary, most data suggest risk variant *APOL1* induce cell death *in vivo*.

Different signaling pathways have been suggested to be involved in *APOL1*-induced cytotoxicity (Figure 3). Most studies failed to observe caspase3 activation and apoptosis following *APOL1* expression [36, 55]. Other mechanisms include the expression of stress kinases such as JNK/SAPK and p38 MAPK as well as mitochondrial damage and energy depletion [88, 91, 92]. While most studies described an increase in LC3II protein expression following exogenous *APOL1* expression, they mostly failed to conclusively demonstrate that autophagy is the mechanism of cell death following *APOL1* expression. Furthermore, our study indicated a decrease in autophagy flux rather than increased autophagy [36]. It appears that non-inflammatory cell death pathways were not consistently associated with *APOL1* induced damage.

Unbiased studies indicate the activation of inflammatory cell death pathways by *APOL1* [55, 93–95]. Studies of HIV nephropathy in human podocytes and in kidneys of HIV-transgenic mice (Tg26) showed increased expression of caspase1, its upstream regulator NLRP3 and IL1 β mRNA and proteins [93–94]. Caspase1 inhibitors decreased cytotoxicity in cultured podocytes and in *APOL1* transfected hepatoma cells [55, 95]. We found an increase in activation and cleavage of caspase1 and downstream IL1 and IL18 expression. Inhibition of

- What are the second hits, either environmental or genetic triggers, which induce kidney disease in APOL1 high-risk genotype people?
- Which intracellular pathway, affected by APOL1 risk alleles, is the best target for therapeutic intervention?

Concise glossary

African trypanosomes

African trypanosomiasis, also known as **sleeping sickness**, is a disease spread by an infected tsetse fly, found in rural areas of sub-Saharan Africa. Sleeping sickness is caused by two different parasite subspecies, *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* and is fatal if left untreated

Chronic kidney disease (CKD) and End stage renal disease (ESRD)

CKD is the gradual loss of kidney function, as manifested by decrease in glomerular filtration rate (GFR). CKD is divided into 5 stages, based on GFR. CKD can result from a variety of causes. Treatment is based on slowing the progression of the kidney damage, usually by controlling the underlying cause, if possible. CKD can progress to ESRD, which is fatal without artificial filtering (dialysis) or a kidney transplant

Podocytes

The kidney's functional unit is the nephron, which consists of a size selective filter (glomerulus) and a long tubule system. Podocytes are specialized epithelial cells in the glomerulus. Leakage of albumin and other proteins (proteinuria or albuminuria) is caused by damage to this filtration barrier. Genetic mutations in podocyte proteins lead to proteinuria.

Tubular cells

epithelial cells that line the tubules, the segment of the nephron that processes the primary filtrate

SNARE protein

a large protein family that mediates vesicle fusion with their target membrane bound compartment

VAMP8

(vesicle associated membrane protein 8) is a SNARE involved in autophagy that mediates the fusion of autophagosome membrane with the lysosome membrane

Pyroptosis

a form of inflammatory programmed cell death pathway, activated mainly by caspase1 and involves the activation of the inflammasome. Pyroptosis is mainly used by the host to control infection by inducing inflammation. Pathologically persistent pyroptosis has been implicated in autoimmune diseases

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Clinician's corner box (box 1)

- African-Americans have an increased risk of developing focal segmental glomerulosclerosis (FSGS), HIV associated nephropathy (HIVAN) and end stage renal disease (ESRD) compared with Americans of European descent
- Much of this increased risk is explained by coding variants in apolipoprotein L1 (APOL1), and two risk alleles, present in about 13% of African-American population confer this risk.
- In kidney transplant patients, a kidney from a high-risk genotype donor increases the risk for graft loss, while recipient genotype does not affect graft survival. Questions remain over whether scoring and kidney allocation should be formally changed to include APOL1 genotype
- As specific treatments are developed, it will become more important to genotype people of recent African ancestry to identify subjects with high risk genotypes for early specific treatment and follow up

Highlights

- Nucleotide variants in the Apolipoprotein L1 (APOL1) gene contribute to the increased risk of kidney disease in people of African ancestry.
- It is believed that the high allele frequency of these variants is due to the resistance they confer against the disease-causing *Trypanosome* species, which is implicated in Human African sleeping sickness.
- The mechanism by which APOL1 causes kidney disease is not fully understood. Experiments suggest that mutant APOL1 proteins create ion channels in membranes, disrupt the endo-lysosomal function, alter autophagy, and induce inflammatory cell death and podocyte damage.

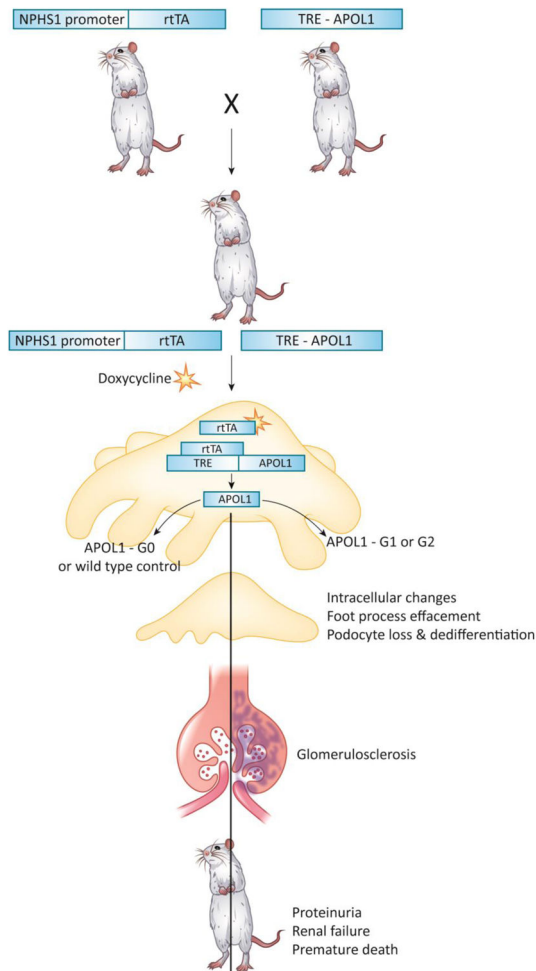


Figure 1. Transgenic expression of podocyte specific *APOL1* induces kidney disease in mice
 The doxycycline inducible (rtTA) system was used to generate mice with podocyte specific conditional inducible *APOL1* expression. Transgene expression was controlled by doxycycline and the nephrin (NPHS1) rtTA promoter was used to drive podocyte specific expression. Either *APOL1* reference (G0) or risk allele (G1 or G2) was expressed (TRE-APOL1). When exposed to doxycycline, transgenic mice expressing risk allele *APOL1* developed kidney disease including functional (proteinuria and renal failure) and structural (glomerulosclerosis and foot process effacement) changes, similar to the human kidney disease. Transgenic mice expressing the reference allele did not develop disease.

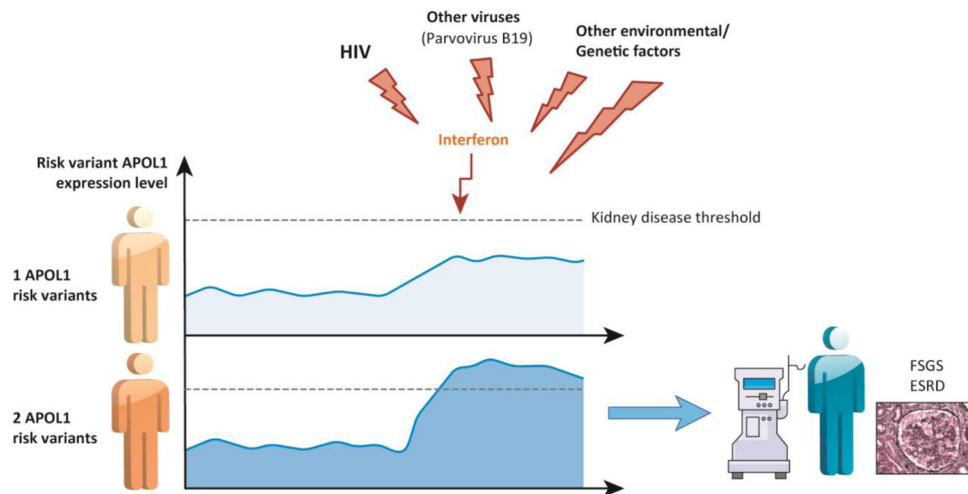


Figure 2. Environmental hits are necessary for disease development

Most people with two APOL1 risk alleles will not develop disease. A second hit is required to induce kidney disease in high risk genotype people. Environmental factors serve as second hits. Viral infections, most likely via an interferon mediated mechanism, present an important second hit for kidney disease development. HIV is a well-established second hit. Multiple studies suggest that environmental factors induce kidney disease by increasing risk variant APOL1 transcript and protein expression in podocytes. Environmental triggers such as viral infections induce interferon expression, which then increases APOL1 protein expression above a certain critical threshold that leads to progressive CKD. People with one risk allele have a lower expression of risk variant that are less likely to reach this threshold.

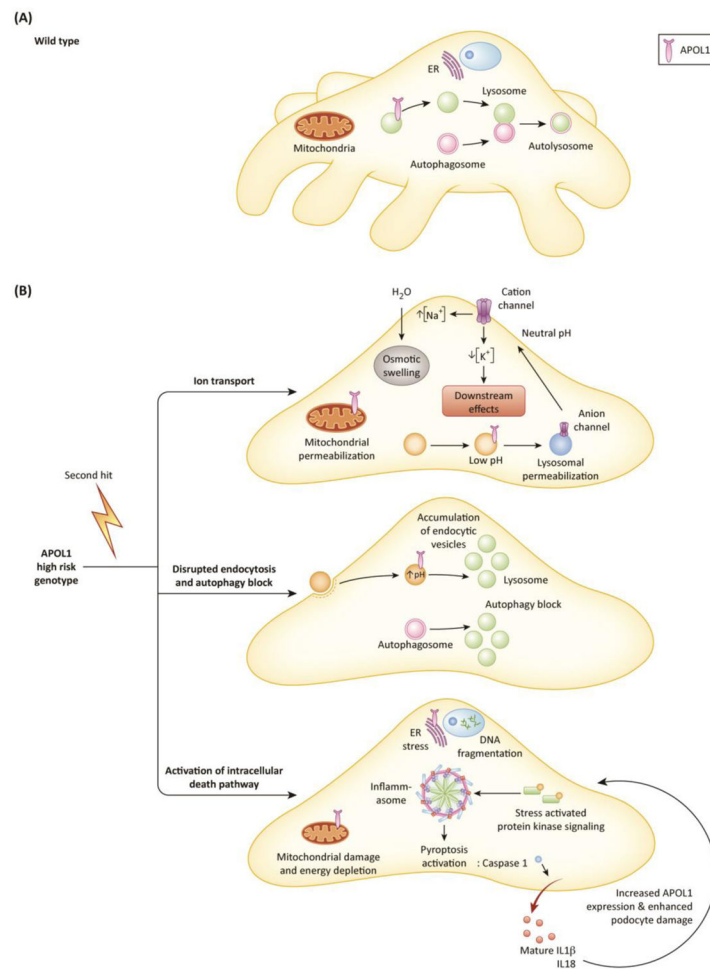


Figure 3. Intracellular mechanisms of APOL1-induced cytotoxicity

(A) In wild type podocytes, reference allele APOL1 does not affect endocytosis and autophagy. Autophagy inhibits pyroptosis, and mitochondrial and ER functions are unperturbed. (B) Upon exposure to a second hit, a cascade of events is initiated in podocytes expressing two APOL1 risk alleles that culminate in podocyte loss and dedifferentiation. APOL1 inserts into membranes and creates anion (when exposed to low pH, e.g. in the lysosome) or cation channel (when exposed to neutral pH, e.g. in the plasma membrane). These channels mediate ion fluxes that induce osmotic swelling and further activate toxic intracellular pathways (top panel). Due to defective endo-lysosomal trafficking and impaired acidification of endocytic vacuoles, there is a defect in autophagosome maturation resulting in an autophagy block and accumulation of endocytic vacuoles (middle panel). Consequently, various intracellular pathways are activated; including stress activated protein kinase signaling, mitochondrial and endoplasmic reticulum damage and inflammasome activation, resulting in caspase1-mediated inflammatory cell death, pyroptosis. Pyroptosis involves the release of IL1 and IL18, DNA fragmentation, and by a positive feedback loop can increase APOL1 protein expression and could further enhance cytotoxicity (bottom panel).