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Apolipoprotein L1 and kidney disease in African Americans

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Abstract

Genetic variants in the Apolipoprotein L1 (APOL1) gene cause high rates of kidney disease in African Americans. These variants, found only in individuals with recent African ancestry, confer enhanced innate immunity against African trypanosomes. Though they are among the most powerful disease-causing common variants discovered to date, we are just beginning to understand how they promote kidney injury. Since APOL1 is only present in a few primate species, much of our current knowledge has come from natural experiments in humans and *in vitro* studies while awaiting the development of transgenic animal models. Understanding more about the function of ApoL1 and how the high-risk variants behave differently from other ApoL1 molecules is a high priority in kidney disease research.

Keywords

ApolipoproteinL1; APOL1; kidney disease; African American

APOL1 risk variants and kidney disease

African Americans develop kidney failure at rates 4–5 times higher than Americans of European descent (1). In 2010, genetic variants in the Apolipoprotein L1 (APOL1) gene were discovered that explained a large fraction of this major health disparity (2, 3). These variants had large effects on multiple different types of renal disease that had been thought of as distinct disease entities. APOL1 kidney risk variants confer increased risk of renal disease associated with hypertension, primary glomerular disease, HIV infection, and other etiologies. The APOL1 risk variants are unusual in that they are common but confer large

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increases on disease risk. The import of APOL1 variants on kidney disease have now been replicated dozens of times but an understanding of how they contribute to a multitude of kidney diseases in African Americans remains elusive.

What is APOL1?

APOL1 is part of a six-membered family of APOL genes clustered on chromosome 22 that arose by gene duplication (4, 5). APOL1 arrived late in mammalian evolution (≈ 30 – 35 million years ago) and a functional gene is present only in a few species in the primate lineage (6). ApoL1 circulates in blood at high levels as part of an HDL complex. It is also expressed widely in tissues, particularly in the lung, placenta, pancreas, liver, and kidney (4, 5, 7). APOL1 is the only member of the APOL family encoding a signal peptide, enabling some splice isoforms to be exported into the serum (6).

Long before ApoL1 had a known role in kidney disease, ApoL1 was primarily known as the trypanolytic factor of human serum, protecting humans and some other primates against African Trypanosomes (see glossary) (8). The APOL1 kidney risk variants have enhanced protective ability against the African trypanosomes that cause the deadliest form of African sleeping sickness (2). Enhanced survival of individuals who carry the risk alleles in the trypanosome belt may explain at least in part the high frequency of these deleterious variants in people of African ancestry (2, 9) (see text box).

Text Box 1

APOL1, innate immunity, and natural selection

Disease-causing variants tend to be strong or common, but not both (9). The APOL1 high-risk variants are both common and powerful because they carry advantages (enhanced innate immunity) in the heterozygous state but only have a powerful deleterious impact when gene frequency rises sufficiently in a population to generate homozygosity (2, 42). In this way, APOL1 resembles sickle cell trait, protecting against malaria in heterozygotes but causing sickle cell disease in homozygotes (72–74). Unlike sickle cell disease, where there is strong evidence for balancing selection, it is unclear whether and where APOL1 risk variants have reached equilibrium between increased survival due to innate immunity and decreased survival due to kidney (or other) disease, or even that the risk variants influence reproductive fitness.

Two human-infective subspecies of trypanosome evolved from *T. brucei brucei* by developing defenses to evade killing by APOL1 (see Figure I). *T. brucei rhodesiense* evolved by repurposing a coat protein into a virulence factor (SRA) that binds to and inactivates APOL1 (8, 68, 75). This parasite causes acute African sleeping sickness in humans. *T. brucei gambiense* evolved into the parasite that causes chronic African sleeping sickness when *T. brucei brucei* developed an anti-APOL1 suite of defenses that impair APOL1 activity by multiple mechanisms entirely distinct from *T. brucei rhodesiense* (76). The kidney risk variants are located at the site where APOL1 is bound by SRA, and these risk variants either prevent the binding of SRA to APOL1 (G2) or reduce its affinity (G1)(2, 42). APOL1 has yet to show protection against *T. b. gambiense* in model systems, but *gambiense* is a much more difficult organism to study. Studies

testing gene frequencies in humans in *T. brucei gambiense* endemic areas are needed to assess APOL1 variant protection against this trypanosome subspecies. It is likely that humans in the trypanosome belt across sub-Saharan Africa who harbored the kidney risk mutation had a survival advantage, leading to rapid spread of these alleles in Africa (2, 9, 42). These APOL1 variants, which emerged on the order of 5,000 years ago, are now found in as many as 90% of individuals in some African tribes, indicating a very rapid selective sweep (2, 9, 42). The risk variants are not found in people without recent (<5,000 years) African ancestry. It is appealing to speculate that SRA hijacked a regulatory site on APOL1 important in keeping APOL1 cellular toxicity in check. Endogenous human proteins that perform this function are eagerly sought as a window into the cell death process that leads to kidney disease.

Evidence suggests that APOL1 risk variants might be advantageous against other pathogens beyond trypanosomes (42, 77). While evidence for positive selection is very strong, it is difficult to attribute precise causes to selection events that happened thousands of years ago when environmental conditions were very different in terms of ecology as well as human population and pathogen distribution. The current distribution of *T. brucei rhodesiense* and heightened frequency of APOL1 risk variants are not currently in alignment (42). While the highly efficient killer of *rhodesiense* trypanosomes, G2, is widely distributed, the less efficient G1 variant is concentrated in West Africa where *rhodesiense* does not currently reside. Moreover, G1 underwent a tremendously robust selective sweep in West Africa when the much more effective *rhodesiense* killer G2 was present in the population. This pattern suggests that something other than *rhodesiense* drove G1 to rise to high frequency in West Africa, and that G1 has enhanced activity against other trypanosomes (*gambiense* type 1 or 2) or other species entirely (42). APOL1 activity against leishmania has been observed, demonstrating that APOL1 is a versatile innate immunity molecule (77).

Recently, evidence has linked APOL1 to anti-viral activity. Interferons and TLR3 agonists induce APOL1, suggesting that it is a component of the viral response program (36). In addition, APOL1 appears capable of inhibiting viral replication through a multiplicity of mechanism (78, 79). Understanding APOL1's role in innate immunity, and the forces that caused selection of APOL1, is of more than academic interest. For example, knowing what kind of pathogens elicit an APOL1 response may help direct us toward the set of environmental factors that we might target to prevent APOL1 kidney disease. The tight link between HIV and APOL1 kidney disease (12, 34), correlations between Parvovirus B19 and collapsing glomerulopathy (80, 81), and patterns of viruria associating with APOL1 kidney disease (82) together suggest that viruses are an important part of the evolving APOL1 story.

Figure 1. Distribution of the G1 and G2 APOL1 variants across Africa

Allele frequencies of the G1 and G2 variants are indicated as blue and green wedges, respectively. Circle size reflects the number of individuals genotyped: small, <10 individuals/20 chromosomes; medium, 10–100 individuals/ 20–200 chromosomes; large, >100 individuals/200 chromosomes. Countries are shaded according to the subspecies of *Trypanosoma 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. Reproduced from reference (42).

The three principal domains in the ApoL1 protein are defined by their role in trypanolysis (10). At the C-terminus is the pore-forming domain that has similarities to channels that bacteria use to punch holes in cell membranes (8). The central region of ApoL1 includes the membrane addressing domain, which is believed to target ApoL1 to the trypanosomal lysosome (10). The ApoL1 risk variants are located near the C-terminus in the Serum resistance-associated protein (SRA)-binding domain, the binding site for SRA, a trypanosomal virulence factor.

The APOL1 risk alleles causing kidney disease are two coding mutations in the C-terminus of ApoL1. The first allele, G1, is two amino acid substitutions (S342G and I384M) that nearly always occur together (i.e. they are in near-perfect linkage disequilibrium)(2, 3). The second allele, G2, is a two amino acid deletion (del388N389Y) in the same functional

domain as G1 (11). The risk alleles have been observed only in individuals with recent African ancestry (2, 12). The transmission of disease risk is consistent with recessive inheritance: the high-risk genotype can be either G1/G1, G1/G2, or G2/G2 (2). One risk allele causes only at most a slight increase in kidney disease risk, whereas two risk alleles (one from each parent) increase risk dramatically (see table 1). About half of all African Americans inherit at least one APOL1 risk allele, while 12–15% are risk homozygotes (13). Since roughly 5 million African Americans with the high-risk genotype have greatly elevated rates of kidney disease, accelerating our understanding of APOL1 biology is an urgent public health priority.

One gene, many disease phenotypes

The APOL1 risk genotypes were originally identified in African Americans with a primary glomerular disease called focal and segmental glomerulosclerosis (FSGS). FSGS is defined by its pattern of injury on kidney biopsy. The accompanying clinical phenotype is typically characterized by substantial protein in the urine, accumulation of interstitial fluid (edema), and loss of kidney function. Traditionally FSGS has been considered a disease of the podocyte, a specialized and highly differentiated epithelial cell that forms a key component of the glomerular filter. It was therefore surprising when multiple other types of kidney disease were also strongly associated with the exact same APOL1 risk variants. Hypertension-attributable end stage renal disease (ESRD), typically thought of as a chronic vascular disease, is also strongly associated with APOL1 risk variants (2, 3). By far the strongest association is with HIV nephropathy, an infectious etiology of kidney disease (12). The same risk variants leading to greatly increased risk of many different types of kidney disease suggests unexpected similarities in disease pathogenesis at a molecular level in these APOL1 nephropathies. Subsequently, lupus kidney disease (14–16) and subtypes of membranous nephropathy (17) have shown associations with the APOL1 risk genotype. The relationship between APOL1 and diabetic nephropathy (DN) remains particularly ill defined. The APOL1 risk genotype appears to associate with progression but not incidence of DN (13, 18, 19). Since most patients with DN do not receive a renal biopsy, and since both APOL1 kidney disease and DN are quite common, there are likely to be many African American individuals included in DN studies who may have APOL1 renal disease misattributed to DN.

These findings have caused rethinking of several long-held tenets of nephrology. For instance, a large fraction of ESRD in African Americans has been attributed to hypertension. The causal molecular overlap of hypertensive (H)-ESRD with FSGS, a primary disease of the renal microvasculature, raises the possibility that in many individuals with the high risk APOL1 genotype hypertension is the symptom of primary renal vascular disease rather than a root cause. This may also explain why control of blood pressure is only modestly effective in preventing progression of kidney failure in hypertension-attributable kidney disease, especially among African Americans (18). APOL1 genotype appear at least as important in predicting decline in kidney function as intensity of blood pressure control (18).

The same variants in APOL1 can contribute to kidney phenotypes that are aggressive or indolent, proteinuric or non-proteinuric, and inflammatory or non-inflammatory. Though

animal models will be essential in studying disease pathogenesis, it is doubtful that any one animal model will ever capture the full range of these phenotypes in this primate-only gene. For this reason, careful study of human disease and natural experiments are an essential part of understanding APOL1 kidney disease. Several such illuminating observations in humans are discussed below.

Are the APOL1 kidney risk variants gain- or loss-of-function mutations?

Risk from APOL1 risk variants is transmitted in a recessive fashion (2). Typically, recessive diseases are caused by loss-of-function mutations. Several factors suggest that the APOL1 risk variants are toxic, gain-of-function mutations instead. First, only humans and a few other primate species even have a functional APOL1 gene (6). APOL1 arose via gene duplication in primates after the divergence of the primate lineage from other mammals, and some primates such as chimpanzees have subsequently lost the gene. This restricted lineage indicates that APOL1 is not required for mammalian kidney development or homeostasis. Secondly, investigators have identified a human completely null for APOL1 after he developed an infection with a trypanosome species (*T. evansi*) that does not normally infect humans (20). This individual has normal renal function (and does not have hypertension or proteinuria) despite absence of the ApoL1 protein, further suggesting that APOL1 is not required for human kidney development or homeostasis, at least under certain environmental conditions (21). Finally, experimental work in human cells and mouse models suggest that ApoL1 is toxic and that the risk variants have markedly enhanced toxicity compared with “wild-type” APOL1 (reviewed in more detail below).

Though kidney disease risk attributable to APOL1 in case-control and population-based studies appears to be inherited in a recessive fashion, with marginal heterozygote effects seen only in the largest studies (2), subtler phenotypes do show excess heterozygote risk when compared with no risk alleles. For example, a single copy of the G1 allele lowers the age of hemodialysis initiation in non-diabetic ESRD to an age intermediate between 0 and 2 risk alleles (22, 23). The reason why human APOL1 risk-heterozygotes have only very small increases in risk of kidney disease (instead of following an additive model where their risk is intermediate) remains an important but unanswered question. It also remains possible that the APOL1 risk variants fail to protect the kidney against some as-yet unknown environmental insults or pathogens consistent with a loss-of-function mechanism, but to date there is little evidence to support this conjecture.

Is circulating APOL1 or APOL1 of kidney origin the disease-causing culprit?

ApoL1 circulates in blood at high concentrations (about 5uM) on the densest high density lipoprotein (HDL) particles (24, 25). The major source of this circulating ApoL1 is presumed to be liver (25). A very recent report has proposed that a substantial fraction of ApoL1 circulates as part of protein complexes largely devoid of lipid rather than as part of HDL3 (28). A more detailed understanding of ApoL1 carriage in blood, filtration at the glomerulus, and handling in the urinary filtrate is needed. Within the kidney, APOL1 is expressed in podocytes and the vascular cells of the kidney, and can also be found in tubular

cells (26, 27), but the fraction of ApoL1 protein in tubular cells due to endogenous expression versus re-uptake from the urinary filtrate is unclear. Increased arteriolar and decreased podocyte ApoL1 staining has been observed with kidney disease (26), though this result may be dependent on which cell types can tolerate high levels of APOL1 expression. To date, no consistent differences in ApoL1 levels or cellular distribution for different APOL1 genotypes have been reported (26), and variation in circulating ApoL1 levels does not appear to impact disease risk (24).

Evidence that kidney cells are the source of disease-inducing ApoL1 comes from the kidney transplantation literature. Three studies demonstrate that risk to the kidney allograft travels with the donor kidney genotype (2, 29–31). Another study found that recipient APOL1 genotype had no effect on survival of kidney allografts (32). Together these observations support the idea that APOL1 originating from the kidney is most likely to be inciting kidney damage. There is no study yet where both donor and recipient APOL1 genotypes have been tested. Other factors such as immunosuppression may also complicate these conclusions. Recent data (discussed below) pointing toward paracrine actions of ApoL1 suggest that some cell types have the capacity for ApoL1 uptake from their surroundings, hinting at the potential for more complex physiology than the transplantation studies alone might indicate.

Why do some individuals with the high-risk genotype develop disease whereas others do not?

About 13% of African Americans have the APOL1 high-risk genotypes. While those with the risk genotype have a greatly increased risk of kidney disease compared to non-risk genotypes, only a minority develops kidney dysfunction. So far, no genetic modifiers have shown a major impact on APOL1 penetrance and several reported associations do not yet appear to be informative about APOL1 disease mechanism (33). Conversely, several environmental factors have been identified. First and foremost, HIV is a tremendously powerful risk factor for APOL1 kidney disease (12, 34). The high-risk genotype increases the risk of a person with HIV developing HIV-associated nephropathy (HIVAN) by 29–89 fold, among the strongest gene-environment combinations observed in common complex disease genetics. Another example is therapeutic treatment with interferon leading to collapsing nephropathy (FSGS with collapsing features). In a case series of interferon-induced collapsing nephropathy, all the patients genotyped had the APOL1 high-risk genotype (35, 36). Moreover, kidney disease started after treatment with interferon and improved after cessation of interferon therapy, suggesting that interferon plus risk genotype was causal in the disease process. In vitro studies showed that multiple types of interferon could all stimulate APOL1 expression by up to 200-fold (36). HIV is also a high interferon state, as are lupus (where APOL1 variants increase risk of kidney disease) and potentially many cases of FSGS where a viral infection is often the inciting trigger that precedes nephrosis (37, 38). Beyond interferons, other inflammatory factors such as toll-like receptor agonists and TNF-alpha can up-regulate APOL1 expression (36, 39) and may drive kidney disease in individuals with the high-risk genotype. Though as yet unproven, a strong argument can be made that both the high risk genotype and increased expression of APOL1 may be required for induction of APOL1 kidney disease.

How does APOL1 kill mammalian cells?

Before the relationship between APOL1 and kidney disease was known, the scant knowledge on ApoL1 function in mammalian cells centered around its ability to kill cells via autophagic cell death. Overexpression of WT APOL1 in HEK293 cells activated autophagy and promoted cell death, while knockout of the key autophagy proteins ATG5 and ATG7 prevented cytotoxicity due to ApoL1 in this system (40, 41). While ApoL1's propensity to cause cell death has been widely replicated (36, 42–46), with the risk variants typically much more lethal than WT ApoL1, the death-promoting mechanism is far from clear and may involve multiple pathways and be cell-type dependent.

Different studies have argued for autophagy, apoptosis, necrosis, and pyroptosis as potential contributing mechanisms to explain APOL1-induced toxicity (25, 40–44, 46). Most studies to date have been performed in human embryonic kidney (HEK) 293 cells, virally transformed podocytes, or liver-derived cells. In one experimental podocyte system, cell death after lentiviral APOL1 delivery lead to cell death with characteristic features of necrosis that included lysosomal damage (and where neither autophagy nor apoptosis is a prominent feature)(46). In this podocyte system, lysosomal acidification inhibitors and the chloride channel blocker DIDS could block APOL1 induced cell death. HEK cells similarly undergo cell death that does not depend on autophagy, but in this cell type there does not appear to be a lysosome dependent step or evidence of lysosomal injury, though only WT APOL1 was considered in these experiments (45). Conversely, in liver-derived cells, investigators proposed that cytotoxicity is largely driven in an autophagy-dependent process, but also with evidence of pyroptosis (25). Risk variant but not WT APOL1 delivery to mouse liver in vivo, however, caused massive cell death that was consistent at the histopathological level with necrosis (42).

Some experiments have demonstrated that certain cell types, such as podocytes, can take up ApoL1 from the extracellular environment (27). In addition, in some cellular systems, ApoL1 either secreted into the media or released from intracellular confinement by cell lysis can kill cells exposed to the ApoL1-containing media (44, 46). The results of transplantation data noted above suggests that a paracrine mechanism, with cellular uptake of ApoL1 made by other kidney cells, is more likely to be a factor in kidney disease than uptake of free circulating ApoL1 or ApoL1/HDL complexes.

While the exact mechanism of cell death in culture, much less human kidneys, is still unclear, many investigators propose that one of the two well-described cell death domains of ApoL1 is a central component in cellular toxicity. The N-terminus of ApoL1 harbors a pore-forming domain (8). The pore-forming domain resembles bacterial colicin-like domains that punch holes in cell membranes. The ApoL1 colicin-like domain kills trypanosomes by creating ion channels in trypanosomal lysosomes leading to swelling and trypanosomal rupture (47, 48). Data supporting the longstanding notion that trypanosome death is caused when ApoL1 creates pores in the lysosome has recently been challenged by evidence of pore formation in the trypanosome's cell membrane or mitochondria (49, 50). The data that support necrosis as the mechanism of ApoL1-induced cell death may be driven by this ion channel activation. Recently, work by Olabisi et al supported a channel-driven cell death

process in mammalian cells (51). In this study, cell death in HEK293 cells driven by G1 or G2 begins with cellular potassium efflux and leads to stress activated kinase initiation of cell death pathways that appear to be autophagy-independent (Figure 1).

Alternatively, ApoL1 also has a BH3-only pro-death domain found in several BCL-2 family members (40, 41). The BH3 domain is an important element in the apoptosis and autophagy pathways (52). While some experiments report the BH3 domain is critical for ApoL1-induced cell death (43), others that rely on disabling its conserved amino acid residues rather than bulk deletion of the BH3 domain suggest that traditional BH3 function itself is not important (43, 53). In addition to the role played by the C-terminus, an N-terminal region has been demonstrated to modulate cell death. The loss of this N-terminal region eliminates WT ApoL1 toxicity and mitigates risk variant ApoL1 toxicity (36, 45).

It remains an important question why two APOL1 risk alleles are necessary for kidney disease if they are gain-of-function mutations. A strict threshold effect for ApoL1 risk variant toxicity, exceeded only in risk homozygotes, seems unlikely given that APOL1 is such a highly inducible gene. ApoL1 multimerization, with rescue of ApoL1 risk variant toxicity by WT ApoL1, has been proposed as an alternative (54). If WT ApoL1 actively suppresses toxicity caused by the risk variants, this biology would create opportunities for therapy based on the mechanism of wild-type rescue.

The large number of different experimental systems used by investigators has lead to a vexing ambiguity in experimental results. Different cell types, different gene delivery systems, and different APOL1 sequences (e.g. splice isoforms) have yielded a wide variety of disparate and often contradictory results. Several emerging animal models may help clarify some of these extremely important mechanistic questions. Ultimately, for results from model systems to be convincing, they must correlate in ways that make sense with genetic, histopathological, and physiologic data from human studies.

Why kidney disease?

The very large increase in disease risk conferred by APOL1 variants appears to be restricted to kidney disease. Some studies demonstrate that the risk variants have substantial impact on several cardiovascular endpoints (55, 56) but other studies have not reinforced these findings (19, 57). It remains difficult to tease apart the direct effects of APOL1 genotype on cardiovascular disease versus an indirect effect through kidney disease, since kidney dysfunction is a major risk factor for cardiovascular disease and markers of kidney dysfunction often lag well behind chronic kidney damage. Various HDL traits associate with APOL1 genotype (58, 59) and APOL1 is a component of a highly anti-oxidative sub-fraction of HDL (59), so potential mechanisms can easily be envisioned.

Podocytes are a highly differentiated cell type with limited capacity for repair or regeneration, so it is conceivable that this specific cell type is exquisitely vulnerable to injury from ApoL1 (or from some insult that is mitigated by WT but not risk variant ApoL1). As terminally differentiated cells, podocytes depend on autophagy for maintenance of health in the absence of renewal (60), and ApoL1's putative role in autophagy could explain a special vulnerability to injury in the presence of risk variant ApoL1 or absence of

WT ApoL1. The podocyte also appears particularly sensitive to autophagy-related processes such as vesicular sorting and endocytic trafficking (61). Alternatively, expression patterns in vivo may lead to especially high levels of APOL1 in kidney in the setting of inflammation, or the kidney may lack expression of some endogenous factor that protects cells from ApoL1-induced injury. These questions will probably not be solved by in vitro experiments and await data from relevant transgenic animal models and human studies.

Lessons learned from trypanosomal biology

A long history of detailed mechanistic studies of ApoL1 function in trypanosomes may also yield vital clues about ApoL1 function in mammalian systems. Humans and other primates are protected from the African trypanosome, *T. brucei brucei*, by ApoL1. Investigators have demonstrated that APOL1 is taken up by trypanosomes as either part of a large lipoprotein complex (TLF1) in a receptor dependent process or as an even larger lipid-poor, IgM-containing complex (TLF2) via an unclear route (62–65). Both complexes also contain another protein, HPR, which helps ApoL1 find its target by engaging a trypanosomal surface receptor (66, 67). After uptake by the trypanosome, ApoL1 appears to traffic to the trypanosomal lysosome, where an acid-dependent step promotes insertion of ApoL1 in the lysosomal membrane (48, 68). Once ApoL1 has reached the lysosome, much debate has focused on whether ApoL1 acts primarily as an anion or cation channel in the trypanolytic process (47, 48). One new report suggests that ApoL1 can traffic to both the lysosome and mitochondria but it is the mitochondrial ApoL1 fraction that initiates cell death (49), while a second implicates oxidation as a necessary step in ApoL1-driven cell death (69).

Recently, a powerful study of ApoL1 activity in lipid bilayers performed in order to understand trypanolysis made several interesting observations that have changed the perspective on ApoL1 channel behavior. In addition to strong support for a cation-centric APOL1 killing mechanism, Thomson and Finkelstein show that full ApoL1 channel activity requires a two-step activation process (Figure 2) (50). First, an acidic environment is required for ApoL1 to insert into a membrane bilayer, where it has measurable but limited ability to flux ions. Subsequently, a return to a neutral pH environment activates a huge increase in ApoL1 ion flux capacity. This finding raises the possibility of acid-dependent activation in the endosome followed by recycling of ApoL1 to the trypanosome (and potentially kidney cell) membrane rather than trafficking from endosome to lysosome. A two-step activation event may introduce additional checkpoints for regulation in human cells where unbridled APOL1 channel activation would likely be detrimental.

Concluding remarks and Future perspectives

The path leading to an understanding of APOL1 biology in the kidney has many formidable impediments. We do not know if a gene that arose 70 million years after the split of the primate and rodent lineages will behave in rodent kidneys in ways that illuminate biology in humans. For example, other gene products not found in mice may be integral to APOL1 disease pathogenesis, or ApoL1 binding partners may have evolved differently in the two species. We also cannot be sure that disease processes that progress over decades in some types of APOL1 kidney disease can be recapitulated in weeks or months in healthy young

mice, or that kidney injury in mice will reflect the same mechanisms that occur in humans. At the molecular level, most investigators have found that ApoL1 protein is very difficult to produce and purify, so our understanding of its structure is still very limited. Progress is expected to accelerate as investigators deploy a wide array of experimental systems including bacteria, yeast, flies, fish, rodents, and primates to solve the many riddles of ApoL1 behavior and its consequences.

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Glossary

African Trypanosome	protozoa of the species <i>Trypanosoma brucei</i> . Humans are immune to <i>T. brucei</i> because APOL1 kills this pathogen. Two subspecies of <i>T. brucei</i> have evolved resistance to ApoL1 and become deadly human pathogens. <i>T. brucei rhodesiense</i> causes acute African sleeping sickness and <i>T. brucei gambiense</i> causes chronic African sleeping sickness in humans. These pathogenic subspecies have evolved entirely different mechanisms to neutralize APOL1
Collapsing glomerulopathy	Most pathologists consider collapsing nephropathy to be a type of FSGS. It is characterized by segments in the glomerulus where the microvessels have collapsed along with robust growth of the cells that surround the glomerular tuft
Colicins	Bacterial toxins made by <i>E. coli</i> that form pores in cell membranes. The N-terminal part of APOL1 is referred to as the colicin-like domain
Diabetic nephropathy (DN)	is a complication of diabetes that can lead to kidney failure. The clinical syndrome is typically defined by albuminuria and progressive decline in the glomerular filtration rate, accompanied by hypertension and high risk of cardiovascular morbidity and mortality
End stage renal disease (ESRD)	is the last stage (stage 5) in kidney disease, when kidney function is below 10 to 15 percent of their normal capacity, resulting in kidney failure. Individuals with ESRD require dialysis or transplantation for survival
Focal Segmental Glomerulosclerosis	A disease of the kidney glomerulus defined by the histological pattern of injury on kidney biopsy. It indicates partial scarring of some glomeruli. It often results in kidney dysfunction, edema (retention of fluids leading to swelling), low serum albumin levels, and elevated cholesterol levels

Glomerulus	The basic filtering unit of the kidney. It allows water, solutes, and small proteins to pass through into kidney tubules where urine is processed but retains cells and larger proteins in the blood
HIV-Associated Nephropathy (HIVAN)	Collapsing glomerulopathy that occurs in the setting of HIV infection
Podocytes	Specialized epithelial cells that surround the capillaries of the glomerulus

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Outstanding Questions

Do APOL1 risk variants have different protein binding partners compared with wild-type APOL1 that alter their trafficking or activity?

How does APOL1 cause cell death in different types of kidney disease? In other words, why do the same genetic variants cause kidney diseases with such different phenotypes?

What makes the kidney particularly susceptible to APOL1-mediated disease, and what are the relevant cell types?

Are APOL1 risk variants solely toxic, gain-of-function mutations or could the risk variants also fail to protect against some pathogen or other environmental insult that promotes kidney disease?

How well will animal models of APOL1 kidney disease reflect human disease given that APOL1 is a primate-only gene/protein?

Trends

Genetic variants in the ApolipoproteinL1 (APOL1) gene account for a large fraction of the high rates of non-diabetic kidney disease in African Americans.

APOL1 risk variants have large effects on several different types of kidney disease previously thought to be distinct entities.

The high-risk APOL1 variants are unusually common for such deleterious genetic variants likely because they conferred a survival advantage in sub-Saharan Africa by enhancing innate immunity against trypanosomes and possibly other pathogens.

Despite the recessive mode of inheritance, evidence suggests that the APOL1 high risk variants are likely to be toxic, gain-of-function mutations.

APOL1 possess two domains that may contribute to kidney cell death: a colicin-like domain that acts as an ion channel and a BH3-only death domain that may influence autophagy or apoptosis.

Figure 1. APOL1 mechanism of cell death

APOL1 causes cell death in kidney HEK293 cells and risk variant APOL1 is far more toxic than WT APOL1. Risk variant cell death is accompanied by cellular potassium depletion and activation of MAP kinase pathways. Replacing media in the extracellular space with high- K^+ media reduces cytotoxicity, whereas replacing extracellular sodium with a large cation that does not cross the cell membrane such as N-methyl-d-glucamine (NMDG) does not reduce cytotoxicity, suggesting that K^+ efflux may be the primary event leading to APOL1-mediated cell death. MAP kinase inhibitors also reduce cell death, pointing toward known pathways, whereby stress caused by K^+ depletion can activate cell death. Whereas K^+ efflux alone would be expected to cause cell shrinkage, cell death is instead accompanied by cell swelling, suggesting that sodium may be entering the cell in response to K^+ efflux. It is not clear whether this occurs through ApoL1 channels or by other routes, and Na^+ influx is not necessary for cell death at least in this model system, so important questions remain to be answered. (51)

Figure 2. Properties of the APOL1 ion channel

ApoL1 acts as an ion channel to kill trypanosomes, and the same mechanism may be important for kidney injury. **Left:** In a lipid bilayer system, ApoL1 channel activity is not seen at normal pH prior to activation by an acid environment. ApoL1 is postulated to form multimers as an explanation for why the protein is a toxic, gain-of-function mutation, yet is inherited recessively. In other words, wild-type ApoL1 may neutralize the toxicity of the risk variant. Whether multimers might form prior to lipid bilayer entry or after entry is unknown. **Center:** At low pH, APOL1 can insert in a lipid bilayer and lead to low-level cation flux. **Right:** Once the APOL1 is inserted in the membrane, a return to neutral pH leads to a huge increase in ion flux capacity. The trypanosome virulence factor SRA does not block the acid-dependent membrane entry but does block the enhanced conductance caused by pH neutralization, posing the hypothesis that the rise in pH leads to the APOL1 C-terminus inserting into the membrane in an SRA-preventable fashion. One leading hypothesis for the greater toxicity of risk-variant than WT APOL1 is that either genotype-specific cellular APOL1 binding partners prevent WT APOL1 from inserting in the kidney cell membrane or that risk variant specific binding partners are necessary for trafficking to the site of toxicity or for channel activation. (50)

Table 1

Effect of the APOL1 risk genotype (2 risk alleles) on kidney disease in individuals of recent African ancestry.

Kidney Disease	Population	Notes	Odds Ratio	Ref
HIVAN	African; Case-control	Biopsy-proven diagnosis	89	(34)
HIVAN	African American; Case-control	Biopsy-proven diagnosis	29	(12)
FSGS	African American; Case-control	Biopsy-proven diagnosis	17	(12)
ESRD, non-diabetic	African American; Population-based (DHS)	based on low frequency of ESRD	11	(13)
ESRD, non-diabetic	African American; Case-control	>1000 cases and >1000 controls tested	7.3	(2)
ESRD, non-diabetic	AA and Hispanic; Case-control	Only G1 considered	6.7	(3)
CKD	African; Case-control	Included some HIV-associated but not diabetes	4.8	(70)
CKD, non-diabetic	African American; Population-based (DHS)	Also highly significant for proteinuria	3.4–3.9	(13)
Lupus-ESRD	African American; Case-control		2.7	(16)
Lupus with collapsing nephropathy	African American; Retrospective biopsy review	Biopsy-proven diagnosis	5.4	(15)
Transplanted kidney grafts	African American; Case-control	Hazard ratios for graft survival	2.0–3.8	(29–31)
CKD, non-diabetic	African American; Population-based (ARIC)	Restricted to no CKD by at least age 45 at study entrance	1.5	(71)
Diabetes and CKD	African American; Population-based (DHS)	No difference in eGFR or albuminuria	0.4–1.0 (NS)	(13, 19)
CKD progression, non-diabetic	African American; CKD only longitudinal (AASK)	Hazard ratio for CKD progression	2.2 (AASK, ESRD)	(18)