Gene of the month: *APOL1*

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ABSTRACT

Apolipoprotein L1 (APOL1) is a protein encoded by the *APOL1* gene, found only in humans and several primates. Two variants encoding two different isoforms exist for *APOL1*, namely G1 and G2. These variants confer increased protection against trypanosome infection, and subsequent African sleeping sickness, and also increase the likelihood of renal disease in individuals of African ancestry. *APOL1* mutations are associated with increased risk of chronic kidney disease, inflammation, and exacerbation of systemic lupus erythematosus-associated renal dysfunction. This review serves to outline the structure and function of APOL1, as well as its role in several disease outcomes.

LOCATION, STRUCTURE, AND FUNCTION

Apolipoprotein L1 (APOL1) is a circulating protein component of HDL (high-density lipoprotein), encoded by the *APOL1* gene. The *APOL1* gene cluster is found on chromosome 22 and is only expressed in humans and several primates, namely gorillas and baboons.[1] Despite being closely related to humans, chimpanzees have lost *APOL1* expression; thus, *APOL1* expression is thought to influence primate adaptation to specific environments.[2] APOL1 is synthesised by the liver and circulates in high levels in the serum, carried on HDL 3 (HDL3) complexes[2] that also include haptoglobin-related protein to form a complex called Trypanosome lytic factor (TLF).[3] HDL3 is an antioxidant with activity against low-density lipoprotein and the level of APOL1 is correlated with this antioxidant activity.[4]

TLF is found in the serum of humans and certain primates and is known to confer immunity to infection by *Trypanosoma brucei brucei*.[5] *APOL1* kidney risk variants (G1 and G2) were identified in 2010[6] and are unusually common in individuals with recent African ancestry (last 5000 years), despite increasing the risk of chronic kidney disease (CKD).[7] This is attributable to the increased protection conferred by the kidney risk variants against African sleeping sickness, caused by trypanosome infection.[2] The *APOL1* high-risk variants are found on the last exon (exon 7) of the *APOL1* gene. The first mutation, referred to as G1, arises from two amino acid substitutions, namely serine for glycine and isoleucine for methionine at positions 342 and 384, respectively. The second mutation (G2) results from the deletion of two amino acids (asparagine and tyrosine at positions 388 and 389, respectively).[8] The mutations do not coexist on the same chromosome and are inherited in an autosomal recessive manner. The commonly encountered *APOL1* isoform is 398 amino acids long, with a molecular weight of 42 kDa.[9] There are three principal domains in the APOL1 protein, defined by functions in trypanolysis (figure 1).[10] First, the N-terminal pore-forming domain creates pores in membranes. This domain also contains a BH3 domain, which functions in autophagy or apoptosis. The pore-forming domain bears similarity to bactericidal pore-forming colicins.[11] Second, the membrane addressing domain functions as a pH sensor and targets the trypanosome lysosome for pore formation. Third, there is a C-terminal serum resistance-associated (SRA)-binding domain, which prevents trypanosome binding, and therefore protects against infection. The C-terminal also harbours the risk variant mutations near the SRA domain.[5 10] The variants act as harmful gain-of-function mutations, as only primates express functional APOL1 and it is not required for normal kidney development in non-primate mammals. Several studies have reported that *APOL1* risk variants (G1 and G2) are more detrimental than the G0 wild type.[1 12–14]

TRYPANOSOME EVOLUTION AND TLF

African trypanosomiasis (African sleeping sickness) in mammals arises from infection with the unicellular *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*. However, humans and certain primates are not susceptible to *T. brucei* infection.[15] This is due to the protection afforded by APOL1 and possibly other TLF components. *T. brucei* requires a host to obtain iron and lipids, and during this process takes up APOL1, which kills trypanosomes by inserting itself into lysosomal membranes. In this manner, APOL1 behaves as an ion channel and induces cellular swelling and rupture.[5] Recent research has further included the possibility of mitochondrial membrane pore formation by APOL1 as a mechanism of action.[16] Since G0 conferred protection against *T. brucei* infection, adaptation by *T. brucei* resulted in two subspecies, which are able to infect humans and cause disease. These subspecies adapted via antigenic variation in variant surface glycoproteins expressed on the trypanosomal membrane. First, *T. b. rhodesiense* adapted a virulence factor, called the SRA-protein, which can bind to and inactivate APOL1 (figure 2).[17] Second, *T. b. gambiense* evolved multiple mechanisms by which to neutralise APOL1 (figure 2).[18 19] Subsequently, *APOL1* adapted to restore the parasite killing ability by mutation of the SRA-binding region of the APOL1 protein (G1 and G2 variants).[7] The G2 variant enables complete escape from SRA binding, regaining the ability to kill trypanosomes, while the G1 variant diminishes SRA-binding affinity.[7] Trypanosomal SRA cannot fully neutralise the risk variants (G1 or G2), thus

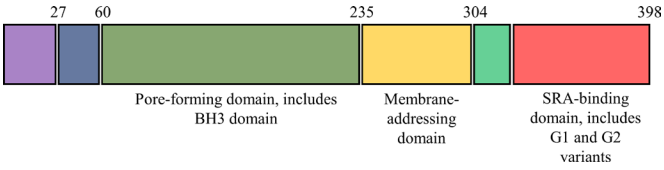

Figure 1 Protein domains of APOL1, including specific domains that contribute to trypanolytic effects.[9] APOL1, apolipoprotein L1; SRA, serum resistance-associated.

allowing the APOL1 variants to confer resistance to African sleeping sickness.

MECHANISM OF ACTION

APOL1 reportedly induces cell death via autophagy, apoptosis, pyroptosis, and necrosis, as evidenced by several studies,[20–22] with the risk variants producing a more destructive effect than wild type APOL1. However, the exact mechanism remains elusive and could be attributed to specific characteristics of different cell types or involve multiple pathways functioning simultaneously. The proposed method of cell death lies in the N-terminus of APOL1, which contains the pore-forming domain, indicating that kidney toxicity could be due to the same mechanism by which APOL1 kills trypanosomes—by punching holes in cell membranes, mitochondria or lysosomes.[5] Several researchers suggest that a loss of N-terminus function could eliminate the toxicity of wild type APOL1 and reduce *APOL1* risk variant toxicity.[12 22] Reports on lysosomal pore formation describe the ion channel behaviour of APOL1.[11] Pore formation results in intracellular K^+ depletion, Na^+ influx, and activation of p38, JNK, and ERK MAPKs (figure 3). These actions induce sodium-mediated cytoplasmic swelling and rupture or cellular toxicity and death via downstream mechanisms (figure 3).[11] Olabisi *et al* reported APOL1-mediated cytotoxicity and autophagy in human embryonic kidney (T-REx-293) cells, while Cheng *et al* reported an autophagic mechanism of APOL1-induced cell death in human hepatocytes and hepatoma cells.[11 20] However, it remains to be determined if APOL1 directly or indirectly triggers autophagy. Another study demonstrated severe hepatic necrosis

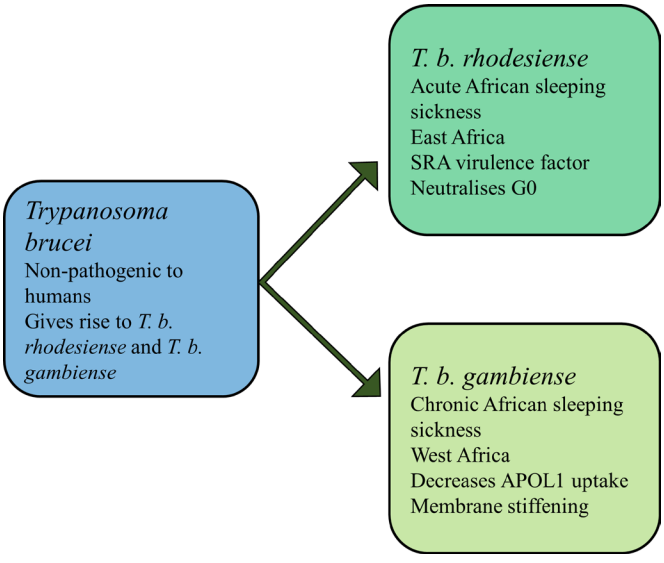

Figure 2 Evolution of *Trypanosoma brucei*, giving rise to APOL1 wild-type resistant *T. brucei rhodesiense* and *T. brucei gambiense*.[9] APOL1, apolipoprotein L1; SRA, serum resistance-associated.

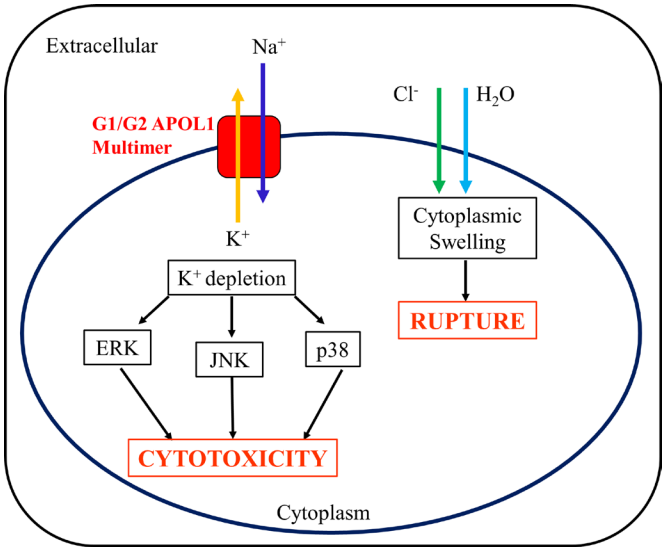

Figure 3 Proposed mechanism of APOL1-induced cell death via ion channel functionality.[11] Adapted from Olabisi *et al*.[11] APOL1, apolipoprotein L1.

following gene delivery of APOL1 high-risk variant G1 to the liver. This effect was less severe with the G2 variant and not observed with G0 expression.[1] It is important to note that a wide range of APOL1-related kidney phenotypes could contribute to disease, resulting in the possibility of multiple mechanisms.

APOL1 ROLE IN DISEASE

The risk variants increase the potential of renal disease associated with increased blood pressure, glomerular disease and HIV infection.[23] APOL1 is also expressed in the lung, placenta, pancreas, liver, and kidney,[23] but the level of circulating APOL1 does not appear to directly influence disease risk.[24]

CHRONIC KIDNEY DISEASE

Individuals with recent African ancestry are four times as likely to develop CKD than Caucasians.[25] The G2 risk variant is more frequently encountered in Sub-Saharan Africa, with a prevalence estimated at 10%.[26] *APOL1* risk genotypes are frequently seen in African Americans who have focal segmental glomerulosclerosis, a primary glomerular disease, which is considered a podocyte disease, and is characterised by kidney injury, proteinuria, interstitial fluid accumulation, and deteriorating kidney function.[6] Podocytes are particularly affected by *APOL1* wild type (G0) and the high-risk variants (G1 and G2), as it is a specialised cell with limited repair capacity and equally limited regeneration, which could increase vulnerability to the autophagic effects of APOL1.[27] Furthermore, APOL1 expression may accumulate in the kidney and lead to increased inflammation.[27] Additionally, an increased incidence of end-stage renal disease, caused by hypertension, is also observed.[7] *APOL1* is strongly associated with HIV nephropathy.[28] As observed in South Africa, HIV-positive patients with *APOL1* high-risk variant genotypes are at greater risk of developing HIV-associated nephropathy.[28 29] The relationship between *APOL1* and diabetic nephropathy (DN) has not been thoroughly elucidated. Risk variants influence progression of DN and not necessarily incidence.[30 31] Further research is required to determine if patients with APOL1-attributed renal disease have been misdiagnosed with DN. Research into a mixed-ancestry population has shown that the risk variants are not commonly observed, but the high risk variants increase the

incidence of renal or cardiovascular complications in patients with diabetes of mixed ancestry.[32]

APOL1 AND INFLAMMATION

The *APOL1* risk variants are associated with systemic lupus erythematosus (SLE)-associated collapsing glomerulopathy. African American patients with SLE are often at higher risk for developing collapsing glomerulopathy, a serious disease that frequently resists treatment.[33 34] Furthermore, inflammation upregulates *APOL1* expression. This is an important consideration, especially in HIV and SLE, which increase inflammatory factors (such as TNFα), and could accelerate CKD in patients with high-risk *APOL1* variants.[12]

CONCLUSION

The structure, functions, and mechanistic involvement of APOL1 remains to be fully elucidated. Results obtained from experiments *in vitro* using different experimental models have been discordant and often contradictory. Further research using transgenic and gene knockout models and more extensive human studies are likely to provide further insights into the function of APOL1 *in vivo*. However, this may be complicated by the fact that APOL1 is only found in primates, and rodent animal models may not be truly reflective of the pathophysiology. It will be insightful to determine why the kidney shows such profound susceptibility, and how and why the genetic variants can cause different forms of renal disease.

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