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APOL1 Toxin, Innate Immunity and Kidney Injury

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Abstract

The discovery that two common *APOL1* alleles were strongly associated with non-diabetic kidney diseases in African descent populations led to hope for improved diagnosis and treatment. Unfortunately, we still do not have a clear understanding of the biological function played by APOL1 in podocytes or other kidney cells, nor how the renal risk alleles initiate the development of nephropathies. Important clues for APOL1 function may be gleaned from the natural defense mechanism of APOL1 against trypanosome infections and from similar proteins (*e.g.* diphtheria toxin, mammalian Bcl-2 family members). This review provides an update on the biological functions for circulating (trypanosome resistance) and intracellular (emerging role for autophagy) APOL1. Further, we introduce a multimer model for APOL1 in kidney cells that reconciles the gain-of-function variants with the recessive inheritance pattern of *APOL1* renal risk alleles.

Keywords

APOL1; renal function; innate defense; autophagy; multimer; toxicity

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The discovery in 2010 that the common *APOL1* G1 and G2 genetic variants are strongly associated with glomerular disease in African descent populations has opened a door that may lead to improved understanding and treatments.[1] Unfortunately, the door has been more difficult for researchers to walk through than many expected. Despite the extensive efforts deployed by the nephrology community, we still do not have a good understanding of how these variants injure podocytes or other kidney cells. This review provides an update on the biological functions for the circulating and intracellular APOL1 forms. We also propose a model for APOL1 renal function (APOL1 multimer weapon with a trigger-lock mechanism

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for safety) that reconciles the gain of apparent deleterious function with the recessive inheritance pattern of *APOL1* risk alleles.

The APOL family

APOL1 is one of the 6 members from the *APOL* gene family (*APOL1-6*), organized in a cluster on human chromosome 22.[2–4] *APOL* gene homologues are found through out the animal kingdom. The *APOL* gene family arose by gene duplication in primates, but only humans, gorillas and baboons retained a functional, expressed *APOL1* gene. The function of the apolipoproteins L (APOLs) is largely unknown. APOL1 was initially discovered complexed in high-density lipoprotein 3 (HDL-3) particles, which were identified as the key component of trypanolytic factor (TLF) in human serum.[5] The exploration of APOL1 trypanolytic activity revealed an organization in three domains: a pore-forming domain, a pH-sensitive membrane-addressing domain and an SRA-interacting domain (Figure 1). The organization of the pore-forming domain directly adjacent to a membrane-addressing domain is similar to that of bacterial colicins, diphtheria toxin and mammalian Bcl-2 family members.[6,7] All APOLs are closely related, although the *APOL5* and *APOL6* genes are evolutionary divergent from the *APOL1-4* gene cluster;[3] it is predicted that the 3 domain organization is conserved in all the APOL family members.

APOL1 is the only secreted member of the family, having acquired an N-terminal signal peptide. The circulation of APOL1 in HDL-3 particles suggests a role in lipid transport and metabolism,[3,8,9] which could be critical in maintaining the plasma membrane of the extensive foot processes of podocytes. However, it remains unclear if APOL1-mediated renal injury is initiated by endogenous kidney-expressed APOL1 or by circulating APOL1. A recent report proposed that the high level of APOL1 protein expression in normal human podocytes is due to both endogenous synthesis and uptake from the circulation,[10] and uptake of APOL1 G1 and G2 renal risk isoforms was shown to contribute to human podocyte injury.[11] These *in vitro* findings contrast with two renal allograft studies that suggested that kidney-expressed APOL1, but not circulating APOL1, damages kidneys: allograft survival was not affected by recipient *APOL1* genotype suggesting no impact of recipient circulating APOL1;[12] however kidneys from donors with two *APOL1* risk alleles had significantly shorter survival time in recipient compared to kidneys from donors carrying one or no risk allele, suggesting a role for donor kidney-endogenous APOL1.[13] Further studies are needed for a definitive answer –particularly renal allograft studies where the *APOL1* genotype of both the kidney donor and kidney recipient are known.

APOL1 and resistance to trypanosome infection

APOL1 is the trypanolytic toxin providing innate resistance against *Trypanosoma brucei* infection, which causes animal and African human trypanosomiasis (African sleeping sickness) in many mammalian species, including African primates.[7,14] The parasite internalizes the APOL1-containing TLF through both fluid phase and receptor-mediated endocytosis[15] and the particle is delivered to the lysosome through the endocytic pathway. The progressive acidification of the environment triggers conformational changes in the membrane-addressing domain of APOL1 resulting in the release of APOL1 from the HDL

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particle within the lysosomal membrane, where APOL1 forms an ionic channel.[6,16] The ion influx then provokes osmotic swelling and death of the parasite. In order to replicate in their hosts, trypanosomes have evolved different mechanisms to lock the trigger of the APOL1 lethal weapon: *T.b. rhodesiense* evolved a serum resistance associated (SRA) glycoprotein that binds to APOL1 within the lysosome to abrogate its toxicity,[7] whereas *T.b. gambiense*-specific glycoprotein (TgsGP) forms hydrophobic β-sheets that stiffen the endo-lysosomal membrane to prevent APOL1 membrane insertion and toxicity.[17] In addition, to fully evade APOL1-mediated trypanolysis, APOL1 uptake is limited and APOL1 degradation is enhanced in *T.b. gambiense*.

The *APOL1* G1 and G2 renal risk alleles are located in the SRA-interacting domain (Figure 1) and restore APOL1-mediated protection against *T.b. rhodesiense* to prevent acute trypanosomiasis in humans.[1,18] APOL1 G1 and G2 variant isoforms are both potent killers of *T.b. rhodesiense*, but intriguingly, they seem to act via different trypanolytic mechanisms (Table 1). The G1 allele is composed of two SNPs in near-perfect linkage disequilibrium ($G1^G$, p.S342G and $G1^M$, p.I384M) but only $G1^G$ can kill trypanosome *in vivo*.[18] Contrary to the G2 deletion, $G1^G$ does not occur within the epitope (leucin zipper 370–392) that is essential for optimal SRA-binding and trypanolytic activity.[19] As a consequence, G2 has a reduced affinity for trypanosome SRA, but not $G1^G$ whose affinity for SRA is similar to the wild-type isoform.[1,18] $G1^G$ is located in a putative α-helix domain and has been hypothesized to stabilize the membrane association of the protein isoform.[18] The G1 and G2 isoforms tend to induce cell death and tissue injury,[11,20] but with different levels of toxicity: G2 is trypanolytic at a lower titer than G1 *in vitro* (10^4-fold dilution *vs.* undiluted),[1] and G1 induces widespread severe liver necrosis in mice whereas G2 caused focal and moderate necrosis.[18] The trypanolytic mechanics appear different for G1 and G2, yet they both efficiently kill *T.b. rhodesiense* and exhibit equivalent effect size for FSGS/HIVAN when comparing individuals carrying G1/G1, G2/G2, and G1/G2 (Table 1).[21]

APOL1 in immunity

Additional studies suggest that APOL1 might play a broader protective role in innate immunity since (1) *APOL* genes are upregulated by pro-inflammatory cytokines such as IFNγ and TNF,[3,11,20,22–25] (2) APOL1 can ameliorate *Leishmania* parasitic infection,[26] and (3) restrict HIV-1 *in vitro* replication in macrophages.[24]

The link between inflammation and APOL1 expression may constitute a modifying factor that might explain the incomplete penetrance of the G1/G2 variants for chronic kidney disease, *i.e.* why only a fraction of individuals carrying two renal risk alleles will develop nephropathy. In particular, the high penetrance of the risk alleles in HIV collapsing nephropathy may be due to elevated and persistent IFNγ levels in response to the virus.[11,27] Interactions of *APOL1* with other genes or other non-HIV viral infections might also act as second hits.[28]

APOL1 and programmed cell death

Among the suggested mechanisms by which APOL1 contributes to glomerulosclerosis are apoptosis,[5,29,30] autophagy,[25,30–32] or endocytosis and lysosomal stimulation.[24] All APOLs

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contain a putative Bcl-2 homology domain 3 (BH3) within the pore-forming domain (Figure 1). Most BH3-only proteins are activators of programmed cell death,[33,34] and in accordance, APOL6 was shown to induce apoptosis,[30,35] and APOL1 can initiate autophagic cell death under certain circumstances.[25,31] This accumulation of evidence combined with the emergence of autophagy as a major pathway in kidney function and glomerular disease[36–40] provides a promising avenue of research for revealing the pathophysiological mechanism of APOL1-mediated renal cell injury. A recent study reported that over-expression of APOL1 G1 and G2 variants in human podocytes drove enhanced lysosomal membrane permeability and cell death.[11] It is notable that both the trypanolytic activity of secreted APOL1 and the autophagy function of intracellular APOL1 converge on endosome and lysosome trafficking, which is coherent with the pH-dependent membrane-addressing function of APOL1.

APOL genes evolution, pressure of selection and binding partners

Functional *APOL1* was only identified in humans, gorillas, baboons and possibly a few other African primates, but was either completely lost (*e.g.* chimpanzee) or pseudogenized (*e.g.* orangutan, macaque) in other African primates, suggesting a fitness cost greater than the benefit conferred against extracellular parasites.[14,29,41] In primates, *APOLs* evolved rapidly and were under positive selection by pathogens (hence reinforcing the likely broader role for these genes in immunity), especially in the functional C-terminal region.[29] For this reason, this domain is thought to be fundamental for APOL function and it is predicted that molecules interacting with the C-terminal domain are essential for preventing APOL-mediated cell death, similar to the APOL1/SRA system in trypanosomes.[19,29] Vanhollebeke and Pays have speculated that mammalian 'SRA-like' proteins are involved in the natural control of APOL toxicity through interaction with the C-terminal helix,[5] and Wan *et al.* have postulated that APOL1 and APOL6 interacting partners, either protein or lipid, might mediate different death-signaling pathways.[32] Efforts to identify APOL1 binding partner(s) have been initiated to reveal the regulatory mechanisms of APOL1 toxicity that could explain kidney injury. The action of a second factor regulating APOL1-mediated cell death could explain why only kidney cells seem to be damaged from G1 and G2 risk alleles when APOL1 expression is quite ubiquitous: the second factor could have a different level of expression in non-kidney cells, or a similar factor with a stronger affinity for APOL1 might be expressed in other cell types to lock its deleterious function. Wan *et al.* demonstrated that APOL1 can bind *in vitro* with high affinity to lipids involved in cell death regulation: phosphatidic acid and cardiolipin that are associated with mTOR/rapamycin autophagy signaling and mitochondria-mediated apoptosis, respectively.[32] Sedor and colleagues recently used a secondary structure-based strategy to identify human proteins with similar structure to trypanosomal SRA and identified VAMP8, YKT6, osteocalcin, VAMP1 and SEC22b as top candidates (O'Toole JF, *et al.*, SA-OR095, ASN annual meeting, Atlanta, GA, 2013; Sedor JR, invited presentation, ASN annual meeting, Philadelphia, PA, 2014; Sedor JR, personal communication). They further explored VAMP8, a SNARE protein of the endo-lysosomal compartment that can anchor SNARE proteins from the autophagosome to trigger fusion between the two compartments.[42] By co-immunoprecipitation and surface plasmon resonance experiments, VAMP8 was shown to interact with APOL1 in a variant-dependent manner to regulate APOL1 toxicity: compared to G0 (wild-type), the APOL1-

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VAMP8 interaction was reduced with G1 and G2 protein isoforms, which impacted APOL1-induced autophagy. Furthermore, molecular dynamics simulations revealed that G1 and G2 are helix-stabilizing variants, and that the G1 and G2 isoforms have less conformational mobility than G0, which would explain why the interaction of APOL1 binding partners would be impaired with G1 and G2 isoforms (Sedor JR, invited presentation, ASN annual meeting, Philadelphia, PA, 2014; Sedor JR, personal communication). Whether phosphatidic acid, cardiolipin and VAMP8 regulate APOL1-induced autophagy or cell death in kidney cells and whether other APOL1 binding partners could modulate any APOL1-related function have not yet been formally demonstrated or related to kidney injury.

APOL1 renal function and the recessive model

Beyond the lack of clarity surrounding the perturbation of kidney cellular function by the G1 and G2 isoforms, any mechanism for renal cell injury must account for the strong recessivity observed for *APOL1* renal risk alleles in epidemiological studies (discussed in[43]). Usually, a recessive model correlates with a loss-of-function mutation. However, APOL1 is not required for kidney development or kidney homeostasis as most mammalian species, including higher primates, lack *APOL1*. Indeed, an Asian Indian who is a homozygous null for *APOL1* was shown to have normal renal function, as did members of his family who were heterozygous carriers of the null mutation.[44,45] This and the evolutionary history of *APOL1* suggest that APOL1 exerts a redundant function. Therefore, if we consider a gain of deleterious function model for G1 and G2, we would expect that an additive or dominant model would best fit the association with glomerular disease (Figure 2, left panel): carrying one copy of the G1 or G2 variant should drive a damaging phenotype, as detrimental as carrying two copies (dominant), or an intermediate phenotype (additive), which is in contradiction with all evidence pointing to a largely recessive model.[43]

Multimerization of APOL1

Based on the multimerization of similar proteins, we propose a model of multimerization for APOL1 in renal cells to reconcile the recessive pattern of inheritance with a gain of deleterious function model (Figure 2). As previously mentioned, APOL domain organization share some structural and functional similarities with bacterial colicins, diphtheria toxin and mammalian Bcl-2 family members (a pore-forming domain adjacent to a pH-sensitive membrane-addressing domain) suggesting a similar activity for all these proteins. Remarkably, the diphtheria toxin pathway through the infected cell is highly comparable to APOL1's pathway in the trypanosome. The diphtheria toxin enters the cell by endocytosis and then travels through the endosome, where the progressive acidification of the environment triggers a conformational change in the membrane-addressing domain, which allows the insertion of the toxin into the membrane, the formation of a ionic pore, and the translocation of the toxic protein fragment into the cytoplasm.[46] Interestingly, diphtheria toxins are able to multimerize in membranes,[47–49] and some bacterial colicins also seem capable of dimerization.[50–52] Finally, dimerization and multimerization are essential for the mammalian Bcl-2 family members harboring a BH3 domain to exert their pro-apoptotic activity.[53–57] In light of this body of evidence in analogous proteins, the multimerization of APOL1 proteins is plausible and warrants investigation. We therefore ran simulations to

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estimate the recessivity for monomers and multimers from dimers to hexamers (Figure 3). In this model, we considered that APOL1 toxicity is antagonized by a second factor (the lipid or protein trigger-lock system, see above) interacting with the C-terminal domain of APOL1 wild-type isoforms, and that this inhibition is either attenuated or abrogated by the G1 and G2 isoforms, due to loss of binding affinity for G1 and/or G2 with the second factor. For multimers carrying one or more wild-type APOL1 isoforms, binding and blocking of toxicity are retained. Our simulations show that multimerization would fit a completely or almost completely recessive model –as observed for G1/G2 associations with CKD and ESKD–, when a monomer-only model would fit an additive model, in contradiction with the epidemiological data.

If we consider that APOL1 exerts its function in a monomeric form (Figure 2, left panel), then we can posit that homozygosity for the wild-type (or G0) allele (0 risk allele) will maintain podocyte (or other renal cell) homeostasis due to the interaction with the second factor that limits toxicity and protects from cell death (locked weapon with the safety engaged). In contrast, individuals with 2 risk alleles would have a decreased affinity for the second factor (actuate the trigger), enhancing the toxicity and cell death that could eventually lead to the development of glomerular injury (weapon discharge). However, if this model were correct, heterozygosity for G1 or G2 (1 risk allele) would also display an increased toxicity that should drive an intermediate phenotype, illustrated by an additive model of inheritance, which is in contradiction with clinical data.

In a dimer scenario (Figure 2, right panel), individuals with no risk allele would maintain cell homeostasis (locked weapon) while individuals carrying two risk alleles would exhibit a high level of podocyte cell death leading to renal injury (weapon discharge). In the case of one risk allele, APOL1 could form homodimers for the wild-type protein, homodimers for the risk protein, and heterodimers for the wild-type and renal risk isoforms. Heterodimer-mediated toxicity would likely be reduced or abrogated by the interaction with the second factor. Individuals with one risk allele might experience an intermediate phenotype (illustrating an additive model), except if APOL1 isoforms tends to preferentially form heterodimers, driving the number of deleterious homodimers down, limiting the toxicity and therefore maintaining cell homeostasis. Alternatively, individuals with one risk allele might also tolerate the increased toxicity (only 1/4 of damaging toxins in a dimer scenario *vs.* 1/2 in the monomer scenario) and maintain cell homeostasis. In the eventuality of a higher order APOL1 multimer (n-mer), the fraction of monotypic/damaging toxins would decrease ($1/2^n$) as the number of APOL1 subunits required to form a functional toxin increases, thereby limiting the effect of the risk variants in individuals with one risk allele (Figure 3). These two models are not mutually exclusive and either would reconcile the recessive model of inheritance with the epidemiological data.

We recently provided evidence by size exclusion chromatography that APOL1 can multimerize *in vitro*. Western blotting and co-immunoprecipitation experiments from cell lysates further confirmed APOL1 multimerization (Dummer PD *et al.*, 10th International Podocyte Conference, Freiburg, Germany, 2014; Kopp JB, personal communication). Whether APOL1 multimerization is important for APOL1 function and is involved in kidney injury remains to be formally demonstrated. Finally, this testable model contains

several caveats and it is likely that the pathophysiologic reality is more complex than pictured in our schematic model, which should raise additional questions: there may be a balance between monomeric and multimeric forms, differences in assembly kinetics, protein abundance, localization, or binding affinities among the various APOL1 isoforms or between the various APOL1 isoforms and binding partner(s). By analogy with *T.b. rhodesiense* restriction, the G1 and G2 isoforms might alter the APOL1 renal function through different mechanisms (toxicity, membrane access and stabilization, and second factor binding).

Conclusions

Advances in understanding the mechanism by which APOL1 G1 and G2 variants cause renal injury have not kept pace with our rapidly expanding knowledge of the genetic epidemiology and disease associations of the *APOL1* risk variants. There is suggestive evidence that APOL1 risk variants interacting with either genetic or environmental factors initiate renal programmed cell death and specific forms of kidney disease. Any mechanism for the pathophysiology underpinning APOL1-mediated disease will have to be consistent with a recessive mode of inheritance and the complementation of G1 and G2 in renal function despite apparent distinct trypanolytic mechanisms. Important clues for how APOL1 G1 and G2 proteins injure the renal cells may be gleaned from the mechanism of APOL1 trypanosome killing (destabilization of the lysosome membrane by pore formation controlled by the SRA binding) and from the function of proteins exhibiting a similar structural organization (multimerization). Deciphering the molecular mechanisms by which APOL1 damages kidney cells is essential for translating *APOL1* genetic associations to effective preventive or therapeutic strategies and expanding the role for genetically informed medicine in the nephrology clinic.

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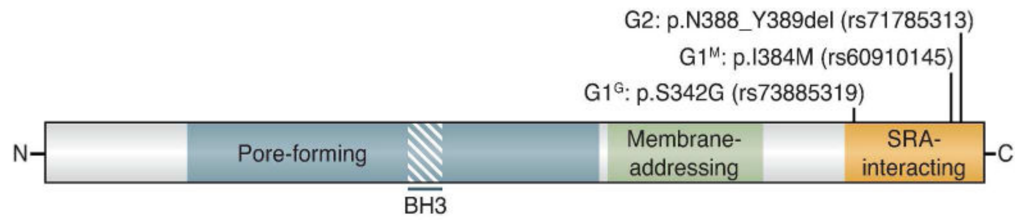

Figure 1. Predicted APOL1 structural domain organization

Pore-forming domain covers residues 60 to 235; BH3 domain = 154–168; membrane-addressing domain = 238–304; and SRA-interacting domain = 339–398. BH3, Bcl-2 homology domain 3.

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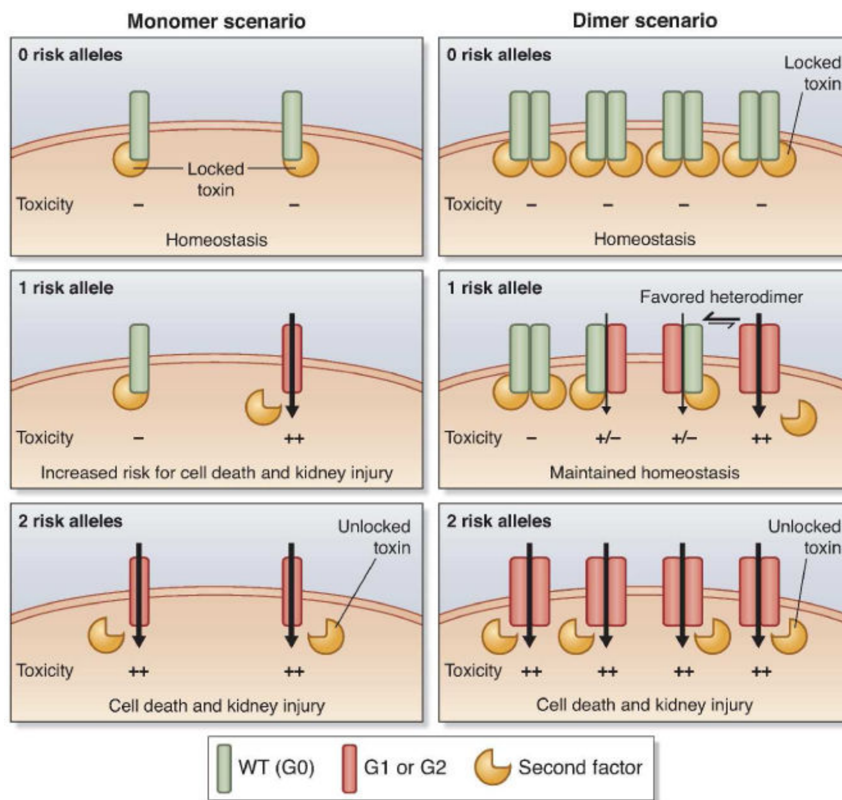

Figure 2. Model of APOL1 multimers in renal cells reconciling a gain of deleterious function model with the recessive pattern of inheritance

Monomer and dimer scenarii are depicted on the left and right panels, respectively. Similarly to its trypanolytic function, we consider that APOL1 wild-type (G0, in green) toxic activity can be inhibited by a yet to be formally identified second factor (the trigger-lock system, in yellow) protecting the cell from death (locked weapon). The inhibition is lifted (actuating the trigger) by the G1 and G2 isoforms (in dark orange), enhancing the toxicity (illustrated by the black arrow) and cell death (weapon discharge) that could eventually lead to the development of glomerular injury. Only the dimer scenario offers a neutral activity for the carriers of one risk allele by limiting the number of damaging APOL1 channels (heterodimers exhibit a low or no toxicity owing to the second factor binding) and therefore reconciles with the recessive model of inheritance.

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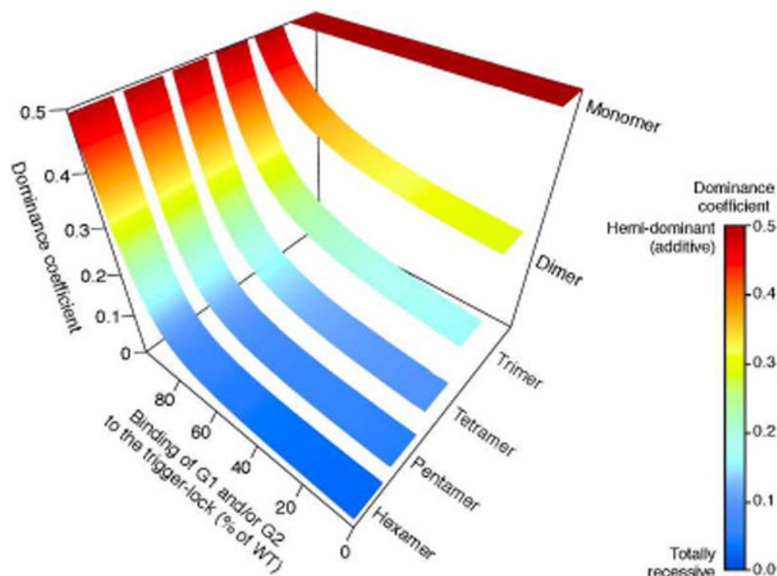

Figure 3. Modeling recessivity as a function of multimerization and loss of binding to the trigger-lock

We assume the following simple model: 1) There is a decrease of probability of having the trigger-lock bound to APOL1 for APOL1 risk isoforms (G1 and G2 are assumed to have equal binding) compared to wild-type (WT); 2) For carriers of 1 risk allele (WT/G1 or WT/G2 heterozygotes), multimers are formed from WT or risk molecules, drawn randomly to give a binary distribution; 3) Binding of the trigger-lock to any of the APOL1 molecules in the multimer blocks multimer activity. For this model, we plot the dominance coefficient as a function of the loss of binding probability for risk allele isoforms to the trigger-lock (expressed as a % of WT binding), from monomers to hexamers. Here, the dominance coefficient ranges from 0.5 predicting an additive model (where one risk allele carriers have half the increased risk of kidney injury of two risk alleles carriers) to 0 for a completely recessive model (where one risk allele carriers have no increased risk).

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Table 1

APOL1 G1 and G2 risk alleles for FSGS are efficient *T.b. rhodesiense* killers through different trypanolytic mechanisms.

	Trypanolytic activity				Toxicity		
APOL1 isoform	***T.b. brucei***	***T.b. gambiense***	***T.b. rhodesiense***	**Trypanosome SRA binding**	**Dilution factor for 100% *in vitro* lysis**	**Mice liver necrosis**	**OR [95%CI] for FSGS, recessive model**
WT (G0)	Lysis	No	No	Yes	–	None	Ref.
$G1^{G}$	Lysis	No	Lysis	Yes	10^{1} or undiluted[a]	Severe and widespread	17 [10–32][b]
$G1^{M}$	Lysis	No	No	Yes		None	
G2	Lysis	No	Lysis	No	10^{4}	Moderate and Focal	25 [9–82][c]

Data from[1,18,21]. WT, wild-type; OR, odds-ratio; CI, confidence interval.

[a] $G1^{M}$ was only considered with $G1^{G}$.

[b] OR=17 when both $G1^{G}$ and $G1^{M}$ are present; OR=16 when only $G1^{G}$ is present.

[c] Compound heterozygotes G1/G2 exhibit a similar effect size (OR=17) as G1/G1 or G2/G2.