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The cell biology of APOL1

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Abstract

The association of variants in the *APOL1* gene, which encodes apolipoprotein L1 (APOL1), with progressive non-diabetic kidney diseases in African Americans has prompted intense investigation into the function(s) of APOL1. APOL1 is an innate immune effector that protects humans from infection by some trypanosomal parasites. We review the data characterizing APOL1 trypanolytic function, which has been a basis for studies of APOL1 function in mammalian cells. Subsequently, we discuss the studies that use animal models, mammalian cell culture models, and kidney biopsy tissue to discover the mechanisms of variant APOL1-associated kidney diseases.

Introduction

The landmark discovery that genetic variations in the *APOL1* gene associate with non-diabetic kidney diseases in individuals with African ancestry^{1,2} has prompted investigation into biologic mechanisms underlying the association. *APOL1* encodes the apolipoprotein L1 protein (APOL1), which primarily circulates bound to HDL particles. This circulating fraction of APOL1 imparts resistance to trypanosomiasis by rapidly lysing infecting trypanosomes of the *Trypanosoma brucei* species³, trypanosome parasites endemic to Africa. However, several *T. brucei* subspecies, *T. b. rhodesiense* and *T. b. gambiense*, which cause African Sleeping Sickness, developed mechanisms to resist APOL1-dependent killing. The *APOL1* kidney disease-associated polymorphisms encode APOL1 proteins that circumvent immune evasion by the parasite and restore parasite killing to the resistant subspecies, but if present on both alleles, strongly increase chronic kidney disease (CKD) risk. The *APOL1* gene (and trypanolytic activity) is only present in humans and several other primate species^{4–6}, which complicates design of *in vitro* and animal models to explore the pathobiology of variant APOL1-associated kidney diseases. In this review article, we focus on data primarily from experimental models, which describe potential mechanisms of APOL1-associated kidney injury. Human kidney biopsy gene expression data addressing APOL1 function are also reviewed. In the following discussion, APOL1-G0 indicates the

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“wild-type” or reference sequence; APOL1-G1 and APOL1-G2 are the kidney disease associated proteins.

APOL1 and trypanolysis

The mechanisms of APOL1's trypanosome-killing activity and the trypanosome's response have been the prime drivers of hypotheses about the pathways mediating APOL1-associated kidney injury (Figure 1). Humans and some higher primates resist trypanosomiasis by *T. brucei*, an arthropod-borne multispecies pathogen that limits agrarian activity in sub-Saharan Africa because livestock are primary hosts. Two trypanosome lytic factors (TLF), constitutively circulating as innate immune effectors, mediate this protection, and both contain the primate-only proteins, APOL1 and haptoglobin-related protein (HPR). Interestingly, genetic variants in the HPR locus associate with circulating APOL1 levels⁷, suggesting the biogenesis of the component proteins comprising TLFs is coordinated. APOL1 is the trypanolytic molecule within both TLFs, and APOL-G0 protects humans from trypanosomiasis by the ancestral *T. b. brucei*. Only TLF-1 will be further discussed; more data about both TLFs are summarized in an excellent, recent review⁸. TLF-1 particles are a subclass of high density lipoprotein particles (HDL), which are lipid-rich and contain apolipoprotein A1 in addition to APOL1 and HPR. To evade the human innate immune response conferred by the TLFs, *T. b. rhodesiense* and *T. b. gambiense*, have adapted by antigenic variation in one of their variant surface glycoproteins (VSG) expressed on the trypanosomal plasma membrane. Trypanosome VSGs are encoded from over 1000 transcription units within the trypanosome genome in 15 expression sites. Transcriptional switching between expression sites and/or by homologous recombination of VSG genes⁸ allows the parasite to elude host humoral immune defenses and other environmental stresses. A VSG, serum response-associated (SRA) protein, is expressed by one of the human disease-causing trypanosomes (*T. b. rhodesiense*) and binds APOL1-G0, blocking its trypanolytic activity. To counter this parasite adaption, *APOL1* sequence variants, encoding APOL1-G1 and APOL1-G2, evolved by positive selection¹. Both variant APOL1 proteins have low affinity for SRA and restore parasite killing and resistance to trypanosomiasis, albeit at increased susceptibility to chronic kidney diseases in humans with two risk alleles.

For trypanolysis to occur, TLF-1 is endocytosed into the parasite by a specific receptor for haptoglobin-hemoglobin, which supplies heme iron necessary for growth and resistance to host's oxidative response⁹. HPR is the ligand for the trypanosomal receptor, facilitating the uptake of TLF-1. A number of studies have demonstrated that TLF-1 traffics through the endolysosomal pathway, ultimately accumulating in lysosomes^{3, 10–12}. However, other evidence has suggested TLF-1 or APOL1 also targets, at least in part, to plasma membrane or the mitochondria, respectively^{13–15}. Early studies suggested SRA bound TLF-1 in the lysosome to block its toxicity and protect the parasite¹¹. More recent data have shown SRA and TLF-1 may actually interact in early endosomes and that SRA accelerates TLF-1 degradation¹⁶.

The mechanism of trypanosomal cell death due to TLF-1 remains unclear. APOL1 is the trypanolytic toxin⁸ in both TLFs, although some studies suggest both HPR and APOA1 are also necessary^{12, 17}. The APOL1 aa 60 – aa235 region has marginal similarity to the pore-

forming domains of the bacterial colicins, which are bactericidal toxins. In an acidic environment found in some endosomes and lysosomes, APOL1 undergoes conformational changes that facilitates its insertion into membranes. The initial report showed lysosomal membrane targeting resulting in chloride influx¹¹. However others demonstrated APOL1 targets the plasma membrane through endosomal recycling and functions as a non-selective, cation channel^{13,14,18}. In contrast to data that have demonstrated low pH allows ion fluxes, other investigators have found that APOL1, inserted into membranes in acid environments, remains inactive, only permitting ion flux when exposed to physiological pH¹⁸. Lysosomal swelling may be the proximate cause of trypanosomal death¹¹. Other data suggest sodium influx through APOL1 pores in the plasma membrane generates osmotic stress with cytoplasmic swelling and trypanolysis^{13,14,18}. APOL1-dependent osmotic lysis can be blocked by the anti-oxidant DPPD, suggesting APOL1-dependent oxidative stress also results from its pore-forming activity¹⁴. Trypanolysis has been dissociated from lysosomal swelling¹⁵, suggesting the lysosomal localization of APOL1 may reflect the lysosome's role as a detoxifying pathway for the parasite rather than key effector of parasite lysis. Rather, some APOL1-containing endosomes escape from the endolysosomal pathway and are trafficked by a trypanosomal kinesin to the mitochondria. Here APOL1 causes mitochondrial membrane permeabilization permitting release of a trypanosomal endonuclease and resulting in DNA fragmentation and trypanolysis.

In aggregate, the data strongly support that APOL1 is the trypanolytic toxin and that it undergoes a conformational change in an acid environment to insert into a lipid membrane. APOL1-G0, -G1 and -G2 have pore-forming or channel like activity in trypanosomes; however, data conflict on the membrane target, channel selectivity, and effect of acid pH on ion flux. Channel activity has been characterized as an acid-activated chloride channel and as an acid-gated cation channel. The specific mechanism of trypanolysis remains unclear; lysosomal membrane disruption, lysosomal swelling, osmotic lysis and apoptosis-like cell death are all mechanisms proposed to cause parasite death. Trypanolytic mechanisms have informed the design of many of the experiments discussed below, which focus on pathways of variant APOL1-dependent kidney injury.

APOL1 tissue and cellular distribution

APOL1 is a circulating trypanolytic toxin, and intriguingly, circulating factors may cause focal segmental glomerulosclerosis (FSGS), a kidney disease associated with *APOL1* G1 and G2 variants^{19, 20}. However, the *APOL1* gene is also expressed in many tissues including the kidney^{6, 21, 22}. This broad tissue distribution suggests that, in addition to its role as a circulating trypanolytic innate immune effector, APOL1 may have a local role in the tissues where it is expressed, either para- or intracellularly. Therefore either circulating variant APOL1 and/or kidney-associated APOL1 could increase the risk for kidney diseases associated with the *APOL1* G1 or G2 variants. Published evidence provides strong support for the premise that kidney-associated APOL1-G1 and/or -G2 mediate disease. Circulating total APOL1 and HDL levels failed to correlate with kidney disease phenotypes in African American HIV subjects followed in the ALLRT cohort²³ or in the Dallas Heart Study⁷. In the latter study, APOL1-G0, -G1 and G2 were quantified by LC-MS. An additional analysis of allele-specific APOL1 with kidney phenotypes, restricted to individuals who carried at

least one copy of the corresponding allele, still failed to show an association with chronic kidney disease, eGFR or albuminuria. In a second approach to assess the contributions of circulating and kidney-associated variant APOL1 to disease, the *APOL1* genotypes of transplanted kidneys and/or the kidney transplant recipients have been ascertained in several cohorts^{24–26}. Risk for graft loss associated with the genotype of the transplanted organ, but not with the genotype of the recipient.

In normal human kidney the APOL1 protein localizes to the podocyte, the proximal tubule and the endothelium of extraglomerular arterioles and small arteries²⁷, a finding subsequently replicated by others^{28, 29}. Interestingly, the amount of APOL1 protein in the podocyte and proximal tubules was decreased in FSGS or HIV associated nephropathy (HIVAN), two kidney diseases associated with APOL1 risk variants^{27, 29}. Although, the APOL1 signal in the vascular endothelium appeared similar in disease and non-disease kidney sections, the vascular media of renal arterioles and small arteries stained positive only in the setting of kidney disease²⁷. Localization of APOL1 to the podocyte is consistent with its central role in the pathogenesis of many of the kidney diseases associated with APOL1. However, the localization of APOL1 to renal cells outside of the glomerulus raises the possibility that these cell types and a functional role for APOL1 within them may contribute to the phenotypes of some renal pathologies such as the proximal tubule in HIVAN or the vascular media and endothelium in hypertension associated kidney disease. The functional role of APOL1 within any of these cell types remains unknown.

APOL 1 abundance and kidney disease

APOL1 expression is induced by TNF- α , an inflammatory cytokine, in cultured endothelial cells²² and cancer cells³⁰. Cytokine induction of *APOL1* expression was subsequently confirmed in cultured kidney microvascular endothelial cells and podocytes^{27, 31, 32}. The *APOL1* promoter contains regulatory elements for the transcription factors STAT2, interferon regulating factor (IRF) 1 and IRF2³², suggesting that *APOL1* is a cellular immune response gene, whose expression is stimulated by viral infection. This speculation is supported by experiments demonstrating increased expression of *APOL1* after treatment of cultured cells with poly I:C, which mimics double stranded RNA and models viral infection³². Together these data suggest that APOL1 is part of an anti-viral response, an idea consistent with the strong association of the *APOL1* G1 and G2 variants with HIVAN³³. APOL1-G0 may prevent uncontrolled HIV infection of the kidney or the APOL1-G1 and-G2 variants in the setting of HIV infection may mediate kidney injury and fail to stop HIV infection.

Although inflammatory mediators consistently increase APOL1 expression *in vitro*, immunohistochemical studies of human kidney sections of individuals with kidney diseases demonstrate decreased abundance of APOL1 protein in the podocyte and proximal tubule^{27, 29}. Subjects with kidney diseases and the *APOL1* risk genotype [two risk alleles] have similar APOL1 abundance by immunohistology compared to subjects with kidney disease and the low risk genotype [one on no risk alleles]. Another study compared *APOL1* transcript levels in glomerular and tubulointerstitial compartments microdissected from human kidney biopsy samples of nephrotic syndrome subjects. Concordant with the

immunohistology, *APOL1* mRNA in both compartments was similar between nephrotic syndrome subjects with and without *APOL1* risk genotypes³⁴. These data suggest that *APOL1*-associated kidney diseases do not result from differential abundance of variant APOL1 compared to APOL1-G0. Of course, both studies used clinically indicated biopsies, reflecting study populations with more advanced kidney diseases that may have obscured variant-dependent differences in APOL1 abundance earlier in the disease course. APOL1 levels have not been compared between kidney diseases associated with *APOL1* variants (e.g. HIVAN and FSGS) and those that are not (e.g. diabetic nephropathy and IgA nephropathy). Further study will be needed to reconcile discrepant assessment of APOL1 abundance between the cell culture models and kidney biopsy observations, and determine if *APOL1* transcription or translation is dysregulated in kidney disease.

APOL1-associated cytotoxicity

Similar to its function as a trypanosome toxin, ectopic expression of both APOL1-G0 and the variant APOL1s in mammalian cells can cause cytotoxicity^{31, 32, 35–38}. As previously mentioned, the APOL1 protein has homology to the bacterial pore-forming toxins of the colicin family¹¹, which have channel-like properties and APOL1-mediated cytotoxicity is dependent upon channel activity^{38, 39}. In these reports the expression of APOL1-G0 or its kidney disease-associated variants are associated with cytotoxicity in stable expression in cell culture³⁸ or when expressed in *Xenopus* oocytes³⁹. APOL1 -G1 and -G2 in cultured cells caused increased cytotoxicity and cell swelling compared to cells expressing APOL1-G0. In contrast, expression of APOL1 in the *Xenopus* oocyte model failed to detect a difference in cytotoxicity between the G0 and the G1 or G2 risk variants. An important piece of data regarding APOL1-mediated cytotoxicity that will need to be addressed in future studies is whether variant-dependent cytotoxicity also occurs in model systems where APOL1 expression is under control of its endogenous promoter and ultimately in APOL1-associated kidney diseases.

Cytotoxicity in extra-renal cells and tissues has also been observed in two published reports. The first examines the stable expression of APOL1-G0, -G1, and -G2 in hepatocytes³⁶. APOL1-G0, -G1 and -G2 expression caused cytotoxicity when stably expressed in rat hepatoma cells without a consistent increase in risk variant cytotoxicity³⁶. The second report utilized hydrodynamic gene delivery (HGD), a technique that allows the expression of a gene of interest in mice resulting in primarily hepatic expression. HGD was used to express the APOL1-G0, -G1, and -G2 variants in the liver and circulation of mice⁴⁰. Although the APOL1 variants have not been associated with liver disease or damage, the authors observed severe hepatic necrosis after injection of constructs for the expression of APOL1-G1, which was less severe for -G2 and not observed with -G0. No renal phenotype was observed in these animals; however, the level of kidney expression of the transgene is much lower than hepatic expression when using the HGD technique.

Two papers have reported kidney phenotypes in model organisms. Morpholino or CRISPR/Cas9 knockdown of zebrafish (*zf*) *apoli* caused pericardial edema, depressed glomerular filtration and podocyte foot process effacement^{29, 41}, which was complemented by human *APOL1* G0 but not G1 or G2⁴¹, suggesting variant APOL1 have lost an activity necessary

for normal function of the pronephric glomerular filtration barrier. zfApoll1 is expressed in podocytes and endothelial cells in the zebrafish pronephros and has 38% identity to human APOL1^{41, 42}. The rapid gene duplication of the human *APOL1* gene cluster makes it difficult to determine homologues outside primate species. However, a case report of an individual with truncating mutations in APOL1 and normal kidney function⁴³ suggests that the function of APOL1 may not be constitutive, but required in response to specific environmental challenges. We created transgenic (Tg) mice using the *Nphs1* promoter to express APOL1-G0 or APOL1-G2 in podocytes⁴⁴. Tg-G0 and Tg-G2 mice did not develop kidney pathology, proteinuria, or azotemia as of 300 days of age, and we could not demonstrate necrotic, apoptotic or autophagic cell death. However, podocyte density was significantly decreased by 200 days of age in Tg-G2 mice compared to age-matched wild-type and Tg-G0 mice. These data suggest APOL1 kidney disease risk variants may compromise the ability of the podocyte to maintain attachment during the hypertrophy of the glomerulus that occurs during normal aging^{45, 46}. In the absence of an additional environmental stressor, the degree of podocyte loss related to the APOL1 risk variants is clinically silent as was observed in the transgenic mouse model (Figure 2) but a “second hit,” like HIV infection, could accelerate podocyte depletion and cause overt kidney disease.

APOL1-activated signaling pathways

Transient and stable expression of APOL1 in cell culture models has been used to study its function. An early report characterized APOL1-G0 as a BH3 domain containing protein and stable expression of APOL1-G0 in cancer cell lines resulted in cell death attributed to autophagy³⁵. These authors found that mutation of the BH3 domain of APOL1-G0 abolished the cell death phenotype. A subsequent report found that this was not related to any functional role of the BH3 domain, but more likely related to altered protein structure resulting from deletion of nine amino acids comprising the BH3 domain³⁹. The role of autophagy in APOL1-mediated cell death has subsequently been examined in a number of reports, which have come to conflicting conclusions^{31, 36–39}. The transient expression APOL1-G0 in HEK293 cells led to an autophagic block due to failure of autophagosomes-lysosome fusion but not autophagy induction and the use of inhibitors of autophagy did not block cell death³⁷. Two studies examined cultured cells stably expressing APOL1 in podocytes³¹ or HEK293 cells³⁸. Both observed variant-dependent differences in APOL1-mediated cell death. Lan et al did not directly measure autophagic flux, but treatment of cells expressing APOL1 with chloroquine, an inhibitor of lysosome acidification, a necessary step in autophagic maturation, attenuated APOL1 variant-dependent cell death³¹. Olabisi et al reported an increase in LC3II/LC3I, a biochemical index of autophagic flux, in cells expressing APOL1-G1, but not in cells expressing APOL1-G0³⁸. However, autophagy inhibitors did not block APOL1-mediated cytotoxicity. APOL1-G0, -G1, and -G2 all increased LC3II and caused cytotoxicity in rat hepatoma cells, which was reduced by blocking autophagy induction with 3-methyladenine, a class III phosphoinositide 3-kinase inhibitor³⁶. Finally, in *Xenopus* oocytes expressing APOL1-G0, -G1 and -G2 caused morphologic toxicity³⁹. Although autophagy was not directly assessed, treatment of oocytes with inhibitors of autophagy induction or maturation did not alter morphological deterioration.

Reconciling these conflicting data is difficult given the diversity of models and experimental designs. Taken together, reference and variant APOL1 can induce autophagy in certain systems. However none of the published data allows a distinction between cause and effect. That is, does APOL1 directly stimulate autophagic pathways or does APOL1-mediated cell death trigger secondary activation of autophagic pathways? APOL1-dependent cytotoxicity results from ion fluxes after its insertion into the plasma membrane^{38, 39}, similar to mechanisms of trypanolysis reported by some but not all investigators^{13, 14}. Pore forming toxins of pathogens can activate autophagy in cells^{47, 48} suggesting that the observed induction of autophagy by APOL1 expression systems may be a cellular response to APOL1 mediated channel activity. Finally, stable expression of APOL1 in cells may poorly model its activities *in vivo*. The APOL1 transgenic mice, described in the previous section, were crossed with another transgenic mouse model ubiquitously expressing a green fluorescent protein-tagged microtubule-associated protein 1 light chain 3 (GFP-LC3)⁴⁴. LC3 labels autophagosomal membranes and GFP-LC3 can be visualized as fluorescent intracellular puncta. Autophagosome numbers in podocytes of wild type, Tg-G0 and Tg-G2 mice were equivalent, suggesting APOL1 expression alone is not sufficient to activate autophagy in healthy kidneys.

The channel activities of APOL1-G0 and variant APOL1s in mammalian cells have been characterized in several papers, again with conflicting results. APOL1 clearly has pore-forming or channel functions when reconstituted in lipid bilayers, which has been variably demonstrated to be a selective chloride channel¹¹ or a non-specific, cation channel that permitted K⁺ efflux and Na⁺ influx^{13,18}. In cultured cells, APOL1-G2, but not APOL1-G0, inserted in the plasma membrane leading to loss of intracellular potassium³⁸. p38 mitogen activated protein kinase (p38-MAPK) is activated by intracellular potassium loss after exposure of cells to pore-forming toxins⁴⁹. Similarly, p-38-MAPK was activated in cells expressing APOL1-G1 or APOL1-G2 but not APOL1-G0. Activation of p38 MAPK is associated with kidney diseases⁵⁰. In addition, both APOL1 variants downregulated gp130-STAT signaling pathways, but the role of this pathway in APOL1-dependent cell death and APOL1-associated kidney diseases is unclear. Some papers suggest STAT3 promotes podocyte differentiation. In contrast, increased STAT3 activity caused podocyte proliferation and dedifferentiation in FSGS and HIVAN^{51–53}, kidney diseases strongly associated with *APOL1* variants^{33, 54}.

HIV-1 infection of renal cells is required for HIVAN pathogenesis^{55, 56}, suggesting APOL1-G0 but not variant APOL1s could block HIV infection in kidney cells. Two papers suggest APOL1-G0 is a HIV restriction factor^{57, 58}, but neither paper assessed differences between APOL1-G0 and APOL1-G1 or -G2 in capability to restrict HIV infection. Taylor et al further explored the mechanism of APOL1-G0 as an HIV restriction factor and found APOL1-G0 caused nuclear translocation of transcription factor EB (TFEB) with subsequent increases in endocytosis and lysosomal biogenesis, which was concordant with a reduction of viral transcription and endolysosomal clearance of the viral proteins Vif and the endocytosis and Gag. Interestingly, a subsequent report found that, while necessary for APOL1 viral restriction function, TFEB nuclear translocation is not required for APOL1-mediated cytotoxicity³⁷.

Currently little data addresses APOL1-G0, -G1 or -G2 function in human tissues where it is expressed under its endogenous promoter. As previously mentioned, *APOL1* transcript levels were similar in glomerular or tubulointerstitial compartments of kidney biopsies from proteinuric African Americans with high and low risk *APOL1* risk genotypes³⁴. In addition, differential expression analysis, comparing transcriptomes from kidney biopsies of subjects with *APOL1* high risk and low risk genotypes, identified only five differentially expressed genes (CXCL9, CXCL11, and UBD in glomerulus; SNOR14B and MUC13 in tubulointerstitium). Finally, these investigators used coregulation analysis to identify gene sets whose expression correlated with *APOL1* transcript levels in the tubulointerstitial compartments of subjects with high risk versus low risk *APOL1* genotypes. Pathway analysis of gene sets, whose expression correlated with *APOL1* transcripts, suggested immune and inflammatory pathways are differentially activated in kidneys of proteinuric subjects with high risk *APOL1* genotypes.

Conclusions

More than 20 million Americans have CKD and more than 500,000 patients have end-stage renal disease (ESRD). Health care for ESRD patients, who comprise less than 1% of Medicare recipients, accounts for over 7% of Medicare expenditures, approximately \$35 billion annually. ESRD patients are at risk for accelerated cardiovascular disease and early death. African American CKD patients in the United States are four times more likely than white patients to progress to ESRD, and a single genetic susceptibility locus (*APOL1*) explains much of this increased risk. In order to improve our prognostic, diagnostic and therapeutic approaches, we need to gain a better understanding of the biologic mechanisms underlying the genetic association of APOL1 variations and kidney disease.

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Figure 1. APOL1 localization and activities in the trypanosome has parallels in human cells
(A) APOL1 enters the trypanosome as a component of HDL3 particles via endocytosis at the flagellar pocket and is trafficked to the lysosome. Independent studies report different mechanisms of trypanosomal killing. Activation of the APOL1 channel activity within the lysosome has been reported to cause lysosomal swelling and trypanolysis¹¹. Others have found trypanolytic activity has localized to the plasma membrane¹³, or the mitochondria¹⁵.
(B) Studies overexpressing APOL1 in human cells have reported that its expression leads to the movement of the transcription factor EB (TFEB) from the cytosol to the nucleus where it induces the transcription of genes necessary for the upregulation of lysosomal biogenesis and increases endosomal trafficking⁵⁷. The upregulation of these pathways contributes to the restriction of HIV-1 infection in this report.
(C) Other investigators found that overexpressed APOL1-G1 and-G2, but not G0, can localize to the plasma membrane where it allows the efflux of potassium driving the phosphorylation and activation of p38 MAPK and inhibition of gp130-STAT3 signaling that may change the cells transcriptional program³⁸. Activation of the p38/STAT3 pathway downstream of channel activity at the plasma membrane was observed only after expression of the kidney disease risk variants of APOL1.

Figure 2. Trajectory of podocyte density change in FVB, Tg-G0 and Tg-G2 mice

Podocyte densities in wild-type, Tg-G0, and Tg-G2 mouse kidneys using a method developed by the Wiggins lab⁴⁵. Data used to construct these trend lines were reported in Bruggeman et al⁴⁴. In rodents and humans, once podocyte density decreases by 40%, glomerular disease becomes progressive⁴⁶. At 40d, podocyte densities were similar in FVB, Tg-G0 and Tg-G2 mice. Subsequently, podocyte density in the Tg-G2 mice were significantly lower but remained above the threshold for overt kidney disease through 300 days of age. We extrapolated the rate podocyte density change from the empirical data and found Tg-G2 mice would not be at risk for overt kidney disease within the normal lifespan of FVB/N mice, which is consistent with the observation that individuals with *APOL1* risk genotypes are not destined to develop kidney disease in the absence of a “second hit” (e.g. HIV infection).