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The biology of APOL1 with insights into the association of APOL1 variants with chronic kidney disease

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Abstract

Recent studies have identified genetic variants in *APOL1* that may contribute to the increased incidence of kidney disease in populations with African ancestry. Herein, we review the biology of APOL1 present in the circulation and localized to the kidney as it may contribute to the pathogenesis of APOL1 associated kidney disease.

Keywords

Genetics; apolipoprotein; HDL; APOL1; kidney; focal segmental glomerulosclerosis; HIV; HIV associated nephropathy; hypertensive nephrosclerosis; chronic kidney disease

Introduction

In 2008 two reports [1,2] provided the first indications that genetic variants on chromosome 22 contribute to the pathogenesis of chronic kidney disease. These groups utilized an approach designed to detect genetic contributors to the increased risk for focal segmental glomerulosclerosis (FSGS) [3] and HIV associated nephropathy (HIVAN) [4] observed in populations with African ancestry. Both groups identified a locus on chromosome 22 associated with non-diabetic kidney disease in African American populations. This 1.2 Mb locus contained 35 genes [1], including *MYH9*, a particularly strong biological candidate, based upon its podocyte expression and previous identification of *MYH9* mutations in individuals with hereditary giant platelet disorders that also have a variably penetrant glomerular pathology; however, work has failed to reveal likely causal variants in *MYH9* [5].

Using data from 1000 genome project, two groups reexamined the chromosome 22 locus for causal variants outside of *MYH9* and identified strongly associated coding variants in the *APOL1* gene, which is adjacent to *MYH9* [6,7]. *APOL1* is a 14.5 kb gene with seven exons, encoding apolipoprotein L1 a 398 amino acid protein. The genetic variants that were identified in *APOL1* were designated G1 and G2 [6]; with the reference sequence designated

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G0 (Figure 1). These variants are common in African populations, but nearly absent from other ancestral populations.

The initial reports associating *APOL1* with non-diabetic kidney disease examined populations with hypertensive kidney disease and FSGS [6,7]. The results of many follow-up genetic studies have supported these initial observations and the evidence supporting the association of *APOL1* variants with kidney disease was recently summarized in an excellent review [8]. These follow-up studies have extended the spectrum of *APOL1* associated diseases and the majority of these studies found the renal pathology and clinical endpoints examined to be associated with two *APOL1* risk variants when compared to zero or one *APOL1* risk variant. These results are consistent with the initial reports describing the association of *APOL1* with non-diabetic kidney disease, where the largest effect sizes were noted using a recessive model of inheritance [6,7]. Whether a single risk variant contributes to the initiation or progression of kidney disease remains uncertain at present, and these studies do not exclude the possibility that there is an effect from a single variant.

The *APOL1* gene is unique to humans and a few non-human primates [9] and is located on a region of chromosome 22 that has undergone positive selection [6,10]. While genetic studies continue to examine the frequencies of *APOL1* variants across different African populations and the factors contributing to this variation [10,11], studies examining the biological function of the *APOL1* protein are now needed to support the genetic association of *APOL1* variants with non-diabetic kidney disease and confirm a causal link between *APOL1* and non-diabetic kidney disease.

APOL1 Protein in the Circulation

Prior to its association with non-diabetic kidney disease, *APOL1* was recognized as the factor in human serum conferring resistance to *Typanosoma brucei brucei* [12], a blood borne parasite endemic to Africa. While the G0, G1 and G2 variants all protect from infection with *Typanosoma brucei brucei*, the G1 and G2 variant also protect from infection by an additional subspecies, *Typanosoma brucei rhodesiense*. A single copy of either the G1 or G2 variant is sufficient to confer this expanded resistance to trypanosomal infection and suggests a possible mechanism contributing to the signal of positive selection detected at this chromosomal location. However, if an individual has a G1 or G2 variant on both alleles of the *APOL1* gene then their risk for non-diabetic kidney disease is increased (Figure 1C). The *APOL1* protein has a secretory signal and circulates in the blood bound to HDL₃ particles that also contain apolipoprotein A1 (APOA1) and the hemoglobin-binding, haptoglobin related protein (HPR). Following endocytosis *APOL1* is localized to the lysosome of the parasite [12], and leads to lysis of the trypanosome by forming pores in the lysosomal membrane mediated by a pore forming domain flanked by membrane addressing domains [13]. The trypanolytic function of *APOL1* clearly demonstrates the importance of circulating *APOL1* in innate immunity.

Circulating *APOL1* was initially identified as a constituent of isolated HDL particles, the majority was associated with HDL₃ particles, but small amounts were also observed in VLDL and HDL₂ particles [14]. Free *APOL1* was not detected in serum. Additional

apolipoprotein components of APOL1-positive particles include apolipoproteins A2, A4, C3, and A1. APOA1 is the major protein component of HDL particles but only 10% of APOA1-containing HDL particles have APOL1 [14], suggesting that the APOL1-positive HDL particles may serve a unique function. Whether this functional niche is entirely explained by the role of APOL1 in innate immune responses or additional functional roles will be identified is an interesting question.

Surprisingly, APOL1 levels in the circulation of normolipidemic subjects is correlated with total cholesterol and triglycerides, but not HDL, the lipid moiety to which it is bound [15]. Increased circulating APOL1 levels have been reported in individuals with hyperlipidemia [15], as well as in subjects with type 2 diabetes where APOL1 levels were correlated with triglyceride levels, but not hemoglobin A1c or fasting glucose, suggesting the increase in APOL1 level was related to the plasma lipid concentrations and not diabetes. Another study examining individuals with coronary artery disease (CAD) [16] did not find an association between circulating APOL1 levels and the progression of CAD or the combined use of the lipid lowering agents, simvastatin and niacin, but again the correlation with VLDL triglycerides was observed. In addition, circulating APOL1 levels were correlated with hyperglycemia in this cohort 18% of whom had impaired fasting glucose or type 2 diabetes. Since this correlation was stronger with frank hyperglycemia than fasting glucose levels, the authors speculate that the correlation is not the result of glucose levels, but another abnormality tightly correlated with hyperglycemia. Diabetes is an important cause of proteinuric kidney disease; however, these reports included no phenotypic data of proteinuria or kidney function. It will be important to learn from future studies if circulating APOL1 levels are influenced by proteinuria in APOL1-associated and unassociated kidney diseases.

A role in lipid metabolism has been postulated for APOL1 based upon the presence of a sterol responsive element in its promoter and the prediction of amphipathic helices in its secondary structure [17]. The contribution of genetic variation in *APOL1* on circulating levels of APOL1 or plasma lipids has been examined in a few studies. The G1 risk allele did not affect HDL cholesterol or estimated GFR in African Americans or West Africans [18]. There was a negative correlation between total HDL cholesterol and eGFR in these populations, where lower total cholesterol was correlated with higher eGFR and this relationship was more negative in individuals with two G1 risk variants compared to individuals with zero or one risk variant. These results imply that HDL particles in individuals with *APOL1* risk variants may deleterious effect on kidney function; however, it should be noted that circulating APOL1 levels were not measured and in other studies APOL1 levels have not been correlated with HDL cholesterol levels [15,16]. Another report found that individuals with two APOL1 risk variants had a reduction in the medium sized HDL particles compared to individuals with zero or one risk variant [19]. Again there was no difference in the total HDL concentration between these two groups, suggesting that APOL1 variants may alter the relative concentration of specific fractions of HDL without altering the total HDL concentration.

Intracellular Distribution and Function of APOL1

The APOL1 protein has recently been localized to specific cell types in normal human kidney sections [20]. Similar to previous reports localizing APOL1 to vascular endothelium [9], APOL1 was detected in the endothelium of extraglomerular vasculature, but not in the glomerular endothelium [20]. APOL1 was also present in the podocytes and proximal tubules. The localization of the APOL1 protein to distinct renal cell types immediately raises the question of whether it is transcribed in these cell types or taken up from the circulation or glomerular filtrate. If however, *APOL1* is expressed in the kidney, a presumption that still awaits experimental confirmation, then a critical question is whether the circulating or kidney-specific fraction of APOL1, is mediating the pathogenesis of kidney disease. This is a particularly important question to answer in the context of renal transplantation and two reports from the transplant literature provide some insight into the answer [21,22]. In the first report kidneys obtained from African American donors with two *APOL1* risk variants had a shorter graft survival when transplanted to ungenotyped recipients than kidneys obtained from African American donors with zero or one risk variant [21]. A subsequent study found no difference in graft survival when ungenotyped kidneys were transplanted to African American recipients with two *APOL1* risk variants compared to recipients with zero or one risk variant [22]. These studies are limited by their retrospective design and the absence of *APOL1* genotypes for the recipients or donors respectively. However, these results do provide interesting early indications that the risk for the initiation and progression of kidney damage may be imparted by the APOL1 expressed in the transplanted kidney, unfortunately, there is no information about circulating levels of APOL1 in these reports. Furthermore, these results highlight the critical need for well designed, prospective, follow-up studies examining the contribution of *APOL1* genotype on allograft survival, as well as, the possible impact of kidney donation on individuals with two *APOL1* risk variant genotypes.

The possible mechanisms whereby APOL1 with risk variants may contribute to the pathogenesis of kidney disease can be broadly divided into two general categories; variant specific changes in protein expression and distribution or variant-specific changes in protein function. We found changes in the distribution of APOL1 in FSGS and HIVAN kidney sections, both APOL1-associated diseases, with reduced signal intensity in the podocytes and proximal tubules and the *de novo* appearance of APOL1 in the vascular media of small arterial vessels of the kidney [20]. The pattern of these changes were the same in sections with and without *APOL1* risk variants, suggesting that the alteration in APOL1 protein distribution is more likely a response to kidney damage than a result of *APOL1* risk variants. It will be important to replicate these results in a larger number of samples and determine if the altered protein distribution varies between APOL1 associated diseases compared to kidney diseases that are not associated with APOL1.

A possible biological function of APOL1 was highlighted in a previous report implicating APOL1 in autophagy pathways mediated by its BCL2 homology domain 3 (BH3) (Figure 1A), which is necessary for this function. Overexpression of APOL1 in cultured cancer cell lines led to the induction of autophagy and autophagic cell death [23] that was abrogated by the overexpression of APOL1 with a deletion of the BH3 domain. This BH3 domain is

embedded in the pore-forming domain of APOL1 (Figure 1A). The pore-forming domain is necessary for APOL1 mediated lysosomal chloride influx and trypanolysis [13], but it is not known if ion flux is also necessary for the induction of autophagy and autophagic cell death or whether APOL1 with or without variants is cytotoxic in the kidney cells where it has been localized. Autophagy pathways have been shown to be important for the maintenance of normal kidney function [24], and it will be interesting to determine if APOL1 variants have a differential effect on autophagy pathways.

Conclusion

Strong genetic support has been established for the association of APOL1 with non-diabetic kidney disease in populations with African ancestry. An improved understanding of APOL1 biology and the mechanisms that underlie its association with non-diabetic kidney disease is clearly important because of the high prevalence of *APOL1* variants and the overrepresentation of APOL1-associated kidney diseases in this population. Understanding the pathogenetic basis of APOL1-mediated kidney disease may also have broader applications to non-African populations through the elucidation of novel pathways and mechanisms of kidney disease. Moving forward the challenge will be the elucidation of the biological mechanisms that underlie this genetic association. Its role in mediating the innate immune response to trypanosomal infection is well recognized and the relationship of APOL1 variants to lipid metabolism is an active area of research. The localization of *APOL1* to specific renal cell types and the functional role of APOL1 in autophagy pathways raise interesting possibilities for ongoing research into the mechanisms explaining the association of APOL1 with progressive kidney damage.

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Figure 1. Sequence variants in Apolipoprotein L1 provide resistance to trypanosomal infection and increase kidney disease risk in African populations

Apolipoprotein L1 is 398 amino acid protein with multiple protein domains shown as a schematic (S=secretory signal, MAD=membrane addressing domain, B=BH3 domain, PFD=pore forming domain, SRA=serum resistance associated binding domain) (A). Both G1 and G2 are located in the last exon of the *APOL1* gene and are coding variants that result in changes to the primary sequence of the encoded protein. G1 is composed of two non-synonymous single nucleotide polymorphisms (SNPs), rs73885319 and rs60910145, that result in amino acid changes to the reference sequence NP_001130012.1, S342G and I384M, respectively. The G2 variant is a 6 base pair in frame deletion that results in the loss of two amino acid residues N388 and Y389. Since these variants are closely positioned in the genome recombination events are unlikely to occur between them. The two SNPs comprising the G1 variant have only rarely been observed individually and the G1 and G2 variants have not been reported to occur on the same allele (*i.e.* they are in perfect linkage disequilibrium) (B). The presence of one *APOL1* risk variant, G1 in the example, expands trypanosomal resistance to include the subspecies *brucei rhodesiense*. The presence of two *APOL1* risk variants continues to protect from *Trypanosoma brucei rhodesiense* infection, but now increases the individuals risk for *APOL1* associated chronic kidney disease (CKD) (C).