The relationship between APOL1 structure and function: Clinical implications

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Abstract

Common variants in the APOL1 gene are associated with an increased risk of nondiabetic kidney disease in individuals of African ancestry. Mechanisms by which APOL1 variants mediate kidney disease pathogenesis are not well understood. Amino acid changes resulting from the kidney disease-associated APOL1 variants alter the three-dimensional structure and conformational dynamics of the C-terminal α-helical domain of the protein, which can rationalize the functional consequences. Understanding the three-dimensional structure of the protein with and without the risk variants can provide insights into the pathogenesis of kidney diseases mediated by APOL1 variants.

Introduction

Population-based studies have established a strong association of two variants in the APOL1 gene with the excess risk of nondiabetic chronic kidney disease (CKD) in individuals of African ancestry. One variant involves the substitution of two amino acids (S342G and I384M; termed G1), and the other the deletion of two consecutive amino acids (N388 and Y389; termed G2) compared to the ancestral nonrisk allele termed G0. APOL1 G1 and G2 variants are common in individuals with African ancestry, with at least 50% of individuals carrying one copy of the risk allele and 15% with two copies of the risk allele. Despite the strong association of APOL1 variants with kidney disease, the molecular mechanisms by which these APOL1 variants contribute to CKD pathogenesis and progression remains unclear. In this review, we discuss the studies that characterized the structural properties of APOL1 and the effect of APOL1-G1 and -G2 variants on the structure.

Biology of APOL1

Apolipoprotein L1 (APOL1) is a member of the six-member APOL gene cluster located in human chromosome 22. APOL1 has unique features compared to other APOL family proteins. APOL1 was initially identified in the human pancreas but is widely expressed with the most abundant expression in
the placenta, lungs, prostate, and spleen.\textsuperscript{9} Unlike the other members of the APOL family, the APOL1 gene is limited to humans and a few non-human primates.\textsuperscript{7,8} Also, while APOL1 is predominantly synthesized in the liver, it is secreted and circulates in the blood in complex with HDL particles.\textsuperscript{9,10} The circulating APOL1-G0 is known to function as an innate immune factor by conferring protection from Trypanosoma brucei brucei, a parasite that causes endemic African sleeping sickness.\textsuperscript{11,12} However, additional species of trypanosomes evolved with a truncated variant surface glycoprotein, called the serum resistance-associated (SRA) protein, that neutralizes the trypanolytic effect of APOL1-G0.\textsuperscript{12,13} In the healthy human kidney, APOL1 is synthesized and expressed in podocytes, glomerular and extraglomerular vascular endothelial cells.\textsuperscript{14-17} APOL1 expression in cultured human podocyte and endothelial cell line is low, but the expression is upregulated by immune stimuli such as cytokines.\textsuperscript{15,18} However, the intracellular function of APOL1 remains to be fully understood, but multiple functions including a role in the regulation of autophagy, intracellular vesicle trafficking, ion channel activity as well as providing a protective effect from HIV infection have been proposed.\textsuperscript{14,19-23}

\textbf{APOL1 variants and risk of kidney disease}

The risk of kidney disease associated with APOL1 variants vary based on the CKD phenotype, and best fits a recessive model.\textsuperscript{1-3} A single allele of APOL1 G1 or G2 exhibits trypanolytic activity against additional subspecies of trypanosomes like \textit{T. brucei rhodesiense} providing a survival advantage.\textsuperscript{1} At the protein level, APOL1-G1 and -G2 variant failed to bind the trypanosomal SRA protein, partially explaining the extended trypanolytic activity and why the variants are positively selected in areas where trypanosomiasis is endemic.\textsuperscript{1,24} However, when two copies of these variants are present (homozygous alleles) there is a greater predisposition to kidney disease risk in addition to the extended trypanolytic activity. This scenario is reminiscent of hemoglobin S (HbS) and sickle cell disease, where a single allele of HbS, which results in sickle cell trait that is partially protective against malaria, while two alleles of HbS results in clinically evident sickle cell disease.\textsuperscript{25,26} Circulating levels of APOL1 did not associate with CKD risk.\textsuperscript{27,28} Hence, it is
hypothesized that the dysregulated cellular homeostasis is caused by variant APOL1 which is synthesized and expressed in the podocyte itself. The risk effect depends on the kidney disease phenotype with HIV associated nephropathy (odds ratio: 29), followed by focal segmental glomerulosclerosis (odds ratio: 17) and hypertension associated kidney disease (odds ratio: 7).\textsuperscript{1-3} Other CKD phenotypes, including SLE-related collapsing glomerulopathy and sickle cell disease-related nephropathy, have also been associated with the presence of high-risk APOL1 genotypes.\textsuperscript{29,30} With about 15\% of African Americans carrying two copies of high-risk APOL1 variants, about 5 million individuals are at risk of developing CKD. However, clinically evident CKD develops in a lower proportion suggesting that -in addition to the background of homozygous APOL1 high-risk variants- a “second hit” is required to manifest CKD.

**Mechanisms of APOL1 mediated kidney disease**

Over the past decade, multiple studies have advanced our understanding of APOL1 variant mediated kidney disease. These studies have established a variable subcellular localization of APOL1 proteins and demonstrated an activation of spatially diverse cell signaling cascades disrupting cellular homeostasis. Cytotoxicity, that results from an enhanced cation channel activity, impaired intracellular vesicular trafficking, induction of stress-activated protein kinase (SAPK) pathways, endoplasmic reticulum stress and reduced mitochondrial respiration rates has been proposed as mechanisms for APOL1-G1, and -G2 induced CKD pathogenesis.\textsuperscript{14,20,21,31-36} A unified mechanism that explains the dysregulated effect of APOL1-G1 and -G2 that leads to CKD pathogenesis and progression is still lacking.

**Structure-function correlation of APOL1 variants: Why is it important?**

Understanding the three-dimensional structure of proteins and the effects of genetic variations on their structure can provide valuable clues to unraveling disease pathogenesis and the identification of potential therapeutic strategies. Even a single amino acid change caused by gene variations can change the structure of a protein, resulting in devastating functional consequences. This is well illustrated in the
structural studies aimed at understanding the biology of hemoglobin and the functional effect of the HbS variation on the structure of hemoglobin. The structure of hemoglobin revealed the allosteric properties of the protein with respect to oxygen binding, resulting in the formation of oxyhemoglobin. Structural studies on HbS revealed that a single amino acid variation from glutamine to valine in the β-chain of hemoglobin results in polymerization of deoxy-HbS which, in turn, is the cause for the sickling of red blood cells. These structure-based studies have successfully guided the development of therapeutic strategies aimed at reversing the abnormal hemoglobin polymerization to treat sickle cell disease. As another example, a comprehensive characterization of aquaporin structure advanced the understanding of the function and development of molecules that can modulate its function in kidney tubules.

The structure of APOL1 has not been experimentally resolved so far. Nuclear magnetic resonance (NMR) spectroscopy, X-ray crystallography, and cryo-electron microscopy (cryo-EM) are the major established methods to determine the three-dimensional structure of proteins experimentally. The advantages of each of these methods that vary with the intrinsic properties of the protein being studied. For example, while the structure and dynamics of proteins can be studied in solution using NMR spectroscopy, it is not a method that is suited to study large size protein structures. X-ray crystallography and cryo-EM, on the other hand, are well suited to study large biomolecular complexes but provide no information about weaker binding interactions or protein dynamics. Our current understanding of the structure of APOL1-G0 and the effect of G1 and G2 variants on the protein structure and dynamics have been obtained from computational modeling, molecular dynamics (MD) simulations, and biophysical studies on recombinant APOL1. Computational modeling to predict a protein’s structure employs three main methods. The first and most effective is comparative or homology modeling, where the three-dimensional structure of an evolutionarily related protein is used as template to generate a structural model for the protein of interest. The second modeling method relies on the observation that proteins from different evolutionary background can have similar structures. Hence in the absence of a closely
related template protein, structure prediction can be achieved by modeling (“threading”) the target protein sequence into many possible known protein structures – the structures which are most compatible with the threaded protein sequences are considered further.\textsuperscript{46} The third and least accurate method is the \textit{ab initio} modeling, where the protein structure is predicted based on physical properties (energy estimates derived from protein sequence employed to predict secondary structures, turns etc.) without using a template and hence this latter methods is computationally exhaustive.\textsuperscript{47,48} The structural model that is predicted from these methods may not accurately reflect the physiological conformation of the protein, which can vary, based on specific cellular location and function. To advance the understanding of a protein structure, MD simulations can be applied to further refine the protein structure models obtained from these methods. MD simulation is a computational method to study the time dependent conformational behavior of biomolecules using the physics of atom movements at a certain temperature.\textsuperscript{14,49-51} APOL1 protein is divided into four domains, and the nomenclature was established in the context of its known function as the trypanosomal killing factor.\textsuperscript{11-13,52,53} The domains include a signal peptide region (M1-R26), a pore-forming domain (M60-W235), a membrane addressing domain (A238-P304) as well as a C-terminal trypanosomal SRA protein-interacting domain (A339-L398). APOL1 G1 and G2 variants are located in the C-terminal SRA interacting domain, and most of the studies have focused on establishing the structure of this region (\textbf{Figure 1}). As the structure of any proteins similar to APOL1 has not been experimentally resolved so far, the structural models proposed have used threading and \textit{ab initio} modeling methods in conjunction with MD simulations.\textsuperscript{12,14,54}

An initial model of the APOL1 C-terminus was published well before its association with kidney disease was discovered. The C-terminal SRA-interacting domain of APOL1-G0 (P340-L398) was shown to form an amphipathic $\alpha$-helix that interacted with the SRA protein in the endosomal compartment of trypanosomes.\textsuperscript{12} The structural model for this interaction provided insights into the neutralization of APOL1 activity by subspecies of Trypanosoma that cause human disease. The structural model suggested
mutations which were then engineered to validate the putative binding interface in the SRA protein. Similarly, naturally occurring kidney disease-associated APOL1-G1 and G2 variants result in an unstable complex formation and hence extended trypanolytic activity of variant APOL1.\textsuperscript{1,12,24} Sharma et al. advanced the structural studies to an extended portion (P340-L398) of the C-terminus of APOL1-G0, -G1, and -G2 using computational modeling.\textsuperscript{54} Their studies showed that the APOL1 C-terminus formed an α-helical hairpin structure. In this model, the amino acid substitution and deletion, corresponding to the G1 and G2 variants, resulted in the loss of inter-helical hydrogen bonds, which then manifested as a higher conformational mobility of the α-helical hairpin (Figure 2). Consistent with these observations, the two-dimensional NMR spectra of G1 varied considerably from those of G0. Our studies modeled a larger fragment of the APOL1 C-terminus (R305-L398) using threading algorithms followed by all-atom MD simulations.\textsuperscript{14} Similar to the other models, the C-terminus of APOL1 formed an α-helical bundle with G1 and G2 variants induced amino acid changes resulted in reduced conformational flexibility of the variant protein. While the initial model of reference and variant APOL1 C-termini proposed by the two latter studies are similar, MD simulations showed different time-dependent conformational behavior. There are multiple explanations for these apparent differences including longer protein fragment (residues 305-398) which added an additional α-helix and a more current force field (computational method to estimate energy between atoms) used in our studies.\textsuperscript{14} Recently, Jha et al. modeled the full-length structure of APOL1 proteins using \textit{ab initio} methods followed by MD simulations.\textsuperscript{55} In addition to confirming the C-terminal helical conformation adopted by APOL1s, the model showed the role of variant residues (S342 and I384 in G1) and (Y389 in G2) in establishing the channel function of APOL1. Overall, the protein conformational changes induced by the G1 and G2 variants could disrupt protein-protein interaction that is necessary for the cellular homeostatic function of APOL1 predisposing to CKD pathogenesis.

APOL1 is a membrane-associated protein with several putative transmembrane domains\textsuperscript{21,56-58} and localizes to multiple cellular membrane environments, including endo-lysosomes, golgi-ER,
mitochondria and plasma membranes. In this membrane environment, full length APOL1 proteins, especially the G1 and G2 formed large molecular weight oligomers as judged by native non-reducing PAGE. Such oligomers may mediate cellular cascade leading to cytotoxicity. Recent studies characterizing the channel function of APOL1 have suggested that the C-terminal α-helix of APOL1 (D337-E355) mediates pH gating and membrane insertion. This group has proposed a model where membrane insertion of APOL1 exposes the C-terminus of the protein to the organelle lumen when APOL1 is localized to endo/lysosomes (in the secretory pathway) and to the extracellular side when the protein is localized to the plasma membrane. While plausible, such a membrane topology will not enable protein-protein interactions of the APOL1 C-terminus with effector proteins and protein domains which are localized to the cytoplasm. Current evidence suggests that kidney expressed APOL1-G1 and G2 is the key mediator of kidney disease pathogenesis. Additionally, APOL1 localizes to subcellular compartments other than endo-lysosomes and plasma membrane. The orientation of proteins on membranes are dynamic and can vary in different organelles due to changes in lipid composition of organelle membranes. Hence, it is tempting to hypothesize that APOL1 inserts to membranes and APOL1 C-terminus is exposed to the cytoplasm where it can participate in coiled-coiled interaction with facilitator proteins. Further studies focused on characterizing APOL1 structure will be critical to understand the topology of APOL1 domains after membrane insertion. To discover the interacting protein partners of APOL1, we searched for proteins with structural similarity to trypanosomal SRA protein, which is the known protein interactor of APOL1 C-terminus. This led to the identification of SNARE family proteins as potential interaction partners of APOL1. SNARE family proteins are integral membrane proteins that constitute the molecular machinery that mediates membrane fusion between cellular compartments and predominantly localizes to the endo-lysosomal compartment. SNARE-mediated membrane fusion and intracellular trafficking contribute to biological functions like autophagy, neurotransmitter release, viral endocytosis etc. Our and other studies showed that APOL1-G0 interacted with the SNARE protein,
vesicle-associated membrane protein 8 (VAMP8) while the presence of G1 and G2 variants attenuated this interaction.\textsuperscript{14,20} VAMP8 is known to be a predominantly endosome-lysosome localized SNARE protein that is involved in cellular functions, including regulation of vesicle trafficking by mediating endosome and autophagosome maturation. The membrane fusion events of VAMP8 and other SNARE proteins are mediated through the coiled-coiled interaction with cognate protein partners via the SNARE domain. This domain has an \( \alpha \)-helical structure, much like the domain at the C-terminus of APOL1. Taken together, these studies suggest that kidney-disease associated variant mediated protein conformational changes could hamper the ability of variant APOL1 to activate podocyte stress-response protein networks leading to CKD development and progression. Whether kidney disease pathogenesis caused by APOL1-G1 and -G2 is secondary to loss of function in presence of a “second hit” or stress to podocytes or a gain-of-function is still debated. APOL1-G0 appears to be dispensable for kidney development and homeostasis and a physiological function except for its trypanolytic activity has not been evident.\textsuperscript{67,68} APOL1-G0 in cell culture models has been shown to provide innate immunity against viral infections like HIV\textsuperscript{22}, a function that is lost by the kidney disease associated variants in murine model of HIV-associated nephropathy (HIVAN).\textsuperscript{69} Hence it is possible that APOL1-G0 related protective cellular processes are “activated” in response to an external second stress, which explains why not all individuals with two copies of APOL1 G1 and/or G2 variants develop kidney disease. However, APOL1-G1 and -G2 appears to change cellular localization and oligomerization pattern\textsuperscript{36} with associated cytotoxicity, which was not rescued by APOL1-G0\textsuperscript{70} in \textit{in vitro} studies suggesting that a dominant gain-of-function could also mediate CKD pathogenesis.

Recent evidence showed the critical importance of the naturally occurring haplotype background of all \textit{APOL1} genotypes when conducting these studies and also suggested that genetic polymorphisms located far from the G1 and G2 sites influence the function of the protein.\textsuperscript{71} Either of these may affect the mechanism of APOL1 folding, if not fold, itself. This underscores the importance of understanding the full-length structure of APOL1 in addition to individual domain structure.
Future directions

Further studies will be needed to characterize the cellular function of APOL1-G0 and disrupted homeostatic pathways triggered by the G1 and G2 variants resulting in kidney disease. One of the major goals will be to translate this information in order to develop therapeutic strategies that will modify the course of APOL1 associated CKD. Understanding the three-dimensional protein structure of APOL1 will provide key insights that will help us in solving this puzzle. However, the cellular properties of APOL1 pose several challenges in conducting these structural studies. The oligomerization of APOL1 into high molecular weight forms is a major hindrance for using nuclear magnetic resonance (NMR) based structural studies to resolve the full-length protein structure as its large size increases spectral overlap and linewidth resulting from the large number of signals and slow tumbling of protein respectively. However, NMR spectroscopy remains a valuable tool for studying the structural properties of individual protein domains and to probe the time-dependent behavior (internal protein dynamics behavior) of the reference and of the variant APOL1s. The membrane interacting properties, post-translational modifications, and cytotoxicity poses limitations for the expression and purification of the natively folded APOL1 protein which is necessary for structural studies, including X-ray crystallography and cryo-electron microscopy. While these challenges exist, efforts to define the structure of APOL1 proteins using multiple methodologies will advance our understanding of APOL1 variant mediated kidney disease and aid the development of druggable targets.

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Figure 1: (A) Schematic representation of APOL1 protein domains. The location of G1 and G2 variants are represented in the C-terminal SRA interacting domain in magenta (G1) and yellow (G2) bars. (B) The amino acid sequence of the C-terminal SRA interacting domain showing the location of G1 and G2 amino acid changes compared to reference protein G0.
Figure 2: Protein structure of APOL1 C-terminus (T336-L398) obtained by computational modeling and molecular dynamics simulation. (A) Overlap of G0 (green) and G1 (blue), (B) G0 (blue) and G2 (orange), and (C) G1 (blue) and G2 (orange) structures showing areas of structural differences highlighted in circled regions. (Reproduced with permission, from Sharma AK et al, FASEB J, 2016)