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How Complicated Can It Be? The Link Between APOL1 Risk Variants and Lipoprotein Heterogeneity in Kidney and Cardiovascular Diseases.

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How complicated can it be? The link between *APOL1* risk variants and lipoprotein heterogeneity in kidney and cardiovascular diseases

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The beginning of human apolipoprotein L1 study (gene: *APOL1*; protein: ApoL1) originated from the identification of circulating ApoL1 as an interacting protein of ApoA1 and a minor component of high-density lipoprotein subfraction 3 (HDL3) in 1997 [1]. In the past 18 years, ApoL1 has been investigated in the context of complex human diseases such as African sleeping sickness, schizophrenia, host innate immunity, cancer, hyperlipidemia, cardiovascular diseases, stroke and

type 2 diabetes [2–10]. A major breakthrough, however, was the documentation of two haplotypes of *APOL1*, harboring three coding-sequence mutations as risk variants associated with non-diabetic chronic kidney diseases (CKD) in African Americans [5, 6]. The first one, termed G1, is a two non-synonymous SNP haplotype [rs73885319 (A > G; p.S342G) and rs60910145 (G > T; p.I384M)]. The second one, termed G2, is a two codon deletion haplotype rs71785313 (6-bp in frame deletion; p.ΔN388Y389) [4, 5]. These two coding-sequence variants have been discovered mainly in kidney disease patients of African ancestry and linked to the pathogenesis of primary focal and segmental glomerulosclerosis, hypertension-attributed kidney disease and HIV-associated nephropathy (HIVAN), under a recessive inheritance pattern [3, 7, 10]. Moreover, these *APOL1* variants and African American recipient ethnicity have been shown to associate with kidney transplant rejection and allograft failure [11–13]. Importantly, the expression of ApoL1 has been detected in renal proximal tubular epithelial cells, podocytes, medium-sized arteries, arteriolar endothelial cells and pre-glomerular vascular structures [3, 14, 15]. Thus, there is no doubt or controversy regarding the notion that the expression of *APOL1* risk alleles in kidney cells is associated with the development and progression of non-diabetic CKD in African Americans. In fact, we and others have shown that interferon-α, -β and -γ and TNF-α can induce the expression of ApoL1 in endothelial cells and overexpression of ApoL1 can lead to autophagy- and/or necrosis-associated cell death in a variety of cell types, including endothelial cells and podocytes [15–19]. However, the role of ApoL1 in the outcome of cardiovascular diseases is a much more controversial topic [20]. Some studies suggest that the *APOL1* risk alleles have an adverse effect [8], while others failed to detect an association between the risk alleles and these cardiovascular events [9], or showed improved survival in patients with two risk alleles [10]. Moreover, the potential role that extracellular/circulating ApoL1 might have on systemic endothelial and/or kidney cells is currently unknown. It is worth noting that besides being a component of HDL, ApoL1 is also a component of very low density lipoproteins (VLDL) and LDL [21]. This fact, however, has been frequently overlooked and the role of ApoL1 in VLDL and LDL has not been explored.

In this issue, Gutierrez *et al.* [22] utilized a case–control sample of African Americas who were part of the Sea Islands Genetics Network (SIGNET) and assessed the relationship between the *APOL1* risk variants, G1 and G2, and the circulating levels of different lipoproteins and sizes of HDL subclasses measured by nuclear magnetic resonance (NMR) spectroscopy. Using this well-established NMR technique, which is based on the assessment of distinct methyl groups of lipid species in plasma samples [23, 24], they found a modest increase of small-size HDL particles (small HDL) in the circulation of patients carrying the *APOL1* G1/G2 risk alleles, independently of age, sex, diabetes and percentage of African ancestry. There were no significant differences in large or medium HDL, VLDL or LDL concentrations observed by APOL1 genotype in this study. Although the classification of large, medium and small of HDL is different from that of HDL 1, 2 and 3 subfractions defined by the density/density centrifugation [25–28], the small HDL should be lipid-poor and high density similar to, if not the same as, HDL3, of which ApoL1 is a component [1]. However, in this study the levels of ApoL1 in plasma samples were not measured and therefore cannot be correlated to the levels of small HDL or other lipoprotein particles.

The results of Gutierrez *et al.* may be clinically relevant, since they suggest that the *APOL1* genotype could play a direct role in determining the circulating concentration of small HDL, which have been associated with renal and cardiovascular diseases [7–10, 27]. However, as discussed in their article, these findings should be interpreted with caution. The authors propose that the *APOL1* risk variants could contribute to the increase prevalence of renal disease by facilitating the formation of circulating HDL subpopulations with pro-atherogenic properties. Nonetheless, the difference in the circulating levels of small HDL between subjects with zero and two *APOL1* risk alleles was modest (0.9 μmol/L), and although a previous study found that changes of this magnitude could be associated with albuminuria [27], no differences were found in the prevalence of CKD, albuminuria or other markers of cardiometabolic status across all the *APOL1* categories in the subjects of this study [22]. In addition, the difference in small HDL concentration between individuals with one risk allele versus two risk alleles was minor (0.2 μmol/L) and very unlikely to be clinically relevant. Moreover, if two *APOL1* risk alleles and higher circulating concentrations of small HDL interact to precipitate CKD, one should ask why two *APOL1* risk alleles do not increase risk of diabetic nephropathy in African Americans with elevated HDL3. If the role of *APOL1* rick alleles is to increase the concentration of small HDL (or HDL3), then adding elevated HDL3 is redundant; if the small HDL (or HDL3) are elevated already due to other factors, then *APOL1* rick alleles cannot play an additional role by increasing small HDL (or HDL3). On the other hand, it is well known that once diabetic nephropathy is established, the progression of the renal disease is accelerated in patients carrying two *APOL1* risk alleles [7]. In summary, given the negligible difference reported between the circulating levels of small HDL in patients carrying one versus two risk alleles (0.2 μmol/L), these changes are very unlikely to explain the increase risk of CKD conferred only by two risk alleles. As an alternative explanation, Gutierrez *et al.* argue that the elevated levels of small HDL could be due to changes in renal metabolic pathways. In this regard, a previous study showed that cubilin (gene: *CUBN*; protein: cubilin), an endocytic receptor highly expressed in renal proximal tubules, mediates the uptake of albumin and filtered forms of ApoA1-HDL [29]. Moreover, *CUBN* heterozygous deficient mice and transgenic mice overexpressing human cubilin showed either decreased or elevated levels of ApoA1, HDL cholesterol and HDL3 particles, respectively [29]. Nonetheless, the subjects carrying two risk alleles of *APOL1* in the Gutierrez study showed only a minor increase in the circulating concentration of small HDL, suggesting that this isolated change is unlikely to be the result of renal metabolism.

Taken together, the findings of Gutierrez *et al.* [22] add more fuel to the ongoing controversy regarding the association of *APOL1* G1/G2 risk status with cardiovascular outcome among African Americans. However, if one speculates that

the *APOL1* risk alleles may directly modify the circulating levels of small HDL and/or other factors and induce a pro-atherogenic state that precipitates CKD and cardiovascular complications, then one should begin to answer the following several questions. (i) Why are the *APOL1* risk variants not associated with an increased prevalence of diabetic nephropathy in African Americans? (ii) Would the *APOL1* risk alleles alter the synthesis and function of other proteins in HDL3, VLDL and VDL? (iii) How do the circulating ApoL1 mutant proteins interact with the plasma membrane and initiate a signal transduction pathway from outside to inside of the targeted cell? (iv) What mechanisms modulate the transport of circulating ApoL1 mutant proteins inside the cells and its interaction with intracellular ApoL1 and/or other intracellular proteins, for example, apolipoprotein L6 (ApoL6), an ApoL1-related protein, which when overexpressed, induces atherosclerotic apoptosis [30]? Finally, as the expression of ApoL1 can be induced by inflammatory cytokines and intracellular accumulation of ApoL1 can initiate autophagy and cell death in endothelia cells, the cross-talk between inflammation, autophagy and cell death mediated by the overexpression of ApoL1 should be much more intensively investigated before one can properly interpret the meaning of these findings reported by Gutierrez *et al.* [22].

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest. The results presented in this paper have not been published previously in whole or part, except in abstract format.

(See related article by Gutiérrez *et al. APOL1* nephropathy risk variants are associated with altered high-density lipoprotein profiles in African Americans. *Nephrol Dial Transplant* 2016; 31: 602–608)

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