THE APOLIPOPROTEIN L1 GENE AND CARDIOVASCULAR DISEASE

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Abstract

Relative to those with European ancestry, African Americans have an excess incidence of nondiabetic chronic kidney disease predominantly due to two coding renal-risk variants in the apolipoprotein L1 gene (*APOL1*). This *APOL1*–kidney disease association is independent of systemic hypertension or blood pressure. Recent reports describe extra-renal effects of the *APOL1* G1 and G2 renal-risk variants on cardiovascular disease (CVD), subclinical atherosclerosis, lipoprotein particle concentrations, and survival. However, results have been less consistent than those seen in kidney disease, and the observed *APOL1* associations with CVD vary from risk to protective. This manuscript reviews the relationships between *APOL1* renal-risk variants and CVD, with an emphasis on study-specific factors that may have contributed to disparate observations. It is possible that *APOL1* renal-risk variants impact the systemic vasculature, not only the kidneys. As novel therapies for *APOL1*-associated nephropathy are developed, APOL1 variant protein effects on large blood vessels and risk of CVD will need to be considered.

Introduction

The increased risk of nondiabetic chronic kidney disease (CKD) in African Americans relative to European Americans had long been unexplained.[1] In contrast to historic reports suggesting that ethnic differences in systemic blood pressures and socioeconomic factors were major contributors, it is now believed that the majority of this ethnic-specific difference relates to biologic variation in the apolipoprotein L1 gene (*APOL1*) on chromosome 22q.[2,3] This breakthrough linked several previously disparate kidney diseases—including idiopathic focal segmental glomerulosclerosis, HIV-associated collapsing glomerulosclerosis (HIV-associated nephropathy), severe lupus nephritis, sickle cell nephropathy, and hypertension-attributed nephropathy (a primary kidney disease manifesting as focal global glomerulosclerosis with renal interstitial and vascular changes and secondary hypertension)—to a single disease spectrum referred to as *APOL1*-associated nephropathy.[4,5]

Several groups have assessed extra-renal effects of *APOL1* renal-risk variants on subclinical atherosclerosis (calcified atherosclerotic plaque determined by computed tomography), myocardial infarction (MI), stroke, altered lipoprotein particle concentrations, and participant survival in large trials and cohort studies. In contrast to the consistently observed autosomal recessive *APOL1* associations with nondiabetic CKD (risk genotypes include G1G1, G2G2, or G1G2), effects on nonrenal outcomes have been inconsistent, and associations have been reported with recessive and nonrecessive (additive and dominant) genetic models.[6] Besides the different cohorts, study designs, and outcomes, *APOL1* associations with kidney disease varied between reports and could have impacted findings. This review summarizes published data on the nonrenal vascular effects of the *APOL1* G1 and G2 renal-risk variants in the admixed African American population with an emphasis on cardiovascular disease (CVD) phenotypes.

APOL1 and Lipoprotein Particle Concentrations

Given the recent interest in the effects of *APOL1* on CVD, it is useful to review studies reporting effects on lipid profiles and lipoprotein particle concentrations. This is especially relevant because APOL1 protein travels in the circulation bound to a subset of trypanosome lytic factors 1 and 2 lipoprotein particles; it does not appear to be bound to typical plasma high-density lipoprotein (HDL) particles as has often been reported.[7,8] To date, there is no evidence of association between *APOL1* genotypes and circulating APOL1 protein concentrations or components of the standard lipid profile, such as low-density lipoprotein (LDL) cholesterol, HDL cholesterol, or triglycerides.[9-13]

A report of more than 2,000 African American participants in the REGARDS study detected positive association between *APOL1* renal-risk variants and small HDL cholesterol particle concentrations.[14] The effect of this observation on risk for CVD or MI is currently unknown. *APOL1* renal-risk variant effects on macrophage cholesterol efflux and other components of the atherosclerotic process also require further study. Finally, Bentley et al. reported that serum HDL cholesterol relationships with CKD differed in African Americans with and without *APOL1* renal-risk variants.[11,15] The impact of this finding on clinical CVD outcomes is under study.

APOL1 and Subclinical Calcified Atherosclerotic Plaque

Calcified plaque in the coronary arteries (CAC), as well as in the aorta and carotid arteries, is widely recognized to be a marker of subclinical atherosclerosis and is associated with heightened risk for CVD events and death.[16-18] The higher risk for CVD in individuals with higher CAC is observed in members of all ethnic groups. In contrast to European Americans, African Americans have markedly lower levels of CAC despite the presence of more severe conventional CVD risk factors such as higher blood pressure, LDL cholesterol, albuminuria, and blood sugars in populations with diabetes.[19-22] The population-based Multi-Ethnic Study of Atherosclerosis (MESA) and the African American-Diabetes Heart Study (AA-DHS) both revealed that African Americans who had higher levels of CAC had higher percentages of European ancestry.[23,24] This demonstrates that African ancestry is protective from the development of subclinical CVD and CAC, whereas European ancestry contributes to risk. This effect is contrary to the *APOL1*-associated risk for CKD based on positive selection for trypanolytic variants of sub-Saharan African origin. It further demonstrates that, in addition to the environment, biologic variation or inherited factors contribute to the risks of CVD and CKD.

Phenotype	Model	Effect	Estimate (SE)	*P*-value	Study
Coronary artery calcified plaque	Recessive	Protective	-6.04 (NA)	0.019	Jackson Heart[28]
Carotid artery calcified plaque	Dominant	Protective	-0.42 (0.18)	0.02	AA-DHS[29]
Coronary artery calcified plaque	Additive	Protective	-0.03 (0.16)	0.08	AA-DHS[29]
Time to death	Additive	Protective	-0.41 (0.14)	0.005	AA-DHS[29]
HDL particle concentration	Additive	↑ small HDL particles	NA	0.004	Renal REGARDS[14]

Table 1. *APOL1* associations with subclinical cardiovascular disease and related phenotypes.
SE: standard error; *APOL1*: apolipoprotein L1; AA-DHS: African American-Diabetes Heart Study.

The clinical relevance of lower levels of CAC in African Americans was evident in studies where both African and European Americans had equivalent access to healthcare. In contrast to the general population, where African Americans have greater risk for CVD than European Americans, African Americans with type 2 diabetes treated by the Veteran's Administration and Kaiser Permanente had 50% lower rates of MI than European Americans.[25,26] Similar lower rates of MI and improved survival rates are seen in African Americans with end-stage kidney disease receiving renal replacement therapy through the Centers for Medicare and Medicaid Services.[27] Therefore, the lower levels of CAC in African Americans relative to European Americans have biologic relevance once the confounding effects of environmental factors, including "differential access to healthcare," are considered.

The Jackson Heart Study (JHS) and AA-DHS measured calcified atherosclerotic plaque with computed tomography in the large blood vessels of African Americans and tested for genetic association with *APOL1* (Table 1). *APOL1* renal-risk variants were associated with lower levels of calcified atherosclerotic plaque in the left main coronary arteries in JHS and with lower levels of calcified plaque in multiple vascular beds in AA-DHS, with results consistent across studies.[28,29] Calcified plaque associations in AA-DHS were strongest in additive and dominant genetic models, whereas JHS reported an association in a recessive model. In the JHS, the significant association between CAC and *APOL1* persisted when removing participants with CKD, supporting a true protective effect of renal-risk variants on subclinical CVD. Despite concordance between calcified plaque associations with *APOL1* in the AA-DHS sample with type-2 diabetes (717 participants with measured CAC) and JHS (1,959 participants; number with CAC not provided), discordant effects of *APOL1* on clinical CVD events were observed despite the expected protection from CVD and MI in those with lower levels of calcified atherosclerotic plaque in the coronary arteries and aorta.

APOL1 Renal-Risk Variants and Cardiovascular Disease

APOL1-*Associated Risk for Cardiovascular Disease and Incident Myocardial Infarction*

Three studies identified an increased risk for CVD or incident MI among African Americans with two *APOL1* renal-risk variants (Table 2); however, *APOL1* was significantly associated with the presence of kidney disease and/or albuminuria in all three reports.[28,30] Although adjustment for CKD was performed, it remains uncertain whether kidney disease, a powerful risk factor for MI, could have confounded results.[31] The initial report by Ito et al. assessed the JHS and African American participants in the Women's Health Initiative (WHI).[28] With the large size of these studies and the national representation of the WHI, CVD events were adjudicated and expected to be highly accurate. Participants with two *APOL1* renal-risk variants in both studies had higher rates of incident MI, stroke, and surgical or endovascular intervention even after statistical adjustment for CKD. Women's Health Initiative participants did not have computed tomography scans to measure CAC or subclinical atherosclerosis. As noted above, JHS participants with two *APOL1* renal-risk variants had significantly lower levels of CAC. Therefore, the paradoxical observation of a lower CAC despite higher CVD rates led the authors to speculate that novel mechanisms might have contributed to CVD outcomes. As stated above, the possibility that CKD was a confounder in this surprising finding may not have been fully excluded by statistical adjustment. In the Cardiovascular Health Study (CHS), Mukamal et al. analyzed incident MI related to *APOL1* in African American participants with a mean age of 73 years.[30] As in JHS and WHI, CVD events were adjudicated. Again, the presence of two *APOL1*

Phenotype	Model	Effect	OR (95% CI) or HR (SE)	*P*-value	Study
Incident myocardial infarction, stroke, surgical or endovascular intervention	Recessive	Risk	2.17 (1.34-3.48)	9.4×10^{-4}	Jackson Heart[28]
Incident major cardiovascular event	Recessive	Risk	1.98 (1.17-3.31)	8.4×10^{-3}	Women's Health Initiative[28]
Incident myocardial infarction	Recessive	Risk	1.80 (1.10-3.00)	0.02	Cardiovascular Health Study[30]
Prevalent myocardial infarction, surgical or endovascular intervention	Additive	None	1.02 (0.82-1.27)	0.86	SPRINT[34]
Death (all cause)	Recessive	Risk	1.30 (1.00-1.70)	0.05	Cardiovascular Health Study[30]
Death (all cause)	Additive	Protective	0.67 (0.14)	0.005	AA-DHS[29]

Table 2. *APOL1* associations with major cardiovascular events and death.
OR (95% CI): odds ratio (95% confidence interval); HR (SE): hazard ratio (standard error); *APOL1*: apolipoprotein L1; SPRINT: Systolic Blood Pressure Intervention Trial; AA-DHS: African American-Diabetes Heart Study; *P*: probability.

renal-risk variants was associated with albuminuria (but not estimated glomerular filtration rate) and MI; association with CVD persisted after adjustment for albuminuria. Therefore, JHS, WHI, and CHS all reported positive association between two *APOL1* renal-risk variants and adjudicated CVD events or MI and also with CKD or albuminuria.[28,30]

APOL1-*Associated Protection from Death and CVD Events*

Consistent findings in JHS, WHI, and CHS provide reassurance as to detected associations. However, it is difficult to completely exclude the confounding effects of *APOL1*-associated CKD on risk for MI. Although the African American Study of Kidney Disease and Hypertension (AASK) was not designed or powered to demonstrate the risk of MI or death, *APOL1* was strongly associated with renal outcomes (doubling of serum creatinine concentration or initiation of dialysis),[32] yet very few deaths were recorded in this population with advanced CKD despite their high risk for CVD.[33] This suggests that *APOL1* might not be associated with a markedly heightened risk for CVD.

In AA-DHS, all participants had type 2 diabetes, and *APOL1* was not associated with diabetic kidney disease. As opposed to conclusions drawn from JHS, AA-DHS linked participant survival in the Social Security Death Index (SSDI) to genotypes (Table 2). In agreement with the *APOL1* association with lower levels of calcified atherosclerotic plaque (and lack of confounding by nephropathy association), increasing numbers of *APOL1* renal-risk variants were associated with longer survival (additive genetic model).[29] As with adjudicated events in JHS and CHS, results were likely accurate given that outcomes were assessed using the SSDI. Although results differed from those in JHS and CHS, AA-DHS evaluated a uniformly diabetes-affected cohort at high risk for CVD, and kidney disease phenotypes (albuminuria and eGFR) did not confound results. Therefore, there are potential explanations for the different directions of association between AA-DHS and the other reports.

Finally, the Systolic Blood Pressure Intervention Trial (SPRINT) enrolled only nondiabetic individuals, many with mild-to-moderate CKD and low level proteinuria (albuminuria below 1,000 mg/day); recruitment specifically targeted the elderly and those with prevalent CVD.[34] SPRINT saw weak association between *APOL1* and CKD in 2,571 African American participants but observed no evidence of association between *APOL1* and prevalent CVD. This was despite large numbers of SPRINT participants with CVD. However, results were cross-sectional. Longitudinal associations will soon be assessed, as SPRINT was halted prematurely due to beneficial effects of a lower systolic blood pressure goal on primary CVD outcomes.

Therapeutic Considerations

As targeted therapies for *APOL1*-associated nephropathy are likely to be developed in the near future, it is critical to elucidate whether the CVD effects of *APOL1* renal-risk variants increase or decrease the risk of cardiac events. Kidney transplantation studies suggest that the nephropathy risk from *APOL1* renal-risk appears principally related to intrinsic gene expression in cells of the kidney and not to circulating APOL1 protein.[35-37] However, it is unknown whether vascular gene expression or circulating proteins are involved in the cardiovascular effects of *APOL1*. Circulating APOL1 protein concentration does not appear to be dependent on *APOL1* renal-risk variant genotypes.[7,38] APOL1 messenger RNA and protein have been detected in vascular cells, which supports the potential for a local vascular effect as with nephropathy risk.[39,40]

If *APOL1* renal-risk alleles exhibit opposing effects on CVD and nephropathy risk, an effect supported by AA-DHS, treatments that improve renal outcomes might have the potential to aggravate CVD. In contrast, if reports from JHS, WHI, and CHS are correct and *APOL1* renal-risk alleles increase the risk for CVD independently from nephropathy, treatments for *APOL1*-associated kidney disease could also improve cardiac outcomes. The conflicting data on *APOL1* associations with increased cardiovascular events (JHS, WHI, and CHS), lower levels of CAC (JHS and AA-DHS), and reduced rates of death (AA-DHS) require additional studies to reach a consensus. Ideally, future analyses can be performed in populations with recent African ancestry where *APOL1* is not (or only weakly) associated with nephropathy to limit confounding between CKD and CVD. This remains a fertile area of research and holds great importance for individuals who possess recent African ancestry.

Key Points:

- Apolipoprotein L1 gene (*APOL1*) renal-risk genotypes contribute to approximately 40% of cases of end-stage kidney disease in the African American population.
- Associations between *APOL1* renal-risk variants with cardiovascular disease and mortality are less consistent than those with nephropathy.
- As targeted therapies for *APOL1*-associated kidney disease are developed, additional studies are required to clearly elucidate the vascular effects of its renal-risk variants.

Conflict of Interest Disclosure: Wake Forest University Health Sciences and Barry I. Freedman have filed for a patent related to *APOL1* genetic testing. Dr. Freedman receives research funding from Novartis and is a consultant for Ionis Pharmaceuticals and AstraZeneca.

Keywords: African Americans, apolipoprotein L1 gene, *APOL1*, atherosclerosis, cardiovascular disease, chronic kidney disease, myocardial infarction

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