APOL1 Genotype, Kidney and Cardiovascular Disease, and Death in Older Adults

Kenneth J. Mukamal, Joseph Tremaglio, David J. Friedman, Joachim H. Ix, Lewis H. Kuller, Russell P. Tracy, Martin R. Pollak

Objective—We sought to evaluate the cardiovascular impact of coding variants in the apolipoprotein L1 gene APOL1 that protect against trypanosome infection but have been associated with kidney disease among African Americans.

Approach and Results—As part of the Cardiovascular Health Study, a population-based cohort of Americans aged ≥65 years, we genotyped APOL1 polymorphisms rs73885319 and rs71785153 and examined kidney function, subclinical atherosclerosis, and incident cardiovascular disease and death over 13 years of follow-up among 91 African Americans with 2 risk alleles, 707 other African Americans, and 4964 white participants. The high-risk genotype with 2 risk alleles was associated with 2-fold higher levels of albuminuria and lower ankle–brachial indices but similar carotid intima–media thickness among African Americans. Median survival among high-risk African Americans was 9.9 years (95% confidence interval [CI], 8.7–11.9), compared with 13.6 years (95% CI, 12.5–14.3) among other African Americans and 13.3 years (95% CI, 13.0–13.6) among whites (P=0.03). The high-risk genotype was also associated with increased risk for incident myocardial infarction (adjusted hazard ratio 1.8; 95% CI, 1.1–3.0) and mortality (adjusted hazard ratio 1.3; 95% CI 1.0–1.7). Albuminuria and risk for myocardial infarction and mortality were nearly identical between African Americans with 0 to 1 risk alleles and whites.

Conclusions—APOL1 genotype is associated with albuminuria, subclinical atherosclerosis, incident myocardial infarction, and mortality in older African Americans. African Americans without 2 risk alleles do not differ significantly in risk of myocardial infarction or mortality from whites. APOL1 trypanolytic variants may account for a substantial proportion of the excess risk of chronic disease in African Americans. (Arterioscler Thromb Vasc Biol. 2016;36:398-403. DOI: 10.1161/ATVBAHA.115.305970.)

Key Words: albuminuria ▪ apolipoproteins ▪ epidemiology ▪ genetics ▪ kidney ▪ myocardial infarction

African American (AA) adults are at risk for several morbidities, including hypertension, peripheral artery disease, and diabetes mellitus.1–3 They are at particularly high risk for several types of chronic kidney disease (CKD), even accounting for associated risk factors.4 Although these findings suggest that genetic variants associated with CKD may exist in AA populations, this hypothesis remains elusive.5

See accompanying editorial on page 219

Recent mapping studies among AA implicate a chromosome 22 locus in a surprising range of CKD among AA.6–7 Subsequent studies have linked specific variants in the gene encoding apolipoprotein L1 (APOL1) to focal segmental glomerulosclerosis, human immunodeficiency virus–associated nephropathy, and hypertension–attributed end-stage renal disease.8–10 Furthermore, these variants seem to have originated within the last few thousand years and been subjected to strong selection pressure.11 These variants are absent in non-AA populations, and their gene products lyse Trypanosoma brucei rhodesiense in vitro, whereas wild-type apolipoprotein L1 does not.9 Because these variants occur on over 30% of AA chromosomes, they potentially explain a large proportion of the excess CKD among AA. Indeed, AA participants without 2 risk alleles seem to have nearly the same rates of albuminuria and CKD as whites.12,13

Given the strong associations of diminished estimated glomerular filtration rate (eGFR) and albuminuria with risks of subclinical atherosclerosis, cardiovascular disease, and death in older adults,14–17 the potential burden of morbidity and mortality related to these APOL1 variants may be substantial and extend well beyond CKD. Indeed, in the Jackson Heart Study and Women’s Health Initiative, individuals with 2 risk variants appeared to have roughly double the risk of cardiovascular disease, although the 2 studies had no information on mortality and included only 12 cases of myocardial infarction (MI) among high-risk subjects.18 Paradoxically, those studies also found that high-risk genotype was associated with less...
coronary artery calcification, but had no information on ankle–brachial index (ABI), which is strongly related to AA race.18

To better understand the associations of these variants with a full range of cardiovascular and renal outcomes and death, we examined \textit{APOL1} variants in a community-living population of older adults with detailed measures of clinical and subclinical vascular disease.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

Among AA participants, the number of participants homozygous wild-type, heterozygous, and homozygous variants at G1 were 513, 249, and 36 (minor allele frequency 0.20; Hardy–Weinberg \( P=0.41 \)). The corresponding figures for G2 were 604, 173, and 19 (minor allele frequency 0.13; Hardy–Weinberg \( P=0.12 \)). A total of 91 (11%) AA participants had 2 risk alleles. As expected, no individual had more than 2 risk alleles.

Table 1 shows participant characteristics according to \textit{APOL1} status. There were no significant differences between AA participants with and without 2 risk alleles in the characteristics shown, including fasting lipids and C-reactive protein. Consistent with expectation, AA participants had a greater prevalence of hypertension and diabetes mellitus and higher body mass index than whites.

Kidney Function and Subclinical Atherosclerosis

Table 2 shows results for kidney function and subclinical atherosclerosis. We observed no difference in mean eGFR between AA participants with zero or one versus 2 risk alleles, nor a difference in the rate of change of eGFR between the groups. Analyses using baseline eGFR or creatinine (rather than cystatin) yielded similar results. There was also no significant association between \textit{APOL1} genotype and eGFR below 60 among AA individuals (adjusted odds ratio 1.4; 95% confidence interval [CI], 0.8–2.4; \( P=0.29 \)). In contrast, \textit{APOL1} genotype was strongly associated with mean albuminuria, with 2-fold higher levels among AA participants with 2 risk alleles but similar levels among other AA and white participants. We also observed a strong positive association of having 2 risk alleles with ascending categories of micro- and macroalbuminuria (adjusted odds ratio 2.9; 95% CI, 1.6–5.1; \( P<0.001 \)).

Similarly, we observed an association of ABI, but not carotid intima–media thickness, with \textit{APOL1} genotype. AA participants had lower mean ABI values than did whites, regardless of genotype, but those with 2 risk variants had significantly lower values than did those with fewer. The prevalence of an ABI below 0.9 was higher among those AA participants with 2 risk alleles than those with fewer (adjusted odds ratio 1.6; 95% CI, 1.0–2.7; \( P=0.001 \)).

Incident Cardiovascular Disease and Mortality

Figure illustrates the association of \textit{APOL1} genotype with mortality. Individuals with 2 \textit{APOL1} variants had the greatest mortality, whereas mortality was virtually identical among white and AA participants with \( \leq 1 \) variants. Median survival

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|}
\hline
\textbf{Nonstandard Abbreviations and Acronyms} & & \\
\hline
AA & African American & \\
ABI & ankle–brachial index & \\
CKD & chronic kidney disease & \\
eGFR & estimated glomerular filtration rate & \\
MI & myocardial infarction & \\
\hline
\end{tabular}
\end{table}
among AA individuals with 2 risk alleles was 9.9 years (95% CI, 8.7–11.9), compared with 13.6 (95% CI, 12.5–14.4) among other AA and 13.3 (95% CI, 13.0–13.6) among whites (log-rank \( P = 0.03 \)).

Table 3 illustrates the adjusted associations of genotype with incident cardiovascular events and death over a median of 13 years of follow-up. MI risk was \( \approx 80\% \) higher among participants with 2 risk alleles than other AA participants; whites and AA participants with \(<2\) risk alleles had comparable risk. Similarly, risk of death was \( \approx 30\% \) higher among those participants with 2 risk alleles compared with other AA participants. The magnitude of higher risk was modestly higher for noncardiovascular than cardiovascular deaths, but the estimates did not differ significantly when modeled simultaneously in competing risk analyses (\( P = 0.72 \)). As with MI, cause-specific mortality was essentially identical between white and AA participants with \(<2\) risk alleles. For both MI and mortality, the observed hazard ratios were not attenuated by adjustment for eGFR. Hazard ratios for specific causes among noncardiovascular deaths are shown in Table III in the online-only Data Supplement.

We did not observe differences in rates of total stroke or congestive heart failure according to \( APOL1 \) genotype. There were also no significant difference between AA participants with \( \leq 2 \) risk alleles in risk of ischemic stroke (n=799 cases; adjusted hazard ratio 1.1; 95% CI, 0.6–2.0), but only 102 cases of hemorrhagic stroke occurred overall and none among AA participants with 2 risk alleles (\( P = 0.41 \) for comparison across 3 race-\( APOL1 \) groups). Similarly, we documented 309 incident cases of clinically significant peripheral artery disease, of which 9 occurred among high-risk \( APOL1 \) individuals. Nonetheless, results largely concord with those seen for ABI. Compared with lower-risk AA, hazard ratios were 1.5 (95% CI, 0.7–3.1) for AA with 2 risk alleles and 0.7 (95% CI, 0.5–0.9) for white participants.

**Sensitivity Analyses**

We examined the dose–response relationships of \( APOL1 \) variants with albuminuria, ABI, and risks of MI and mortality. In all 4 instances, higher risk was limited to individuals with 2 risk alleles (data not shown). Although power was limited, we also observed generally similar associations with albuminuria and ABI for homozygosity at either G1 or G2.

We repeated our primary analyses within subgroups defined by age or diabetes mellitus and observed generally consistent positive associations (Table I in the online-only Data Supplement). Similarly, genotype did not interact with age or diabetes mellitus on albuminuria (\( P > 0.10 \)) or with age on ABI (\( P = 0.99 \)), but did so nominally with diabetes mellitus on ABI (\( P = 0.02 \)). The difference in ABI between AA participants with 2 risk alleles and those with fewer was 0.00±0.02 U among nondiabetic individuals (\( P = 0.99 \)) and 0.11±0.03 among diabetic participants (\( P < 0.001 \)).

In analyses of categorical outcomes (albuminuria, low ABI, MI, and death) adjusted for commonly collected clinical characteristics (Table II in the online-only Data Supplement), we found no consistent attenuation of the associations across outcomes with this additional adjustment. We also repeated our analyses among the subset of 781 AA participants with available markers of ancestry. We found no attenuation with adjustment for albuminuria. Estimates for ABI, MI, and death were attenuated by \( \approx 20\% \) with adjustment.
The APOL1 genotype was not consistently associated with retinal vascular caliber; it was marginally associated with smaller arteriolar diameter but not venular diameter, although both differed significantly between AA and white participants (Table IV in the online-only Data Supplement).

Finally, we compared the associations with mortality of 2 APOL1 variant alleles with 2 APOE4 alleles. Among 5396 participants with available data on both loci, the mutually adjusted hazard ratios for mortality were 1.3 (1.0–1.7) for APOL1 and 1.4 (1.1–1.8) for APOE4 (P=0.78 for comparison of APOL1 and APOE).

**Discussion**

In this prospective study of older adults over 2 decades, APOL1 genotype was related to albuminuria, peripheral atherosclerosis, and risk of MI and death. AA participants with 2 risk alleles had a median survival 3 years lower than other AA individuals or whites, and AA participants with fewer risk alleles had levels of albuminuria and risk of MI essentially identical to whites.

Humans are innately immune to Trypanosoma brucei infection for all but 2 subspecies—gambiens and rhodesiens—which cause African sleeping sickness. This immunity results from apolipoprotein L1, a Bcl-2-like protein carried on dense high-density lipoprotein particles that enters the parasitic lysosome after endocytosis, where it interacts with the C-terminal helix of apolipoprotein L1 in the lysosome to limit its pore-forming activity and induce resistance. As a consequence, T. brucei infection poses a serious burden to individuals at or below child-bearing age in Africa.

APOL1 variants seem to have emerged relatively recently and become prevalent among African populations because they may protect against sleeping sickness. Much like those hemoglobinopathies that confer protection from malarial infection, the protein products of these APOL1 variants retain trypa-nolytic activity against T. brucei rhodesiens (but not gambiens) species, albeit at an apparent cost of long-term kidney and related chronic disease. The G2 variant, which encodes a two-amino-acid deletion in the C-terminal domain, prevents serum resistance–associated protein from binding to apolipoprotein L1 in vitro. The mechanism by which the G1 variant retains pore-forming activity is less clear, but both amino acid substitutions seem to be necessary for maximal effect.

Our results expand the risks associated with APOL1 genotype. Previously, the G1 and G2 variants at this locus had been associated in AA with hypertension-associated end-stage renal disease, focal segmental glomerulosclerosis, and human immunodeficiency virus-nephropathy. Among nondiabetic individuals in the Dallas Heart Study, APOL1 genotype was also associated with microalbuminuria and eGFR <60 mL/min per 1.73 m², with a prevalence of non-diabetic CKD among AA without 2 risk alleles (1.7%) that matched that of whites (1.5%). Among AA with end-stage renal disease, APOL1 risk variants seem to confer a lower age at initiation of dialysis and more rapid progression of existing CKD. The mechanism by which these variants contribute to kidney disease remains uncertain, although indirect genetic evidence suggests that the APOL1 locus is likely to be causal.

In Cardiovascular Health Study, APOL1 genotype was strongly associated with albuminuria, a strong risk factor for mortality. It was not associated with mean eGFR, either cross-sectionally or longitudinally, much as AA race is associated with albuminuria but not with eGFR in Cardiovascular Health Study, although we observed a trend toward higher risk of eGFR <60 mL/min per 1.73 m². We previously observed a parallel finding in the Dallas Heart Study, with a strong association with low eGFR but not with mean eGFR. Other studies have also found a lack of association with eGFR, suggesting that albuminuria is a more sensitive marker of the nephrotoxic effects of APOL1 genotype.

### Table 3. Hazard Ratios for Incident Cardiovascular Disease and Mortality According to APOL1 Genotype

<table>
<thead>
<tr>
<th></th>
<th>AA 2 Risk Alleles</th>
<th>AA 0–1 Risk Alleles</th>
<th>White (N=4964)</th>
<th>P Value (0–1 vs 2 Risk Alleles)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cardiovascular disease</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocardial infarction, N</td>
<td>19</td>
<td>84</td>
<td>779</td>
<td></td>
</tr>
<tr>
<td>Adjusted hazard ratio (C)</td>
<td>1.8 (1.1–3.0)</td>
<td>Referent</td>
<td>1.1 (0.9–1.4)</td>
<td>0.02</td>
</tr>
<tr>
<td>Stroke, N</td>
<td>12</td>
<td>114</td>
<td>846</td>
<td></td>
</tr>
<tr>
<td>Adjusted hazard ratio (C)</td>
<td>0.9 (0.5–1.7)</td>
<td>Referent</td>
<td>0.9 (0.7–1.1)</td>
<td>0.80</td>
</tr>
<tr>
<td>Congestive heart failure, N</td>
<td>23</td>
<td>182</td>
<td>1407</td>
<td></td>
</tr>
<tr>
<td>Adjusted hazard ratio (C)</td>
<td>1.0 (0.6–1.5)</td>
<td>Referent</td>
<td>0.9 (0.7–1.0)</td>
<td>0.96</td>
</tr>
<tr>
<td><strong>Mortality</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total mortality, N</td>
<td>61</td>
<td>401</td>
<td>3366</td>
<td></td>
</tr>
<tr>
<td>Adjusted hazard ratio (C)</td>
<td>1.3 (1.0–1.7)</td>
<td>Referent</td>
<td>1.0 (0.9–1.1)</td>
<td>0.05</td>
</tr>
<tr>
<td>Cardiovascular mortality, N</td>
<td>23</td>
<td>159</td>
<td>1334</td>
<td></td>
</tr>
<tr>
<td>Adjusted hazard ratio (C)</td>
<td>1.3 (0.8–2.0)</td>
<td>Referent</td>
<td>1.0 (0.8–1.1)</td>
<td>0.31</td>
</tr>
<tr>
<td>Noncardiovascular mortality, N</td>
<td>38</td>
<td>240</td>
<td>2026</td>
<td></td>
</tr>
<tr>
<td>Adjusted hazard ratio (C)</td>
<td>1.4 (1.0–1.9)</td>
<td>Referent</td>
<td>1.0 (0.8–1.1)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

AA indicates African American; and CI, confidence interval.
The prevalence and magnitude of effect of APOL1 genotype is noteworthy. The combined minor allele frequency was approximately one-third, and >10% of AA individuals had a high-risk genotype, suggesting that over 4 million Americans may have this genotype. Further, the high-risk 2-allele genotype was associated with >80% higher risk of MI, higher than any single nucleotide polymorphism identified in genome-wide association studies to our knowledge.28 The magnitude of the association with albuminuria also exceeds that observed for polymorphisms found in genome-wide association studies.27 Thus, APOL1 seems to parallel APOE as among the few loci that harbor common polymorphisms with strong effects on the widespread chronic diseases of older age.

Our results suggest that a potentially large proportion of the excess risk of morbidity and mortality among AA beyond kidney disease alone relates to APOL1 genotype. The 90% of AA participants with no or one risk allele had virtually identical cardiovascular and noncardiovascular mortality and kidney function to white participants. However, our results suggest that APOL1 genotype does not fully explain the excess risk of peripheral atherosclerosis observed among AA adults,29 for ABI remained higher even among those without 2 risk alleles, and genotype was not associated with carotid intima–media thickness, hypertension, or congestive heart failure. Furthermore, genotype clearly does not explain racial disparities in access to health care30 or risk of mortality unrelated to chronic disease,31,32 and hence, race remains more than a simple genetic construct33 even when race-specific disease loci are identified.

Although we genotyped nearly 800 AA individuals, the number of high-risk AA participants limited some analyses. For example, because we measured albuminuria 4 years after most AA participants enrolled, we could not reliably estimate whether excess mortality associated with APOL1 genotype was mediated by albuminuria; the high-risk stratum included 54 individuals and 27 subsequent deaths at that point. Clarification of that point will be important in future studies because proteinuric kidney disease remains a likely mechanism by which APOL1 genotype might influence vascular disease and mortality. Similarly, we had limited ability to examine subgroups in which genotype might be most important, although we found no clear differences by age or diabetes mellitus.

In summary, APOL1 genotype was strongly associated with albuminuria, ABI, and risks of MI and mortality in older AA adults. Mean albuminuria and risks for MI and mortality did not differ between AA with no or one risk alleles and whites, suggesting that this locus may account for much of the excess risk of these conditions among AA adults.

Acknowledgments
A full list of principal Cardiovascular Health Study investigators and institutions can be found at http://www.chs-nhlbi.org. Dr Mukamal had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Disclosures
Dr Friedman, Dr Pollak, and the Beth Israel Deaconess Medical Center have filed for patents related to APOL1. The other authors report no conflicts.

References


**Significance**

Recently described APOL1 risk variants seem to protect against trypanosomiasis but have been associated with specific forms of kidney disease among individuals of African descent. In a population-based study of older adults, we find that rates of kidney disease, myocardial infarction, and total mortality were identical among whites and African Americans who did not have the high-risk genotype, suggesting that potentially all of the excess risk of mortality, albuminuria, and myocardial infarction among African Americans may be attributable to their carriage of these alleles. Also, the high-risk genotype was associated with both total mortality and peripheral atherosclerosis, highlighting the importance of this locus for cardiovascular health among African Americans.
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Methods and Materials

Study Population and Design

The Cardiovascular Health Study (CHS) is a population-based prospective study of 5,888 men and women aged 65 years or older who were recruited from Medicare-eligibility lists in Pittsburgh, PA, Sacramento, CA, Hagerstown, MD, and Forsyth County, NC who have now been followed for two decades. Participants were not institutionalized or wheelchair-dependent, did not require a proxy for consent, were not under treatment for cancer at the time of enrollment, and were expected to remain in their respective regions for at least three years. In 1989-1990, 5201 predominately white and AA participants were recruited and examined (the original cohort); in 1992-1993, an additional 687 predominately AA participants were included, for a total AA sample size of 924.

The CHS study design and objectives have been published previously. The baseline examination included standardized medical history questionnaires, physical examination, and laboratory examination; these procedures were generally repeated in the original cohort in 1992-1993 when the AA cohort was added. Follow-up contact occurred every six months, alternating between telephone calls and clinic visits through 1999 and telephone calls thereafter.

Participants gave written informed consent upon enrollment. The institutional review boards at each field center and the central data coordinating center at the University of Washington approved the original protocol and its ongoing conduct.

APOL Genotyping

We genotyped two variants in the APOL1 locus, rs73885319 and rs71785313, at the CHS Central Laboratory at the University of Vermont. The rs73885319 single nucleotide polymorphism (SNP) encodes one of two nonsynonymous polymorphisms in near-perfect
linkage disequilibrium that define an allele referred to as G1. The rs71785313 variant, also called the G2 allele, encodes a two amino-acid deletion in the same C-terminal domain of apolipoprotein L1 as G1. We used the TaqMan allelic discrimination system with custom probes for genotyping. We only genotyped AA participants (original or new cohort), as these variants are essentially absent in other populations; we also excluded 104 individuals who did not provide informed consent for use of their genetic material, leaving 820 AA eligible for genotyping.

We successfully genotyped all individuals for G1. The G2 indel could not be genotyped in 24 (3%) participants; of these, two were homozygous for G1 and included in our analyses, for a final sample size of 798 AA and 4964 white participants.

As previously noted, the G1 and G2 alleles are defined by SNPs in close proximity and recombination between them is absent. As a result, any individual can have zero, one, or two risk alleles. Based upon previous findings, we assumed a recessive/compound heterozygote model, in which risk was elevated only in the presence of two risk alleles, although we tested this assumption in sensitivity analyses.

**Measures of Kidney Disease and Subclinical Atherosclerosis**

All laboratory measurements were made at the University of Vermont. Cystatin C has been measured in CHS using a BNII nephelometer using stored samples from the 1989-1990, 1992-1993, and 1996-1997 visits. We estimated eGFR using the equation: eGFR = 76.7 × [cystatin C]⁻¹.¹⁹ Coefficients of variation ranged from 2.0 to 2.8% (intra-assay) and from 2.3 to 3.1% (interassay). Serum creatinine was measured at identical points with the Kodak Ektachem 700 Analyzer.

Urine was collected in 1996-1997 as a single morning void. The Central Laboratory measured urine albumin by rate nephelometry using the Beckman Array 360 CE Protein
Analyzer and urine creatinine using the Ektachem 700 Analyzer. Albuminuria was quantified as urine albumin-to-creatinine ratio (ACR), expressed in milligrams per gram and evaluated as both a continuous (log-transformed) and a categorical variable, using thresholds of 30 and 300 mg/g.

At baseline, participants underwent measurement of ankle-brachial index (ABI) and carotid intima-media thickness (IMT), as previously described.³⁴ We calculated side-specific ABI values as the ratio of the average of two blood pressure measurements in the right arm and each lower extremity and used the lower side-specific ABI for each individual. Because high ABI values reflect arterial stiffness, we excluded individuals with values ≥1.4 in analyses of ABI. With high-resolution B-mode ultrasonography, technicians acquired one longitudinal image of the common carotid artery and three longitudinal images of the internal carotid artery, which a central reading center evaluated. As previously, we standardized internal and common carotid measurements, averaged the standardized values, and report combined IMT in standard-deviation units.

In 1997-1998, participants underwent standardized retinal photography of a randomly-selected eye. The diameters of all vessels coursing through a specified area one-half to one disc diameter from the optic disc were estimated with a computer-assisted program. Arteriolar and venular calibers are expressed as central retinal arteriolar equivalents and central retinal venular equivalents.⁵⁶

**Determination of Incident CVD and Mortality**

Cases of MI, stroke, congestive heart failure, clinically-significant peripheral artery disease, and death in CHS are adjudicated by central committees. Details of the protocols and algorithms for confirmation of these events have been published.⁷ In brief, participants were questioned regarding hospitalizations and other acute events every six months. Discharge
summaries and diagnoses were obtained for all hospitalizations. For all potential incident events, additional information, such as cardiac enzyme levels, serial electrocardiograms, and cranial imaging studies, was collected. To be categorized as a stroke, a new neurologic deficit had to persist for 24 hours, or if less than 24 hours, a lesion appropriate to the clinical deficit must have been detected on brain imaging studies. Congestive heart failure required a physician diagnosis along with treatment with a diuretic and vasodilator or diagnostic radiographic findings. Deaths were identified through reviews of obituaries, medical records, death certificates, the Centers for Medicare and Medicaid Services health care utilization database, and interviews of contacts and proxies. Using categories defined by CHS, we grouped cause of death as cardiovascular or not and, among non-cardiovascular deaths, as due to cancer, infection, dementia, kidney disease, respiratory disease, fracture/trauma, and other. We followed individuals for a maximum of 18 years of follow-up in these analyses.

**Covariates**

Race was self-reported at baseline as white, black, American Indian/Alaskan Native, Asian/Pacific Islander, and other. Technicians measured blood pressure, weight, and height at baseline; hypertension included a blood pressure $\geq 140/90$ or use of antihypertensive medication recorded in a validated medication inventory.\(^8\)\(^9\) Similarly, we measured fasting serum glucose at baseline and defined diabetes as a value of 126 mg/dl or higher or use of hypoglycemic medication. We previously assessed $APOE$ genotype.\(^10\) To account for population stratification, we used the top 10 principal components derived by eigenvalue decomposition from a genome-wide association study conducted in CHS using 97,404 SNPs genotyped on the Illumina Omni 1M platform.

**Statistical Analysis**
In all analyses, we categorized participants as AA with no or one risk allele, AA with two risk alleles, or white; our primary comparisons were among AA. For analyses of log-transformed albuminuria, ABI, and IMT, we performed linear regression with adjustment for age, sex, and clinic site; we present least-square means (back-transformed as appropriate) for interpretability. We also performed standard (for eGFR below 60 ml/min/1.73 m$^2$ and ABI below 0.9) and ordinal logistic regression (for micro and macroalbuminuria) to test the robustness of our findings. For eGFR, we used generalized estimating equations with an exchangeable correlation matrix to incorporate the multiple measures within-individual; we evaluated both average eGFR (adjusting for year of measurement) and trend in eGFR (i.e., change in eGFR in ml/min/1.73 m$^2$/year). We used similar approaches for analysis of retinal vascular diameters in sensitivity analyses among the subset of 1,989 participants with available measurements.

For incident cardiovascular disease and mortality, we constructed Kaplan-Meier curves tested with log-rank tests. We conducted multivariable Cox proportional hazards analyses with age and sex-specific hazards and adjustment for clinic site. In these models, we evaluated MI, stroke, congestive heart failure, and death as individual events. We also evaluated cardiovascular and non-cardiovascular death and ischemic and hemorrhagic stroke in secondary analyses with both standard Cox and Lunn and McNeil competing risk methods, and clinically manifest peripheral artery disease as a complementary outcome to ABI. We also evaluated cause of death among non-cardiovascular deaths in secondary analyses. For incident events other than death, we excluded individuals with prevalent diagnoses at baseline. We found no violation of the proportional hazards assumption using interaction terms of genotype and log-transformed follow-up time (global p > 0.15).
Although genotype is necessarily unconfounded by traits established late in life, we tested whether \textit{APOL1} genotype was associated with risk even after additional adjustment for readily measured, potentially mediating risk factors, including marital status, diabetes, prevalent coronary heart disease and congestive heart failure, systolic blood pressure, antihypertensive use, body-mass index, pack-years of smoking, creatinine, C-reactive protein, cystatin, and total cholesterol. We similarly compared regression coefficients with and without adjustment for population eigenvectors in supplemental analyses.

In prespecified analyses, we examined whether associations were similar among strata defined by age (<75 versus \( \geq 75 \) years) and diabetes\textsuperscript{13} and tested their multiplicative interactions. To provide context with a comparator with established single-locus genetic effects, including an estimated lifetime risk of Alzheimer disease of 50-60\%,\textsuperscript{14} we compared the association with mortality of having two \textit{APOL1} variant alleles with that of having two \textit{APOE4} alleles.
References


Supplemental Table I. Adjusted hazard ratios for incident myocardial infarction and mortality according to *APOL1* status stratified by age and diabetes.

<table>
<thead>
<tr>
<th></th>
<th>AA 2 Risk Alleles</th>
<th>AA 0-1 Risk Alleles</th>
<th>White</th>
<th>P-interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Myocardial Infarction</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;75 years (564 cases)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted Hazard Ratio (CI)</td>
<td>2.1 (1.1-1.4)</td>
<td>Referent</td>
<td>1.3 (1.0-1.7)</td>
<td></td>
</tr>
<tr>
<td>≥75 years (318 cases)</td>
<td></td>
<td></td>
<td></td>
<td>0.78</td>
</tr>
<tr>
<td>Adjusted Hazard Ratio (CI)</td>
<td>1.4 (0.6-3.3)</td>
<td>Referent</td>
<td>0.8 (0.6-1.2)</td>
<td></td>
</tr>
<tr>
<td><strong>Non-Diabetic (574 cases)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted Hazard Ratio (CI)</td>
<td>1.8 (0.9-3.5)</td>
<td>Referent</td>
<td>1.1 (0.8-1.5)</td>
<td></td>
</tr>
<tr>
<td><strong>Diabetic (303 cases)</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.42</td>
</tr>
<tr>
<td>Adjusted Hazard Ratio (CI)</td>
<td>2.0 (0.9-4.4)</td>
<td>Referent</td>
<td>1.4 (0.9-2.1)</td>
<td></td>
</tr>
<tr>
<td><strong>Total Mortality</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;75 years (2133 deaths)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted Hazard Ratio (CI)</td>
<td>1.3 (0.9-1.9)</td>
<td>Referent</td>
<td>0.8 (0.7-1.0)</td>
<td></td>
</tr>
<tr>
<td>≥75 years (1695 deaths)</td>
<td></td>
<td></td>
<td></td>
<td>0.95</td>
</tr>
<tr>
<td>Adjusted Hazard Ratio (CI)</td>
<td>1.3 (0.9-2.0)</td>
<td>Referent</td>
<td>1.2 (1.0-1.4)</td>
<td></td>
</tr>
<tr>
<td><strong>Non-Diabetic (2529 deaths)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted Hazard Ratio (CI)</td>
<td>1.2 (0.8-1.7)</td>
<td>Referent</td>
<td>1.0 (0.9-1.2)</td>
<td></td>
</tr>
<tr>
<td><strong>Diabetic (1269 deaths)</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.45</td>
</tr>
<tr>
<td>Adjusted Hazard Ratio (CI)</td>
<td>1.4 (0.9-2.1)</td>
<td>Referent</td>
<td>1.0 (0.8-1.2)</td>
<td></td>
</tr>
</tbody>
</table>

P-value for interaction tests the multiplicative interaction between genotype and either age or diabetes among AA participants in models adjusted for sex, site, and the main effects of age or diabetes.
Supplemental Table II. Change in age-, sex-, and site-adjusted estimates associated with two *APOL1* risk alleles among African-American participants with adjustment for clinical characteristics or ancestry.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Age-Sex-Clinic Adjusted Risk Ratio</th>
<th>+Clinical Characteristics</th>
<th>Δ in Regression Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial Infarction (N=698, 93 events)</td>
<td>1.8</td>
<td>1.7</td>
<td>-5%</td>
</tr>
<tr>
<td>Death (N=760, 439 events)</td>
<td>1.3</td>
<td>1.2</td>
<td>-21%</td>
</tr>
<tr>
<td>Micro/Macro-Albuminuria (N=439, 99 cases)</td>
<td>2.7</td>
<td>3.3</td>
<td>+20%</td>
</tr>
<tr>
<td>Ankle-Brachial Index &lt;0.9 (N=732, 156 cases)</td>
<td>1.7</td>
<td>1.7</td>
<td>3%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Age-Sex-Clinic Adjusted Risk Ratio</th>
<th>+Ancestry Eigenvectors</th>
<th>Δ in Regression Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial Infarction (N=717, 99 events)</td>
<td>1.6</td>
<td>1.4</td>
<td>-23%</td>
</tr>
<tr>
<td>Death (N=781, 453 events)</td>
<td>1.3</td>
<td>1.2</td>
<td>-17%</td>
</tr>
<tr>
<td>Micro/Macro-Albuminuria (N=455, 102 cases)</td>
<td>2.7</td>
<td>2.6</td>
<td>-3%</td>
</tr>
<tr>
<td>Ankle-Brachial Index &lt;0.9 (N=749, 159 cases)</td>
<td>1.6</td>
<td>1.4</td>
<td>-23%</td>
</tr>
</tbody>
</table>

Risk ratios include hazard ratios for myocardial infarction and death and odds ratios for albuminuria (ordinal) and low ankle-brachial index (binary). Clinical characteristics adjusted for include marital status, diabetes, prevalent coronary heart disease and congestive heart failure, systolic blood pressure, antihypertensive use, body-mass index, pack-years of smoking, creatinine, C-reactive protein, cystatin, and total cholesterol.
Supplemental Table III. Hazard ratios for specific causes of death among African-American participants with 2 APOL1 risk alleles, compared with 0-1 risk alleles.

<table>
<thead>
<tr>
<th>Cause</th>
<th>Hazard Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer (N=16/99)</td>
<td>1.3 (0.8-2.2)</td>
</tr>
<tr>
<td>Dementia (N=6/40)</td>
<td>1.3 (0.5-3.1)</td>
</tr>
<tr>
<td>Infection (N=2/35)</td>
<td>0.4 (0.1-1.7)</td>
</tr>
<tr>
<td>Respiratory (N=5/19)</td>
<td>2.9 (1.1-8.0)</td>
</tr>
<tr>
<td>Fracture (N=1/4)</td>
<td>3.2 (0.3-28.7)</td>
</tr>
<tr>
<td>Kidney Disease (N=4/15)</td>
<td>2.9 (0.9-9.3)</td>
</tr>
<tr>
<td>Other Causes (N=27/189)</td>
<td>1.2 (0.8-1.9)</td>
</tr>
</tbody>
</table>

Numbers of cases are shown among participants with 2 / 0-1 risk alleles.
**Supplemental Table IV. Measures of retinal caliber in 1997-1998 according to *APOL1* status.**

<table>
<thead>
<tr>
<th>Measure of Retinal Caliber</th>
<th>AA 2 Risk Alleles (N=91)</th>
<th>AA 0-1 Risk Alleles (N=707)</th>
<th>White (N=4964)</th>
<th>P-value (0-1 vs. 2 Risk Alleles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central retinal venous equivalents</td>
<td>197 (190-204)</td>
<td>199 (197-201)</td>
<td>189 (188-190)</td>
<td>0.73</td>
</tr>
<tr>
<td>Central retinal arteriolar equivalents (trunk)</td>
<td>157 (151-164)</td>
<td>165 (162-167)</td>
<td>163 (163-164)</td>
<td>0.05</td>
</tr>
<tr>
<td>Central retinal arteriolar equivalents (branch)</td>
<td>163 (155-170)</td>
<td>168 (166-171)</td>
<td>165 (165-166)</td>
<td>0.17</td>
</tr>
</tbody>
</table>