Combined Effects of GSTM1 Null Allele and APOL1 Renal Risk Alleles in CKD Progression in the African American Study of Kidney Disease and Hypertension Trial

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ABSTRACT
Apolipoprotein L-1 (APOL1) high-risk alleles and the glutathione-S-transferase-μ1 (GSTM1) null allele have been shown separately to associate with CKD progression in the African American Study of Kidney Disease and Hypertension (AASK) trial participants. Here, we determined combined effects of GSTM1 null and APOL1 high-risk alleles on clinical outcomes in 682 AASK participants who were classified into four groups by GSTM1 null or active genotype and APOL1 high- or low-risk genotype. We assessed survival differences among these groups by log-rank test and Cox regression adjusted for important clinical variables for time to GFR event (change in GFR of 50% or 25-ml/min per 1.73 m² decline), incident ESRD, death, or composite outcomes. The groups differed significantly in event-free survival for incident ESRD and composite outcomes (P<0.001 by log-rank test). Compared with the reference GSTM1 active/APOL1 low-risk group, other groups had these hazard ratios for the composite outcome of incident ESRD and change in GFR: GSTM1 active/APOL1 high-risk hazard ratio, 2.13; 95% confidence interval, 0.76 to 5.90 (P=0.15); GSTM1 null/APOL1 low-risk hazard ratio, 2.05; 95% confidence interval, 1.08 to 3.88 (P=0.03); and GSTM1 null/APOL1 high-risk hazard ratio, 3.0; 95% confidence interval, 1.51 to 5.96 (P=0.002). In conclusion, GSTM1 null and APOL1 high-risk alleles deleteriously affect CKD progression among blacks with hypertension, and subjects with both GSTM1 null and APOL1 high-risk genotypes had highest risk of adverse renal outcomes. Larger cohorts are needed to fully explore interactions of GSTM1 and APOL1 genotypes in other subgroups.

Blacks account for only 13.2% of the general population, but their proportion among those receiving dialysis treatments in the United States in 2011 was 36.8%. The incident rate for starting dialysis was 940 per million in blacks, which is 3.4 times greater than the 280 per million found among whites. Higher poverty and more limited access to health care among blacks do not entirely account for their higher ESRD incidence rates, suggesting that this faster kidney disease progression must be partly caused by genetic factors.
The deletion allele is common; 50% of whites and 27% of blacks are homzygous carriers.13 In the African American Study of Kidney Disease and Hypertension (AASK) cohort, we found that black individuals who are hypertensive and carry the GSTM1 null allele have faster CKD progression compared with those homozygous for the active allele.9 More recently, APOL1 renal risk variants were shown to also be associated with faster decline in kidney function in the AASK.14,15 Thus, we set out to study the influence of the joint effects of APOL1 and GSTM1 genes in the AASK trial participants.

RESULTS

The AASK was a multicenter, randomized clinical trial designed to test the effect of three different antihypertensive medications and two different levels of BP control on the progression of hypertensive kidney disease among blacks with a GFR between 20 and 65 ml/min per 1.73 m2 with no other identified causes of CKD. Primary outcomes were reduction in GFR defined as 50% reduction or an absolute 25-ml/min per 1.73 m2 decline (∆GFR), reaching dialysis, death, or their composite outcomes. Doubling of the protein-to-creatinine ratio to >0.22 g/g was a secondary outcome in the AASK.

We assessed the survival differences among the four genotype groups by log-rank test and in the Cox regression for the primary outcomes used in the AASK. On the basis of a priori consideration, the Cox regression analyses were performed sequentially to adjust for the risk factors in the following orders. Model 1 adjusted for age; sex; mean arterial BP (MAP); history of CVD; assigned to amlodipine, metoprolol, or ramipril during the randomized treatment phase; and the degree of European ancestry. Model 2 adjusted for the all of the variables used in model 1 and baseline GFR, whereas in model 3,

<p>| Table 1. Baseline characteristics of the four genotype groups in the AASK among all 682 patients included in the study |
|---------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|-----------|</p>
<table>
<thead>
<tr>
<th></th>
<th>GSTM1 Active/APOL1</th>
<th>GSTM1 Null/APOL1</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td>113 (16.56)</td>
<td>30 (4.39)</td>
<td>0.001</td>
</tr>
<tr>
<td>Age at randomization, yr</td>
<td>54.45 (10.04)</td>
<td>51.21 (12.02)</td>
<td>0.03</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>117.61 (17.68)</td>
<td>112.06 (11.79)</td>
<td>0.01</td>
</tr>
<tr>
<td>Protein-to-creatinine ratio, g/g creatinine</td>
<td>0.20 (0.67)</td>
<td>0.50 (0.67)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Baseline creatinine, mg/dl</td>
<td>1.90 (0.67)</td>
<td>1.92 (0.67)</td>
<td>0.002</td>
</tr>
<tr>
<td>GFR, ml/min per 1.73 m2</td>
<td>49.39 (14.28)</td>
<td>47.95 (12.99)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Men, %</td>
<td>57.52</td>
<td>61.31</td>
<td>NS</td>
</tr>
<tr>
<td>CAD history, %</td>
<td>51.33</td>
<td>53.77</td>
<td>NS</td>
</tr>
<tr>
<td>Ramipril therapy, %</td>
<td>44.25</td>
<td>40.63</td>
<td>NS</td>
</tr>
<tr>
<td>Metoprolol therapy, %</td>
<td>34.51</td>
<td>39.90</td>
<td>NS</td>
</tr>
<tr>
<td>Amlodipine therapy, %</td>
<td>21.24</td>
<td>19.46</td>
<td>NS</td>
</tr>
<tr>
<td>Low-BP goal group, %</td>
<td>47.79</td>
<td>51.34</td>
<td>NS</td>
</tr>
</tbody>
</table>
| Subjects who had two copies of high-risk APOL1 variants were classified as having the APOL1 high-risk genotype; those with no copies or one copy were categorized as being in the APOL1 low-risk group. Subjects who were homzygous for the GSTM1 active alleles were considered to have the GSTM1 active genotype, whereas those carrying the GSTM1 null allele were classified as having the GSTM1 null genotype. The mean values are followed by SDs in parentheses. We used the chi-squared test for categorical variables and one-way ANOVA for continuous variables. CAD, coronary artery disease.
additional adjustment was made for the urine protein-to-creatinine ratio. Because proteinuria can mediate the effect of genotype on the outcome, to avoid overadjustment, we chose model 2 as our final model. The use of model 2 as our final statistical model is in line with the analysis performed in the previous studies addressing the effect of APOL1 alone in the AASK cohort.14,15

Table 1 summarizes the baseline characteristics of 682 subjects who were included in the study. Overall, approximately 79% of the cohort carried the GSTM1 null allele (27% as homozygous carriers and 52% as heterozygous carriers), and 23% had the APOL1 high-risk genotype. The expected genotype frequencies in the general black population are approximately 76% for the GSTM1 null allele, assuming Hardy–Weinberg equilibrium for the GSTM1 null and active alleles, and 13% for the APOL1 high-risk genotype.5,13 At baseline, the APOL1 low-risk groups, regardless of GSTM1 genotype, were older (P=0.03) and had higher MAP (P=0.01). Among the four genotype groups, the GSTM1 null/APOL1 high-risk group had the lowest baseline renal function (P=0.003), despite having the lowest MAP. Over the 5-year follow-up period, of 682 subjects, 12 subjects (1.8%) died before progressing to ESRD, 80 subjects (11.7%) reached incident ESRD, and overall, 147 subjects (21.6%) met the criteria for the composite outcome (ΔGFR, dialysis, or death). The protein-to-creatinine ratio was available for 680 of 682 subjects. During the 5-year follow-up period, 421 of 680 subjects experienced a secondary outcome of doubling of the protein-to-creatinine ratio to a value >0.22.

The results of the Kaplan–Meier analysis for the four genotype groups are displayed in Figure 1. The survival curves were not only significantly different among the four groups for the composite outcome of ΔGFR, incident ESRD, or death (Figure 1A), but also, they were also significantly different with regards to the composite outcome of ΔGFR and incident ESRD (Figure 1B) and incident ESRD (Figure 1C).
**GSTM1** active/**APOL1** low–risk genotype had relatively the best survival from incident ESRD or either of the composite outcomes, whereas the **GSTM1** null/**APOL1** high–risk group had relatively the lowest survival from incident ESRD or either of the composite outcomes over the 5-year follow-up period. There were only 30 subjects with the **GSTM1** active/**APOL1** high–risk genotype; thus, no firm conclusion can be made from comparison of the **GSTM1** active versus null groups within the **APOL1** high–risk group. However, those with **APOL1** low–risk genotypes seemed to do worse if they also had the **GSTM1** null allele.

The results of Cox regression analyses for the four genotype groups are depicted in Table 2. Compared with the **GSTM1** active/**APOL1** low–risk genotype group as the reference, subjects in **GSTM1** null/**APOL1** low–risk and **GSTM1** null/**APOL1** high–risk groups had two and three times higher risk, respectively, to have composite events. For incident ESRD, only the **GSTM1** null/**APOL1** high–risk group was significantly different from the reference.

We then performed a Cox regression analysis for time to doubling of the urinary protein-to-creatinine ratio and found no significant differences among the four genotype groups. We also performed the Cox regression analysis for the four genotype groups using statistical model 3, which adjusts for proteinuria. Compared with the reference **GSTM1** active/**APOL1** low–risk group, the hazard ratios (HRs) for the composite outcome of incident ESRD and ΔGFR were 1.99 (95% confidence interval [95% CI], 1.03 to 3.84; *P*=0.04) and 2.01 (95% CI, 1.00 to 4.04; *P*=0.05) for the **GSTM1** null/**APOL1** low–risk and the **GSTM1** null/**APOL1** high–risk groups, respectively. The HR for the **GSTM1** active/**APOL1** high–risk group was 1.18 (95% CI, 0.41 to 3.38; *P*=0.75). We note that adjusting for proteinuria reduced the HR from 2.99 to 2.01 for the **GSTM1** null/**APOL1** high–risk group. This HR is virtually the same as the HR of 1.99 that we see for the **GSTM1** null/**APOL1** low–risk group in the same analysis, suggesting that the strength of the effect of **APOL1** depends on proteinuria and that there is overadjustment when using model 3.

To address the complex relationship between genotypes and outcomes, we also compared Kaplan–Meier survival within genotype subgroups for the composite outcome of ΔGFR, incident ESRD, and death (Supplemental Figure 1). In the
APOL1 high–risk genotype subgroup (Supplemental Figure 1A), the event–free survival difference between those with GSTM1 null and GSTM1 active genotypes was not statistically significant, with a log–rank \( P \) value of 0.20. However, in the APOL1 low–risk genotype subgroup (Supplemental Figure 1B), those with GSTM1 null genotype had a significantly worse event–free survival compared with those with the GSTM1 active genotype, with a log–rank \( P \) value of 0.04. Similarly, in the GSTM1 null genotype subgroup (Supplemental Figure 1C), those with the APOL1 high–risk genotype had a significantly worse event–free survival compared with those with the APOL1 low–risk genotype, with a log–rank \( P \) value of 0.003. Finally, no significant difference in event–free survival between those with APOL1 high– and low–risk genotypes was found in the GSTM1 active group (Supplemental Figure 1D), with a log–rank \( P \) value of 0.21. Overall, both the GSTM1 null and the APOL1 high–risk genotypes resulted in worse outcomes unless the comparison involved the GSTM1 active/APOL1 high–risk group, which only had 30 participants.

In addition, we performed a gene–gene interaction analysis between the GSTM1 and the APOL1 genes for the composite outcome of \( \Delta \)GFR, incident ESRD, and death and adjusted for the same set of covariates as those used in model 2. Both the GSTM1 null and the APOL1 high–risk genotypes are significantly associated with faster progression of CKD when analyzed independently. The HRs are 1.87 \( (P=0.02) \) and 1.56 \( (P=0.02) \) for the GSTM1 null and the APOL1 high–risk genotypes, respectively. However, when their interaction term was added to the Cox regression model, the APOL1 high–risk genotype lost statistical significance. The GSTM1 null genotype alone reaches statistical significance, but the interaction with APOL1 high–risk genotype also failed to reach statistical significance. We postulate that the interaction term must have absorbed some of the effect that was previously attributed to the APOL1 high–risk genotype. Overall, we could not conclusively show or reject a gene–gene interaction.

In the four-group analyses presented above, the GSTM1 null group is defined by carrying the GSTM1(0) null allele [GSTM1(1/0) and GSTM1(0/0)]. We chose a dominant model to determine the effect of GSTM1 on kidney disease outcomes, because in our original study, we had examined the effects of the 0/0 to 1/0 and 1/1 genotypes for GSTM1 on CKD.
progression in the AASK and found a stepwise effect; GSTM1 (1/0) and GSTM1(0/0) groups each had significantly worse outcomes than GSTM1(1/1) (active), whereas the GSTM1(1/0) group was not significantly different compared with the GSTM1(0/0) group, suggesting a dominant effect. In addition to the dominant model for GSTM1(0), we also examined its effect using an additive model, which includes the GSTM1(0/0) to GSTM1(1/0) and GSTM1(1/1) genotypes separately and either APOL1 low or high risk, yielding six groups.

The baseline characteristics of the six genotype groups can be found in Table 3. At baseline, the APOL1 low–risk groups, regardless of GSTM1 genotype, had higher MAP (P=0.02). Among the six genotype groups, the GSTM1(0/0) and APOL1 high–risk and the GSTM1(1/0) and APOL1 high–risk groups had the lowest baseline renal function (P=0.02). The APOL1 high–risk groups, regardless of GSTM1 genotype, had significantly higher amounts of proteinuria at baseline (P<0.001).

The results of the Kaplan–Meier analysis for the six genotype groups are displayed in Figure 2. The survival curves were not only significantly different from each other for the composite outcome of ΔGFR, incident ESRD, or death (Figure 2A), but they were also significantly different with regards to the composite outcome of ΔGFR and incident ESRD (Figure 2B) and incident ESRD alone (Figure 2C) as well. Mirroring the results seen in the four genotype groups, the GSTM1(1/1) and APOL1 low–risk group had relatively the best survival from incident ESRD or either of the composite outcomes, whereas the GSTM1(0/0) and APOL1 high–risk group had relatively the lowest survival from incident ESRD or either of the composite outcomes in the unadjusted survival analysis. In both those with APOL1 high– and low–risk genotypes, there was a graded separation of the survival curves on the basis of the GSTM1 genotype. Subjects with the GSTM1(1/1) genotype had relatively the best survival, with the relatively worst survival seen in those who had the GSTM1(0/0) genotypes, whereas subjects with the GSTM1(1/0) genotype closely tracked the GSTM1(0/0) group.

The results of the Cox regression analyses for the six genotype groups are depicted in Table 4. Compared with the reference GSTM1(1/1) and APOL1 low–risk group, patients who had the APOL1 low–risk genotype and carried the GSTM1 null allele [GSTM1(0/0) and APOL1 low– and GSTM1(1/0) and APOL1 low–risk groups] had approximately two times higher risk to have composite events, whereas those participants who had the APOL1 high–risk genotype and carried the GSTM1 null allele [GSTM1(0/0) and APOL1 high– and GSTM1(1/0) and APOL1 high–risk groups] had three times higher risk to have composite events. For incident ESRD, only the GSTM1(0/0) and APOL1 high– and GSTM1 (1/0) and APOL1 high–risk groups were significantly different from the reference group. The four- and six-group comparisons suggest that the GSTM1(0) allele has adverse effects on clinical outcomes, regardless of the use of either the dominant or additive model.
Table 3. Baseline characteristics of the six genotype groups in the AASK among all 682 patients included in the study

<table>
<thead>
<tr>
<th></th>
<th>GSTM1(1/1)/APOL1 Low Risk</th>
<th>GSTM1(1/1)/APOL1 High Risk</th>
<th>GSTM1(1/0)/APOL1 Low Risk</th>
<th>GSTM1(1/0)/APOL1 High Risk</th>
<th>GSTM1(0/0)/APOL1 Low Risk</th>
<th>GSTM1(0/0)/APOL1 High Risk</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td>113 (16.56)</td>
<td>30 (4.39)</td>
<td>7 (11.29)</td>
<td>131 (19.20)</td>
<td>51 (7.47)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at randomization, yr</td>
<td>54.45 (10.44)</td>
<td>51.21 (12.02)</td>
<td>51.67 (11.38)</td>
<td>55.6 (9.79)</td>
<td>52.84 (12.08)</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>117.61 (17.68)</td>
<td>112.06 (11.79)</td>
<td>114.37 (16.38)</td>
<td>109.13 (14.06)</td>
<td>113.81 (15.93)</td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>Protein-to-creatinine ratio, g/g creatinine</td>
<td>0.2 (0.37)</td>
<td>0.52 (0.69)</td>
<td>0.27 (0.47)</td>
<td>0.49 (0.63)</td>
<td>0.22 (0.39)</td>
<td>0.52 (0.64)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Subjects who had two copies of high-risk APOL1 variants were classified as having the APOL1 high-risk genotype; those with no copies or one copy were categorized as being in the APOL1 low-risk group. Subjects who were homozygous for the GSTM1 active alleles were considered to have the GSTM1(1/1) genotype, whereas those heterozygotes carrying the GSTM1 null allele were classified as having the GSTM1(1/0) genotype. Those homozygous for the GSTM1 null alleles were classified as having the GSTM1(0/0) genotype. The mean values are followed by SDs in parentheses. We used the chi-squared test for categorical variables and one-way ANOVA for continuous variables. CAD, coronary artery disease.
with higher circulating levels of malondialdehyde, a reactive aldehyde, in patients with epilepsy. In patients with HIV, those with the active GSTM1 allele have higher CD4 cell counts, reduced oxidative stress, and lower HIV viral loads compared with those with the GSTM1 (0) allele. Here, we show that having no copies or only one copy of the active allele of GSTM1 seems to amplify the effect with the APOL1 high-risk genotype in kidney disease progression in the AASK. Taken together, it is reasonable to speculate that the faster CKD progression observed in those having both the GSTM1 null and the APOL1 high-risk genotype is also attributable to oxidative stress.

We did not observe any association between GSTM1 genotype and progression of proteinuria; however, it is intriguing to note that adjustment for proteinuria diminishes the effect of the APOL1 genotype on the adverse kidney outcomes. Perhaps this can be viewed as a piece of circumstantial evidence that the deleterious effect of the APOL1 genotype on renal outcomes is at least partially mediated by proteinuria.

Our study allows for comparisons between the effects of GSTM1 null and APOL1 high-risk genotypes on CKD progression simultaneously in the same cohort. As previously published, the GSTM1 null allele reaches significance for the composite outcome of incident ESRD and GFR change, or death; (B) the composite outcome of incident ESRD and GFR change; or (C) incident ESRD alone. GFR event was defined as follows: an absolute 25-ml/min per 1.73 m² decline or a 50% reduction in the GFR. The numbers of subjects at risk at yearly time points by subgroups are shown at the bottom of each panel.
among 479 subjects. However, the total frequency of the null allele (homozygous and heterozygous states) was not reported. By Hardy–Weinberg equilibrium, we assume that the frequency of the null allele is $0.519 (0.519 \times 0.519 = 0.27)$ in blacks, and therefore, approximately 76% of the general black population are carriers of the null allele in either the homozygous or the heterozygous state, which is in line with the frequencies reported in other populations. We estimated the relative effect of the $GSTM1$ null and the $APOL1$ high–risk genotypes on CKD progression on the population level among blacks who are hypertensive using the population–attributable risk fractions (PARs) on the composite outcome of ESRD and ΔGFR adjusted for covariates and time at risk. The PARs were 38.4% (95% CI, 7.9% to 62.4%) and 12% (95% CI, 0.8% to 22.9%) for the $GSTM1$ null and the $APOL1$ high–risk genotypes, respectively. Our estimation showed that the $GSTM1$ null and the $APOL1$ high–risk genotypes have comparable effects on CKD progression in this population. One also needs to take into account that, although the $APOL1$ high–risk alleles are specific to those of African ancestry, the $GSTM1$ null allele is highly prevalent across racial groups.

Our observations generate clinically important questions. First, are the effects of $GSTM1(0)$ and $APOL1$ high–risk alleles generalizable to CKD attributable to disease processes other than hypertension–associated kidney disease? Second, do $GSTM1(0)$ and $APOL1$ high–risk alleles have additive effects on the circulating and urinary levels of markers of oxidative stress? Interpretation of our findings is limited to the AASK, a single cohort of subjects with hypertension–associated kidney disease. Additional studies in a similar cohort and those with other kidney diseases with longitudinal follow-up are needed to confirm these findings. The possibility of selection bias is another limitation of our study, because we could ascertain the genotypes of only 682 of the original 1094 AASK participants, although patient baseline data for this study were similar to published data for the entire AASK trial.

In conclusion, the $GSTM1$ null allele and $APOL1$ high–risk alleles both have deleterious effects on disease progression among blacks with kidney disease associated with hypertension. Subjects with both the $GSTM1(0)$ and $APOL1$ high–risk alleles had the highest risk of adverse renal outcomes.
cohorts are needed to confirm these findings and further explore the interactions between GSTM1 and APOL1.

**CONCISE METHODS**

**Design, Participants, and Outcomes**

We performed a retrospective cohort study among the AASK trial participants. The AASK was a multicenter, randomized clinical trial designed to test the effect of three different antihypertensive medications and two different levels of BP control on the progression of hypertensive kidney disease in a three by two factorial design. Briefly, the AASK trial included 1094 blacks ages 18–70 years old with clinical diagnosis of hypertension and an iothalamate GFR between 20 and 65 ml/min per 1.73 m² with no other identified causes of CKD. Between February of 1995 and September of 1998, participants were randomly assigned to one of two MAP goals (102–107 mmHg [usual; n=554] or ≤92 mmHg [lower; n=540]) and initial treatment with a β-blocker (metoprolol, 50–200 mg/d; n=411), an angiotensin–converting enzyme inhibitor (ramipril, 2.5–10 mg/d; n=436), or a dihydropyridine calcium channel blocker (amlodipine, 5–10 mg/d; n=217). Open-label agents were added as needed to achieve the assigned BP goals. The amlo-
dipine arm was halted early in September of 2000 on the basis of the recommendation of the Data and Safety Monitoring Board (subjects were switched to open-label medications), whereas the other two arms were followed up to September of 2001 as planned. Primary outcomes were reduction in GFR define as ≥50% reduction or absolute 25-ml/min per 1.73 m² decline (ΔGFR), reaching dialysis, death, or their composite outcomes. Doubling of the protein-to-creatinine ratio to ≥0.22 g/g was a secondary outcome. Of the 1094 subjects in the AASK, 850 patients consented to provide DNA samples. The institutional review boards of the individual clinical sites had approved use of the DNA samples and clinical data from the AASK trial.

The GSTM1 genotype was successfully determined in 682 of the 693 participants with known APOL1 genotype. Thus, we included in our study 682 of the 1094 original AASK participants.

**Genotyping and Classification**

GSTM1 genotyping was performed using a real–time PCR method. APOL1 genotyping was done by TaqMan assays (Life Technologies,
The APOL1 high-risk group was defined by having G1/G1, G1/G2, or G2/G2 genotypes, whereas the GSTM1 null group is defined by carrying the GSTM1(0/0) null allele [GSTM1(1/1) and GSTM1(0/0)]. The G2 allele, by definition, is homozygous for the active allele [GSTM1(1/1)].

**Population Admixture**

To adjust for the degree of European ancestry in the AASK and ensure that the AASK participants were comparable in genetic background, our analyses were corrected for population stratification using the multidimensional scaling approach as previously performed.

Specifically, individual genotypes of a panel of 126 biallelic markers were used to construct an identity by state distance matrix, and the two most significant multidimensional scaling components (C1 and C2) were extracted and used as covariates in regression analyses.

**Statistical Methods**

Demographics and baseline risk factors were summarized in proportions and means±SDs as appropriate. Differences among the four and six genotype groups were compared using the chi-squared test for categorical variables and one-way ANOVA for continuous variables. The survival differences among the four and six genotype groups were assessed by log-rank test and in the Cox regression for time to the event of doubling of the protein-to-creatinine ratio, ΔGFR (50% reduction or a 25-ml/min per 1.73 m² decline), dialysis, death, or their composite outcomes. On the basis of a *priori* consideration, the Cox regression analyses were performed sequentially to adjust for the risk factors in the following orders. Model 1 adjusted for age; sex; MAP; history of CVD; assigned to amlodipine, metoprolol, or ramipril during the randomized treatment phase; and degree of European ancestry. Model 2 adjusted for all of the variables used in model 1 and baseline GFR, whereas in model 3, additional adjustment was made for the urine protein-to-creatinine ratio. Because proteinuria can mediate the effect of genotype on the outcome, to avoid overadjustment, we chose model 2 as our final model.

When performing the Cox regression for doubling of the protein-to-creatinine ratio, we adjusted for the same variables as used in our model 2, with the exception of the baseline protein-to-creatinine ratio.

To assess the potential effect of the GSTM1 null and APOL1 high-risk genotypes on the outcomes of interest, the partial PAR was used to estimate the proportion of events that may have been avoided if the participants did not have these risk genotypes. The partial PAR and its 95% CI for the GSTM1 null genotype were calculated from a Cox model that was adjusted for the confounding risk factors and presence of the APOL1 high-risk genotype. Likewise, the partial PAR for the APOL1 high-risk genotype was calculated from a Cox model that was adjusted for the confounding factors and the presence of the GSTM1 null genotype. A P value <0.05 was considered to be statistically significant for all statistical results. All statistical analyses were performed using SAS software, version 9.3 (SAS Institute Inc., Cary, NC).
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DISCLOSURES

None.

REFERENCES


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