

APOL1, Acid Load, and CKD Progression



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Introduction: High dietary acid load and metabolic acidosis are associated with an accelerated decline in kidney function and may contribute to the observed heterogeneity in end-stage renal disease (ESRD) risk according to *APOL1* genotype. Our objective was to examine the associations of metabolic acidosis and dietary acid load with kidney disease progression, according to *APOL1* genotype, among individuals with chronic kidney disease (CKD).

Methods: We studied 1048 African American participants in the Chronic Renal Insufficiency Cohort. Metabolic acidosis was defined as blood levels of serum bicarbonate less than 22 mEq/L, and dietary acid load was quantified by potential renal acid load (PRAL) using data from the Diet Health Questionnaire. *APOL1* status was defined as having 2 risk variants, consisting of either possible combination of the G1 and G2 risk alleles. We tested associations of *APOL1* and dietary and metabolic acidosis with CKD progression, defined as time to ESRD or 50% decline in eGFR.

Results: During a median follow-up period of 7 years, 379 participants had an incident CKD progression event (6.4 events per 100 person-years). After full adjustment, among participants with 2 *APOL1* variants, the analysis failed to detect an association between metabolic acidosis or dietary acid load and CKD progression (hazard ratio [HR], 1.03; 95% confidence interval [CI], 0.96–1.11 per 1 mEq/L higher serum bicarbonate and an HR, 1.03; 95% CI, 0.92–1.15 per 10 mEq/L higher PRAL). Similar associations were noted among participants without the *APOL1* high-risk genotype.

Conclusion: In a population at high risk of developing ESRD, metabolic acidosis and dietary acid load were not associated with CKD progression.

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KEYWORDS: acidosis; *APOL1*; chronic kidney disease; dietary acid load; end-stage renal disease

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See Commentary on Page 911

Blacks have a 3.7-fold higher prevalence and a 3-fold higher incidence of ESRD compared with whites.^{1–3} This disparity only partly is explained by known determinants of ESRD, including hypertension, diabetes, socioeconomic factors, and the presence of *APOL1* risk variants.^{4–7} Polymorphisms in *APOL1*, absent among whites, have been shown to increase the odds of developing ESRD in African Americans.⁸ *APOL1* risk allele frequency estimates have been reported to be 21% (G1, composed of 2 missense variants), 13% (G2, in-frame deletion of 2 amino acids),

and 13% with 2 high-risk alleles in African Americans.⁸ However, not all individuals carrying the risk alleles show evidence of kidney disease, suggesting that environmental risk factors may influence the biological effects of *APOL1* on ESRD.²

Dietary acid load is a measure of balance between acid-inducing foods (e.g., animal sources of protein, metabolized to phosphorous and sulfate) and base-inducing foods (e.g., fruits and vegetables). High consumption of acid-inducing foods, along with diminished renal acid excretion, can induce metabolic acidosis (serum bicarbonate levels <22 mEq/L), which may be a risk factor for CKD progression. Observational studies have found that lower circulating bicarbonate levels and high dietary acid load are associated with adverse kidney outcomes, including a faster decline in estimated glomerular filtration rate (eGFR) and higher risks of incident CKD and ESRD.^{9–14}

Among patients with CKD, dietary acid load is associated more strongly with risk for ESRD among

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blacks than among whites.¹⁵ However, a recent study suggested that the *APOL1* high-risk genotype was associated more strongly with CKD progression among blacks with low net endogenous acid production (NEAP).¹⁶ We investigated whether the association of dietary acid load and metabolic acidosis on kidney disease progression is modified by *APOL1* genotype among black participants within the Chronic Renal Insufficiency Cohort (CRIC) Study. Dietary acid load and metabolic acidosis are modifiable risk factors that may be future targets for intervention and prevention for patients with a high-risk genotype.

METHODS

Study Population

The CRIC Study was a prospective observational study of risk factors for progression of kidney and cardiovascular disease among 3939 individuals with CKD.^{17,18} Participants were recruited between 2003 and 2008 at 13 centers across the United States and underwent extensive clinical evaluation at baseline and annual clinic visits and via telephone at 6-month intervals. The reporting of this study conforms to the Strengthening the Reporting of Observational Studies in Epidemiology guidelines.¹⁹

For this study, we restricted analyses to 1658 CRIC participants of self-reported black race. We further excluded participants who had an eGFR at baseline of less than 15 ml/min per 1.73 m² (n = 2), had missing *APOL1* status (n = 129), or did not have available dietary data for analysis (n = 479), leaving a final analytic sample of 1048. Compared with participants with missing dietary data, our study population had a larger proportion of women, and fewer had diabetes and congestive heart failure at baseline.

Metabolic Acidosis

Serum bicarbonate (CO₂) was measured using an enzymatic procedure with phosphoenolpyruvate carboxylase on the Ortho Vitros (Raritan, NJ) platform at the University of Pennsylvania Core Laboratory. The total CO₂ content includes serum bicarbonate as well as available forms of carbon dioxide. Serum bicarbonate comprises approximately 95% of the total CO₂ content; thus, the serum CO₂ measurement can be used as an estimator of serum bicarbonate.²⁰ Circulating CO₂ was evaluated dichotomously according to clinically relevant and previously published categories (i.e., <22 vs. ≥22 mEq/L [reference group], and continuously per mEq/L).

Dietary Acid Load

Food intake was quantified using the National Cancer Institute's Diet History Questionnaire, a food frequency questionnaire administered at baseline and targeting the intake of 255 common food items over the past

year.^{21,22} Food intake data were converted into daily nutrient intake using Diet*Calc software (available: <http://appliedresearch.cancer.gov/DHQ/dietcalc>).²³

The PRAL and net endogenous acid production (NEAP) describe the acid load of an individual's diet by estimating the production of nonvolatile acids and bases produced during digestion based on known nutritional content. We calculated the PRAL from dietary data using the Remer and Manz^{24,25} equation as follows: (PRAL = 0.49 * protein [g] + 0.037 * phosphorus [mg] - 0.021 * potassium [mg] - 0.026 * magnesium [mg] - 0.013 * calcium [mg]). Participants were classified into quartiles (Q) by PRAL (Q1, < -12.8; Q2, -12.8 to -1.6; Q3, -1.5 to 9.3; and Q4, >9.3).

We estimated NEAP using the previously published equation: (NEAP = 54.5 * [dietary protein {g/d}/dietary potassium {mEq/d}] - 10.2).^{26,27} Participants were classified into quartiles by NEAP (Q1, <30.3; Q2, 30.3-39.3; Q3, 39.4-51.7; and Q4, >51.7).

Genotyping and *APOL1* Risk Group Definition

Participants were genotyped for the *APOL1* G1 (rs73885319 or rs60910145) and G2 (rs71785313) risk alleles using ABI TaqMan (Applied Biosystems, Foster City, CA). High-risk *APOL1* status was defined as having 2 risk variants, consisting of either possible combination of the G1 and G2 risk alleles (i.e., G1/G1, G1/G2, or G2/G2 variants).

Outcomes

Kidney disease progression was defined as a 50% reduction in eGFR from baseline using the CKD-Epidemiology Collaboration (EPI) equation,²⁸ or the development of ESRD (initiation of dialysis therapy or kidney transplantation) during follow-up evaluation until June 30, 2009. ESRD was ascertained through active follow-up evaluation and linkage of the cohort with the US Renal Data System.

Measurement of Covariates

Demographic information, medical history, medication use in the past 30 days, height, weight, and blood pressure were collected at baseline by questionnaire and examination. Diabetes was defined at baseline by a fasting blood glucose level of 126 mg/dl or greater or use of diabetic medications. Urine albumin and creatinine concentrations from a 24-hour urine sample were measured at a central laboratory using standard assays. Baseline levels of serum creatinine were used for estimation of eGFR using the CKD-EPI equation.²⁸

Ancestry-informative genetic markers were based on the IBC Illumina chip array panel (San Diego, CA). A total of 1053 ancestry-informative markers, shared between the genotyping platform and HapMap3 reference

panel, were used to derive global African continental genetic ancestry measures.²⁹

Statistical Analysis

Participant characteristics were tabulated with respect to categories of serum bicarbonate. Unadjusted incidence rates were calculated as the number of events divided by person-years at risk. Participants were considered at risk from the date of their baseline examination until the first occurrence of kidney disease progression, death, or their data were censored owing to loss of follow-up evaluation or end of data collection on June 30, 2009.

We used Cox proportional hazards regression models to test for *APOL1* genotype status-specific associations of serum bicarbonate (categorically as <22 vs. ≥22 mEq/L and continuously per mEq/L), and PRAL (in study-specific quartiles and continuously per 10 mEq/d) with the relative hazard of CKD progression. Subgroup-specific HRs were calculated via linear combination of regression coefficients for main effect and cross-product terms. Analyses completely stratified by *APOL1* variant status yielded similar results.

Covariates for adjustment were chosen *a priori* based on the suspicion that they may confound the association between acid load and CKD progression. The first model included age at enrollment, sex, and the percentage of African ancestry. The second model added education (< high school, high school, or higher), income, body mass index (in kg/m²), baseline eGFR, prevalent diabetes, smoking status (current, former/never), history of cardiovascular disease, and 24-hour urine albumin to creatinine ratio (log-transformed). Models examining PRAL additionally adjusted for the total caloric intake (kcal/kg per d, log-transformed).

Functional forms of the *APOL1* genotype-specific associations of serum bicarbonate and PRAL with the hazard of CKD progression were examined graphically using adjusted penalized smoothing splines with evenly spaced knots among the inner 97.5% of serum bicarbonate or PRAL concentrations.

Multiplicative interactions were tested by the Wald test of a product term between *APOL1* genotype and serum bicarbonate or PRAL. We also tested for departure from an additivity of effects of *APOL1* genotype and serum bicarbonate by the relative excess risk owing to interaction.³⁰ The 95% CI of the relative excess risk owing to interaction was calculated as proposed by Hosmer and Lemeshow.³¹ Because associations of *APOL1* and CKD progression are reported to differ by diabetes status, analyses were repeated with stratification by diabetes at baseline.

The CRIC Study enrolled 3939 participants, of whom 1048 met our inclusion criteria. Given 281 CKD

Table 1. Baseline characteristics by category of serum bicarbonate

	Total	CO ₂ < 22 mEq/L	CO ₂ ≥ 22 mEq/L
N	1048	163	876
Demographics			
Age (yr)	58.5 (10.5)	57.2 (11.1)	58.8 (10.4)
Female sex	568 (54.2)	90 (55.2)	473 (54.0)
Education: high school graduate	793 (75.7)	120 (73.6)	668 (76.3)
Income			
<\$20,000	391 (37.3)	84 (51.5)	301 (34.4)
\$20,001–\$50,000	279 (26.6)	29 (17.8)	248 (28.3)
\$50,001–\$100,000	147 (14.0)	17 (10.4)	129 (14.7)
>\$100,000	47 (4.5)	7 (4.3)	40 (4.6)
Do not wish to answer	184 (17.6)	26 (16.0)	158 (18.0)
Current smoker	202 (19.3)	48 (29.4)	151 (17.2)
African ancestry, n (%)	77.3 (9.3)	77.3 (8.8)	77.3 (9.5)
Clinical values			
Body mass index	33.5 (8.1)	31.8 (8.1)	33.9 (8.1)
uACR			
<30	410 (40.4)	38 (23.8)	366 (43.3)
30–300	272 (26.8)	52 (32.5)	219 (25.9)
>300	333 (32.8)	70 (43.8)	261 (30.9)
eGFR	44.0 (15.0)	34.6 (13.5)	45.7 (14.7)
Systolic blood pressure	132.4 (22.9)	132.3 (23.0)	132.4 (22.9)
Diastolic blood pressure	73.6 (13.6)	72.4 (12.6)	73.7 (13.9)
APOL1 status			
0–1 risk variants	837 (79.9)	127 (77.9)	702 (80.1)
2 risk variants	211 (20.1)	36 (22.1)	174 (19.9)
Dietary characteristics			
Potential renal acid load (mEq/d)	-1.9 (21.4)	1.2 (22.1)	-2.6 (21.3)
Protein (g/d)	69.4 (38.3)	67.8 (42.4)	69.7 (37.7)
Phosphorous (mg/d)	1111.3 (539.9)	1084.9 (538.0)	1115.6 (541.1)
Potassium (mg/d)	2913.0 (1390.4)	2711.5 (1266.5)	2953.6 (1412.8)
Magnesium (mg/d)	301.8 (140.6)	286.7 (132.7)	304.5 (141.8)
Calcium (mg/d)	659.2 (362.2)	634.4 (346.5)	663.1 (364.5)
Total energy intake (kcal/kg per d)	20.7 (11.2)	21.5 (11.4)	20.5 (11.1)
Total protein intake (g/kg per d)	0.8 (0.4)	0.8 (0.5)	0.7 (0.4)
Net endogenous acid production (mEq/d)	41.9 (16.8)	43.8 (18.2)	41.4 (16.5)
Comorbidities/medications			
Stroke	145 (13.8)	25 (15.3)	120 (13.7)
Cardiovascular disease	398 (38.0)	56 (34.4)	340 (38.8)
Heart failure	128 (12.2)	17 (10.4)	110 (12.6)
Anti-acidosis medications	11 (1.1)	3 (1.8)	8 (0.9)
Diabetes	525 (50.1)	81 (49.7)	441 (50.3)
Hypertension	978 (93.3)	156 (95.7)	813 (92.8)

APOL1, apolipoprotein 1; CO₂, serum bicarbonate; eGFR, estimated glomerular filtration rate; uACR, urine albumin-to-creatinine ratio. Means (±SD) or N (%) are shown.

progression events in the subgroup of participants with 0 to 1 *APOL1* high-risk variants, at a type I error rate of 5%, we estimated that we had 77%, 91%, and 97% power to detect HRs of 1.2, 1.25, and 1.3, respectively. In the subgroup of participants with 2 high-risk genotypes, we had 62%, 74%, and 83% power to detect HRs of 1.3, 1.35, and 1.4, respectively.

The nominal level of significance was defined as a *P* value less than 0.05 (2-sided). All analyses were

Table 2. Number of events, incidence rates, and HRs with 95% CIs for serum bicarbonate in the chronic renal insufficiency cohort

	N	Events, n	Incidence rate ^a	Unadjusted HR (95% CI)	Model 1 HR (95% CI) ^b	Model 2 HR (95% CI) ^c
Overall						
<22 mEq/L	163	86	12.1	2.12 (1.67–2.70)	2.20 (1.72–2.81)	1.25 (0.95–1.64)
≥22 mEq/L	876	290	5.6	1.0 (ref)	1.0 (ref)	1.0 (ref)
Per mEq/L	–	–	–	0.91 (0.89–0.94)	0.91 (0.89–0.94)	1.00 (0.96–1.03)
APOL1: 0–1 risk variants						
<22 mEq/L	127	66	11.7	2.24 (1.70–2.96)	2.28 (1.72–3.03)	1.28 (0.94–1.74)
≥22 mEq/L	702	215	5.1	1.0 (ref)	1.0 (ref)	1.0 (ref)
Per mEq/L	–	–	–	0.90 (0.87–0.94)	0.91 (0.87–0.94)	0.98 (0.94–1.02)
APOL1: 2 risk variants						
<22 mEq/L	36	20	13.5	1.74 (1.06–2.86)	1.92 (1.17–3.17)	1.24 (0.74–2.08)
≥22 mEq/L	174	75	7.6	1.0 (ref)	1.0 (ref)	1.0 (ref)
Per mEq/L	–	–	–	0.94 (0.89–0.99)	0.94 (0.88–0.99)	1.03 (0.96–1.11)
<i>P</i> for interaction				0.495	0.736	0.715

APOL1, apolipoprotein 1; CI, confidence interval; CO₂, serum bicarbonate; HR, hazard ratio.

^aPer 100 person-years.

^bModel 1: adjusted for age, sex, and percentage of African ancestry.

^cModel 2: adjusted for age, sex, percentage of African ancestry, diabetes, systolic blood pressure, body mass index, income, education, estimated glomerular filtration rate, 24-hour albumin to creatinine ratio, smoking, and history of cardiovascular disease.

HRs and 95% CIs were derived from Cox proportional hazard models.

conducted using Stata Version 14.2 (College Station, TX)³² and R version 3.4.1 (Vienna, Austria).

RESULTS

Participant Characteristics

At baseline, the mean age and eGFR were 59 years (SD, 10.5 yr) and 44.0 ml/min per 1.73 m² (SD, 15.0 ml/min per 1.73 m²), respectively. Approximately 20% of the study population had 2 *APOL1* risk variants. The mean PRAL was -1.9 mEq/d (SD, 21.4 mEq/d), serum bicarbonate was 24.8 mEq/L (SD, 3.3 mEq/L), and NEAP was 41.9 mEq/d (SD, 16.8 mEq/d). PRAL and NEAP were highly correlated with a Pearson correlation coefficient of 0.88. Serum bicarbonate also was correlated inversely and weakly with PRAL and NEAP (correlation coefficients, -0.07 and -0.06, respectively).

Participants in the lowest category of serum bicarbonate (CO₂ < 22 mEq/L) had a lower body mass index, lower eGFR, and were more likely to be current smokers and have heavy albuminuria (Table 1). NEAP was similar across categories of serum bicarbonate, and participants with the lowest serum bicarbonate levels had higher PRAL (Supplementary Table S1), with a mean of 1.2 (SD, 22.1) compared with -2.6 (SD, 21.3) in those with the highest levels of serum bicarbonate.

APOL1 Risk Variants and CKD Progression

Over a median follow-up period of 6.6 years (range, 0.1–9.7 yr), 379 participants experienced CKD progression, yielding an incidence rate of 6.4 cases per 100 person-years. Among participants with 2 *APOL1* risk variants, the incidence rate of CKD progression was 8.4 per 100 person-years; among those with 0 to 1 risk variants, the incidence rate was 5.9 per 100 person-years (Supplementary Table S2).

Serum Bicarbonate and Outcomes

Circulating serum bicarbonate levels less than 22 mEq/L were associated with CKD progression, relative to a serum bicarbonate level greater than 22 mEq/L, after adjustment for age, sex, and African ancestry (model 1), in both *APOL1* genotype subgroups; HRs were 2.28 (95% CI, 1.72–3.03) and 1.92 (95% CI, 1.17–3.17) among individuals with 0 to 1 and 2 *APOL1* risk variants, respectively (Table 2). Associations were attenuated in model 2, and the 95% CI no longer excluded the null (HR, 1.28; 95% CI, 0.94–1.74 and HR, 1.24; 95% CI, 0.74–2.08) among individuals with 0 to 1 and 2 risk *APOL1* variants, respectively. The magnitude of associations was similar comparing participants with 0 to 1 risk variants with those with 2 risk variants (*P* value for multiplicative interaction = 0.736, *P* value for additive interaction = 0.98) (Table 2). An evaluation of the association of serum bicarbonate with risk of CKD progression using splines showed similar findings (Figure 1). Results were similar when examining serum bicarbonate continuously (Table 2) and when stratifying by diabetes at baseline (Supplementary Table S3).

Potential Renal Acid Load, NEAP, and Outcomes

Participants with higher PRAL were more likely to be older, male, and current smokers (Supplementary Table S1). PRAL was not associated with CKD progression in either *APOL1* genotype subgroup (HR, 0.98; 95% CI, 0.93–1.04 and HR, 1.03; 95% CI, 0.92–1.15) per 10 mEq/L higher PRAL, among individuals with 0 to 1 and 2 *APOL1* variants, respectively (Table 3). However, visual inspection of the splines suggested that a higher PRAL may be associated more strongly with the risk of CKD progression among participants with 2 *APOL1* variants (Figure 2).

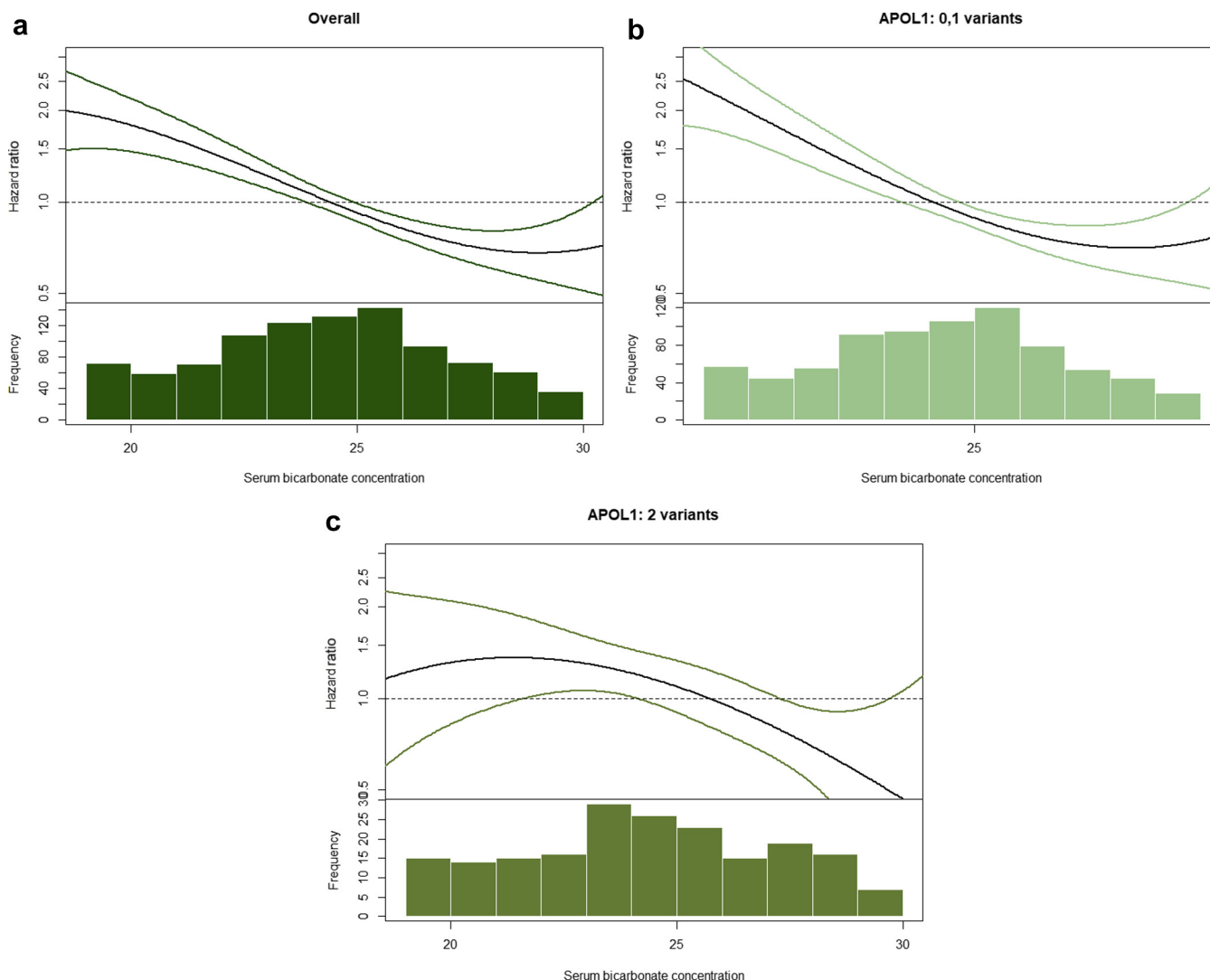


Figure 1. Association between overall serum bicarbonate concentrations (a), serum bicarbonate concentrations in participants with 0 to 1 *APOL1* risk variants (b), and serum bicarbonate concentrations in participants with 2 *APOL1* risk variants with the hazard of chronic kidney disease progression (c). Adjusted penalized smoothing splines with evenly spaced knots were used.

Similar findings were noted for NEAP (data not shown).

DISCUSSION

Among a cohort of African Americans at high risk for ESRD, acidosis was associated with CKD progression in crude but not in fully adjusted analyses. The magnitude of associations was small and generally similar across *APOL1* risk variant groups, although associations of PRAL trended stronger among individuals with 2 *APOL1* variants compared with those with 0 or 1. These findings add to the growing body of literature examining gene–environment interactions or second-hit factors in the risk of ESRD associated with high-risk *APOL1* variants.

The association between *APOL1* high-risk genotype and ESRD is well established. Blacks with 2 *APOL1* risk

variants have a 2- to 6-fold higher odds of developing ESRD.⁸ In the Atherosclerosis Risk in Communities Study, carrying 2 risk alleles was associated with a faster decline in eGFR and a higher risk of developing CKD and ESRD.³³ Similarly, among individuals with CKD, black patients in the *APOL1* high-risk group have higher risks of renal outcomes and a faster decline in eGFR.²⁹ Although the *APOL1* high-risk genotype has been shown to be associated with a higher risk of ESRD, unexplained variance remains. In a recent gene-by-*APOL1* association study, Langefeld *et al.*³⁴ found no interactions between *APOL1* risk variants and any single-nucleotide polymorphism, suggesting that *APOL1* and environmental interactions may be of greater importance.

Previously, in a study of CRIC participants of all race/ethnicities, Dobre *et al.*³⁵ reported that the risk of developing a renal end point was lower with a higher

Table 3. Number of events, incidence rates, and HRs with 95% CIs for potential renal acid load in the chronic renal insufficiency cohort

	N	Events, n	Incidence rate ^a	Unadjusted HR (95% CI)	Model 1 HR (95% CI) ^b	Model 2 HR (95% CI) ^c
PRAL						
Overall						
Q1	262	87	5.9	1.0 (ref)	1.0 (ref)	1.0 (ref)
Q2	262	96	6.4	1.09 (0.81–1.45)	1.10 (0.82–1.48)	1.01 (0.74–1.38)
Q3	262	93	6.2	1.05 (0.78–1.41)	0.96 (0.71–1.30)	0.88 (0.64–1.20)
Q4	262	103	7.2	1.23 (0.92–1.63)	1.06 (0.79–1.43)	1.01 (0.74–1.38)
Per 10 mEq/d	–	–	–	1.02 (0.98–1.08)	1.00 (0.95–1.05)	1.00 (0.95–1.05)
APOL1: 0–1 risk variants						
Q1	204	66	5.6	1.0 (ref)	1.0 (ref)	1.0 (ref)
Q2	208	75	6.4	1.14 (0.82–1.59)	1.13 (0.81–1.59)	1.05 (0.74–1.50)
Q3	215	70	5.5	0.99 (0.71–1.39)	0.91 (0.65–1.30)	0.96 (0.67–1.38)
Q4	210	72	6.2	1.11 (0.79–1.55)	0.97 (0.69–1.37)	0.92 (0.64–1.32)
Per 10 mEq/d	–	–	–	1.01 (0.95–1.06)	0.98 (0.93–1.04)	0.98 (0.93–1.04)
APOL1: 2 risk variants						
Q1	58	21	6.9	1.0 (ref)	1.0 (ref)	1.0 (ref)
Q2	54	21	6.4	0.92 (0.50–1.68)	0.99 (0.53–1.85)	0.88 (0.47–1.65)
Q3	47	23	9.4	1.34 (0.74–2.42)	1.20 (0.66–2.21)	0.67 (0.36–1.26)
Q4	52	31	11.7	1.67 (0.96–2.90)	1.48 (0.83–2.63)	1.33 (0.74–2.41)
Per 10 mEq/d	–	–	–	1.09 (0.99–1.20)	1.07 (0.97–1.19)	1.03 (0.92–1.15)
P for interaction				0.086	0.109	0.384

APOL1, apolipoprotein 1; CI, confidence interval; HR, hazard ratio; PRAL, potential renal acid load; Q, quartile.

^aPer 100 person-years.

^bModel 1: adjusted for age, sex, and percentage of African ancestry.

^cModel 2: adjusted for age, sex, percentage of African ancestry, diabetes, systolic blood pressure, body mass index, income, education, estimated glomerular filtration rate, 24-hour albumin to creatinine ratio, smoking, history of cardiovascular disease, and kilocalories per weight per day.

HRs and 95% CIs were derived from Cox proportional hazard models. Q1, <12.97 mEq/d; Q2, –12.97 to –1.881 mEq/d; Q3, –1.880 to 8.96 mEq/d; and Q4, >8.96 mEq/d.

serum bicarbonate level. In another cohort study of 1781 participants from the Modification of Diet in Renal Disease Study, Menon *et al.*³⁶ found that a lower serum bicarbonate level was associated with an increased risk of long-term outcomes in nondiabetic patients with CKD. In our study, consistent with previous studies but restricted to participants of black race, CKD progression incidence rates were higher among participants with metabolic acidosis. However, after full adjustment for potential confounders, including baseline kidney function, the analysis failed to detect an association between metabolic acidosis and CKD progression, overall or within *APOL1* risk variant groups. Because eGFR is associated very strongly with both serum bicarbonate levels and CKD progression, adjustment for this factor attenuated the magnitude of the HR and rendered the estimate no longer statistically significant; our study lacked the statistical power necessary to detect an effect of this magnitude.

Dietary acid load is a modifiable risk factor that has been shown to be associated with CKD and ESRD. In a study of 15,055 participants in the Atherosclerosis Risk in Communities Study, Rebholz *et al.*¹⁰ found that a higher dietary acid load (PRAL and NEAP) was associated with a higher risk of incident CKD. Similarly, among individuals with CKD in the National Health and Nutrition Examination Survey III, a higher dietary acid load, measured by net acid excretion, was associated with a higher risk of ESRD and this association

was stronger among blacks than among whites.^{15,37} In the African American Study of Kidney Disease and Hypertension, a higher NEAP was associated with a more rapid decline in kidney function.¹⁴ In healthy individuals, an increase in dietary acid load was accompanied by an increase in urinary ammonia excretion. Among persons with CKD, however, lower acid excretion was more likely a reflection of the impaired ability of the kidney to excrete ammonia than of a decreased acid load. Along these lines, in a previous analysis within CRIC, higher net acid excretion, examined in 980 participants using urinary biomarkers, was associated with a lower risk of ESRD among participants with diabetes.³⁸ Similarly, among participants from the Nephro Test cohort, a lower urinary ammonia level was associated with a higher risk of CKD progression.³⁹

Visual inspection of the associations of dietary acidosis raise the possibility of differential effects of PRAL on CKD progression according to *APOL1* genotype. Although we cannot be certain, in our study the lack of a statistical interaction between *APOL1* variants, PRAL, and CKD progression may have been owing to limited statistical power. It would be premature to conclude that dietary modification may be particularly beneficial among individuals with high-risk *APOL1* genotypes. High consumption of acid-inducing foods can induce metabolic acidosis (serum bicarbonate levels ≤ 22 mEq/L), which, among CKD

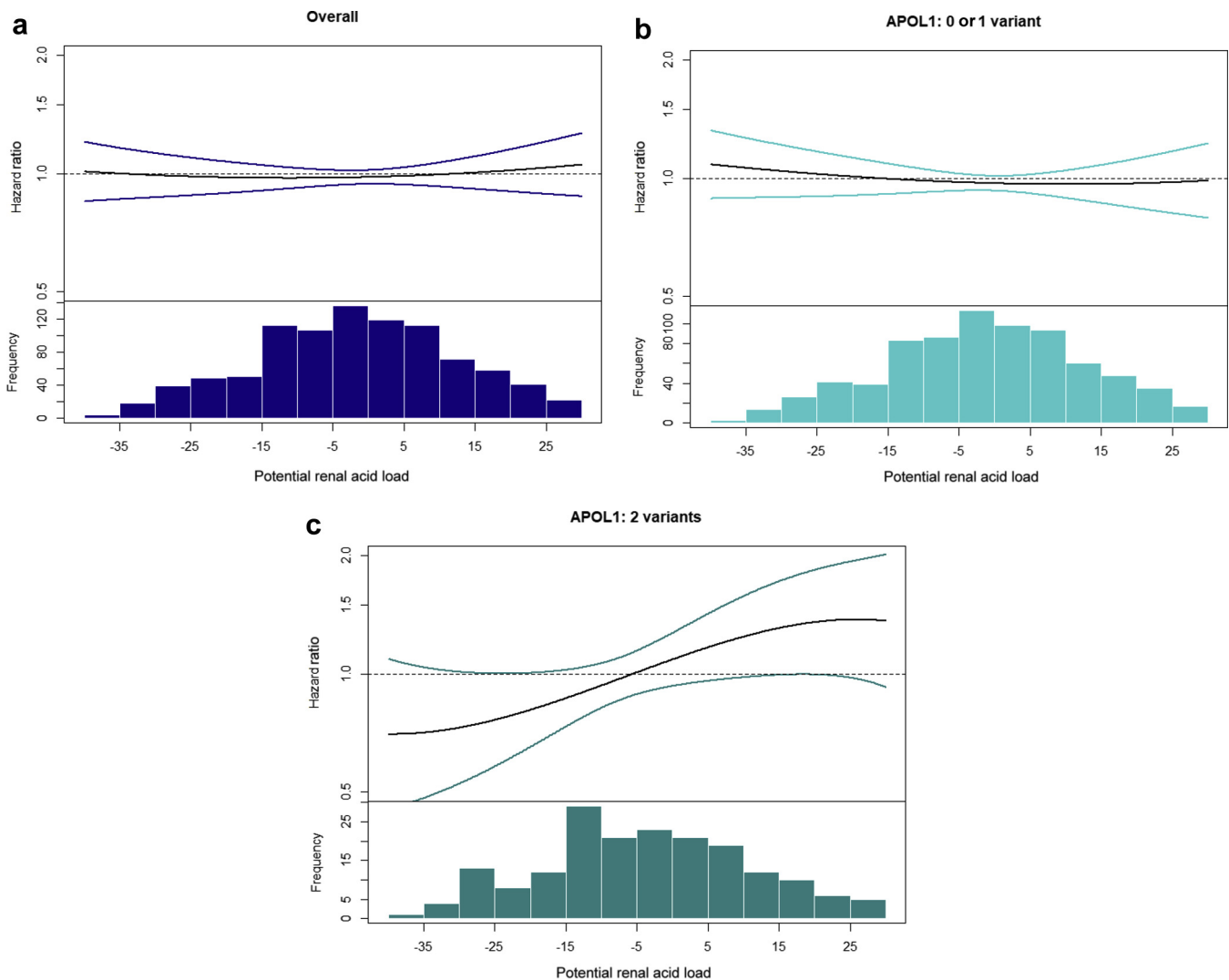


Figure 2. Association between overall potential renal acid load (PRAL) concentrations (a), PRAL concentrations in participants with 0 to 1 *APOL1* risk variants (b), and PRAL concentrations in participants with 2 *APOL1* risk variants with the hazard of chronic kidney disease progression (c). Adjusted penalized smoothing splines with evenly spaced knots were used.

patients, is a risk factor for disease progression, bone loss, and muscle wasting. Small single-center randomized trials have shown benefits of alkali therapy (sodium bicarbonate or a diet high in fruits and vegetables), even in patients with normal serum bicarbonate levels, on progression of CKD, bone health, and general nutritional status.^{40–43} In a recent randomized trial of 20 adults with moderate CKD and a moderate serum bicarbonate concentration, Scialla *et al.*⁴⁴ found that treatment with sodium bicarbonate had a strong effect on the tricarboxylic acid cycle and additional effects on propanoate metabolism, pyruvate, and branched-chain amino acid metabolism. From these findings, the investigators suggested that sodium bicarbonate treatment may be beneficial in a population with CKD. Dietary acid load and metabolic acidosis are modifiable risk factors that could be used to increase the focus on individuals with the highest risk of ESRD

and may be important for developing individualized interventions to reduce ESRD risk in African Americans. Although our study did not find a significant association between dietary acid load and CKD progression, further research is needed to examine the relationship between dietary acid load and *APOL1*.

This study examined the association between *APOL1* risk variants, metabolic acidosis, dietary acid load, and ESRD. Strengths of our study included the prospective design and the unique cohort of individuals with a high risk of ESRD. Another important strength of this study was the ascertainment of acid load using a validated measure (i.e., the Diet Health Questionnaire²³). Although we were powered to detect subgroup-specific associations ranging from 1.2 to 1.3, our study had limited power to detect associations of such small magnitude. The CKD progression rate in CRIC is slower than would be expected for a cohort

with these comorbidities and clinical characteristics.^{45,46} It is possible that the frequent follow-up evaluations and criteria for entry drew patients with a more favorable prognosis into the CRIC study. An additional limitation was that dietary acid load was calculated from self-reported food frequency questionnaire results, which may not reflect intake. Kidney dysfunction at baseline, in combination with measuring dietary and metabolic acidosis at a single time point, impeded us from fully elucidating the interplay, through time, of changes in diet and the progression of CKD. Although we attempted to alleviate this concern by adjusting for measured subclinical disease and baseline kidney function, a fully comprehensive view of the time course of CKD progression and changes in diet over time would be ideal.

In conclusion, in a population at particularly high risk for ESRD, the associations between metabolic acidosis, dietary acidosis, and CKD progression were inconclusive. If there truly are differences in the population in the association of acidosis and CKD progression between *APOL1* variant groups, they are likely to be small. We were underpowered to detect the very small differences in associations between groups in our sample; the clinical relevance of such small differences is unclear. As such, whether dietary acid load and metabolic acidosis should be targets for future interventions in those at the highest risk of ESRD has yet to be determined. These observations do motivate future larger studies of individuals with high-risk *APOL1* genotypes to better evaluate and assess risk factors for ESRD.

DISCLOSURE

All the authors declared no competing interests.

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Institute of Diabetes and Digestive Kidney Diseases Central Repositories, or the National Institute of Diabetes and Digestive Kidney Diseases.

SUPPLEMENTARY MATERIAL

Table S1. Baseline characteristics by quartiles of potential renal acid load. Means (\pm SD) or N (%) are shown.

Table S2. Number of events, incidence rates, and hazard ratios with 95% confidence intervals for serum bicarbonate in the Chronic Renal Insufficiency Cohort stratified by diabetes.

Table S3. Number of events, incidence rates, and hazard ratios with 95% confidence intervals for potential renal acid load in the Chronic Renal Insufficiency Cohort stratified by diabetes.

Supplementary material is linked to the online version of the paper at www.kireports.org.

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