Materials and Methods

Study Design and Population

The Chronic Renal Insufficiency Cohort (CRIC) Study is a multi-center prospective observational study designed to examine risk factors for progression of renal insufficiency and cardiovascular disease (CVD) in adults with mild to moderate chronic kidney disease (CKD). The CRIC Study has been described extensively\(^1\textsuperscript{-}^4\) and will be briefly summarized here. A total of 3939 men and women were recruited from seven clinical centers located across the United States between 2003 and 2008. Participants aged 21-74 years were enrolled according to age-based estimated glomerular filtration rate (eGFR) inclusion criteria: 20-70 mL/min/1.73 m\(^2\) for persons aged 21-44 years, 20-60 mL/min/1.73 m\(^2\) for persons aged 45-64 years, and 20-50 mL/min/1.73 m\(^2\) for persons aged 65-74 years. Exclusion criteria included: New York Heart Association class III or IV heart failure, cirrhosis, HIV/AIDS, multiple myeloma, renal carcinoma, polycystic kidney disease, recipient of organ transplant, previous dialysis, history of immunotherapy for renal disease or vasculitis, and history of chemotherapy. The institutional review board at each study site approved the study protocol. All participants provided written informed consent.

Data Collection

As has been previously described,\(^1\textsuperscript{,}^2\) participants completed an in-person baseline visit, during which blood samples were obtained and 24-hour urine collections were initiated. Participants returned annually for in-person follow-up visits with repeat blood samples taken. Plasma lipids were measured on fasting blood samples prior to freezing using standard laboratory assays; Lp(a) was measured using a latex-enhanced immunoturbidimetric assay.
(Pointe Scientific, Canton, MI). The eGFR was calculated using an equation derived from CRIC participants using serum creatinine and cystatin-C, age, sex, and race. Participants were contacted by telephone at six-month intervals between clinic visits to collect interim health status updates.

A total of 3680 CRIC participants were genotyped using the Illumina HumanOmni1-quad Array Platform (Illumina, San Diego, CA). The genotype data for 3635 participants passed initial quality control metrics. The single nucleotide polymorphisms (SNPs) of interest, based on prior studies that showed an association with Lp(a) level in the general population, were: rs3798220, rs10455872, and rs9457951. Variants of the rs3798220 SNP, which is found mostly in Hispanics, was not found among the genotyped CRIC participants (<12% Hispanic), and was not further analyzed. The rs9457951 SNP was not located on the chip, so a proxy, rs6930542, determined to be in perfect linkage disequilibrium ($r^2 = 1$), was used in its place.

The CRIC genome wide association study data are uploaded to dbGAP (Study Accession: phs000524.v1.p1). CRIC data is uploaded to the National Institute of Diabetes and Digestive and Kidney Diseases repository according to pre-established requirements and timelines.

**Assessment of Outcomes**

Hospitalizations ascertained through participant self-report were confirmed by study personnel who reviewed medical records for the presence of International Classification of Diseases, Ninth or Tenth Edition (ICD-9/ICD-10) and Current Procedural Terminology (CPT) codes suggestive of atherosclerotic cardiovascular events, which were then adjudicated by two physicians. Primary endpoints were defined as myocardial infarction (MI), death, and a
composite of the two events. Deaths were ascertained from reports of next of kin, death certificates, obituaries, hospital records, and the Social Security Death Master File.

Statistical Analysis

Baseline characteristics were described using means (standard deviations [SD]) or medians (interquartile ranges [IQR]) for continuous variables, and with frequencies (%) for categorical variables. Skewed variables were natural-log transformed. Group comparisons were conducted using Kruskal-Wallis tests for continuous variables and $\chi^2$ tests for categorical variables. All analyses were performed using Stata software, version 14 (StataCorp). All tests were 2-sided, with $P < 0.05$ considered significant.

In participants with genotype data, information for the rs10455872 and rs6930542 SNPs were selected using PLINK version 1.90. For each SNP, participants were stratified into two categories based on presence of the minor allele: zero (non-carrier) versus at least one copy (carrier). A linear regression model, first unadjusted and then adjusted for age, gender, race, clinical site, systolic blood pressure, body mass index (BMI), tobacco use, diabetes mellitus (DM), and statin use, was used to determine the association between carrier status for each SNP with change in the log-transformed Lp(a) level. The models were repeated to determine the association between baseline eGFR (above/below 45 mL/min/1.73 m$^2$) with change in the log-transformed Lp(a) level. To test for interaction, an interaction term with the dichotomous eGFR variable was included into the regression model for each SNP and a likelihood ratio test was used to determine the significance of the interaction.

Participants with available baseline Lp(a) levels were stratified into quartiles by Lp(a) level. Cumulative event curves were constructed for each quartile for the composite outcome
using the Kaplan-Meier method. Crude event rates were compared with the log-rank test. A Cox proportional hazards model was used for each outcome to determine cause-specific hazard ratios (HR) with associated confidence intervals (CIs) for each quartile of Lp(a). The quartile with the lowest event rate was used as the reference category. The proportional hazards assumption was met based on Schoenfeld residuals. Participants were censored either at time of withdrawal from the study, time of last study visit (if they did not withdraw) or upon database lock in mid-2013, whichever occurred first.

A tiered approach was used to study the association between Lp(a) level and the outcomes. Covariates were selected a priori on the basis of previously described risk factors for CVD development. First, the association was evaluated using an unadjusted, univariate analysis (Model 1). Model 2 adjusted for age, gender, race, and clinical site. The final model, Model 3, additionally adjusted for education level, tobacco use, DM, history of prior CVD, systolic blood pressure, BMI, statin use, total cholesterol, LDL-C, high-density lipoprotein cholesterol (HDL-C), and triglycerides. Total cholesterol and LDL-C were both corrected for the Lp(a) contribution according to compositional data which shows that approximately 30% of Lp(a) is cholesterol.9 Thus, Lp(a) was multiplied by 0.3 and this value was subtracted from the total cholesterol and LDL-C for each participant as has been done in prior studies.10,11 In sensitivity analyses, Model 3 was additionally adjusted for eGFR and 24-hour urine protein. However, this was not considered the final model and thought to be an overadjustment because renal dysfunction is hypothesized to be on the causal pathway for the elevations in Lp(a) levels. To explore for effect modification, the Model 3 for the composite outcome was repeated after stratifying by subgroups of age, gender, race, tobacco use, presence/absence of DM, baseline eGFR, and baseline urine protein excretion, and tested for interaction by subgroup.
In further sensitivity analyses, participants who, at time of enrollment into the study, had a history of MI, cardiac revascularization or a cerebrovascular event, were excluded from the survival analyses. Cerebrovascular events from the medical history were not further classified into subtypes, so all were excluded, given that over 80% of strokes would have been expected to be ischemic in etiology.\textsuperscript{12,13}

References


