1.a. Full Title: CHEK2 and genetic susceptibility to kidney damage and albuminuria

b. Abbreviated Title (Length 26 characters):

2. Writing Group: Nora Franceschini
   Writing group members: KE North, Lynda Kao, Joe Coresh, Anna Kottgen, Eric Boerwinkle, JS Pankow, D Arnett, L Baird, MF Leppert, CC Gu, CE Lewis, RH Myers, Steven Turner, Alan Weder, Holly Kramer, SC Hunt

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. _NF____ [please confirm with your initials electronically or in writing]

First author: Nora Franceschini, MD, MPH
   Research Assistant Professor
   Department of Epidemiology
   University of North Carolina Chapel Hill
   Bank Of America Center
   137 E. Franklin St., Suite 306
   CB #8050
   Chapel Hill, NC 27514
   (919) 966-2148 (Voice)
   (919) 966-9800 (Fax)
   (919) 671-1029 (Cell)
   kari_north@unc.edu

Corresponding/senior author (must be an ARIC investigator for the proposal but can be different in the published paper; correspondence will be sent to both the first author & the corresponding author):
Address:

Phone: Fax:
E-mail:
3. **Timeline**: 1-2 years

4. **Rationale**: Increased urine albumin excretion (albuminuria) results from increased glomerular capillary permeability and is associated with incipient renal disease. In population studies, microalbuminuria (albuminuria levels between 30 and 299 mg/g creatinine) is associated with older age, minority race, type 2 diabetes, and the presence of hypertension, but also is present in at least 5% of the low-risk population (Jones, Francis et al. 2002). Albuminuria is also an independent risk factor for stroke, myocardial infarction, new-onset heart failure and death (Gerstein, Mann et al. 2001; Hillege, Fidler et al. 2002). Genetic and environmental factors likely contribute to the increased albuminuria excretion. A high heritability of albuminuria has been noted in twin studies (0.45 ± 0.07) (Rao, Wessel et al. 2007). A genetic component for kidney damage has also been identified in studies of type 1 and type 2 diabetic study participants (Fogarty, Hanna et al. 2000; Fogarty, Rich et al. 2000; Imperatore, Knowler et al. 2001; Iyengar, Fox et al. 2003; Krolewski, Poznik et al. 2006), in hypertensive study participants (Chung, Ferrell et al. 2003; Freedman, Beck et al. 2003; Turner, Kardia et al. 2006; Leon, Freedman et al. 2007) and in the general population (Fox, Yang et al. 2004; Fox, Yang et al. 2005). The identification of genes for albuminuria may give insights into the genetic susceptibility to kidney damage and to cardiovascular disease susceptibility.

Programmed cell death or apoptosis of podocytes in response to injury has been described in experimental animal models and in humans (Shankland 2006). Recent research suggests that reactive oxygen species and DNA damage induce podocyte apoptosis in the membrane attack complex C5b-9 injury (passive Heymann nephritis)
(Pippin, Durvasula et al. 2003), in puromycin nephrosis (Marshall, Pippin et al. 2006) and in cisplatin nephrotoxicity (Pabla, Huang et al. 2007). DNA damage leads to activation of the checkpoint pathways, which mediate cell cycle arrest and apoptosis (Niida and Nakanishi 2006; Bartek and Lukas 2007). Therefore, genes in the DNA repair and cell cycle checkpoint pathways are excellent candidates for evaluation of association with albuminuria.

The checkpoint 2 gene (CHEK2) is an important transducer in DNA damage signaling pathways in response to environmental injury. We have performed analyses of the association of CHEK2 single nucleotide polymorphisms (SNPs) with albuminuria in the Family Blood Pressure Program (FBPP). Two CHEK2 SNPs, rs5762764 and rs2346397, were independently associated with albuminuria among white participants of the Hypertension Genetic Epidemiology study (HyperGEN). rs2346397 and rs4035540 were also independently associated with albuminuria among African American HyperGEN participants. However, these SNPs were not associated with albuminuria among participants of the Genetic Epidemiology Network of Arteriopathy (GENOA). We propose to use the ARIC data for replication of our findings in the FBPP.

5. **Main Hypothesis/Study Questions:** Our main hypothesis is that the CHEK2 gene, an important transducer in DNA damage signaling pathways in response to environmental injury, contains one or more polymorphic variants (SNPs) that are associated with albuminuria excretion. We propose to extend our analyses to the population-based ARIC study for replication of our findings in the FBPP.
6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).
We will use data from the Visit 4 examination of 8,250 genotyped white and 2,025 genotyped African American individuals with available albuminuria data.

Study design: cross-sectional race-stratified analyses of the association of \textit{CHEK2} polymorphisms with albuminuria at ARIC Visit 4.

Exposure: Five \textit{CHEK2} SNPs. See genotyping details below.

Outcome: We used the urine albumin-to-creatinine ratio (ACR) as a measure of urinary albumin excretion (2002).

Genotyping: Briefly, SNPs were chosen using the HapMap Phase I CEU sample and a pairwise analysis of correlation ($r^2 = 0.65$). An additional SNP, rs2346397, located 200 kb from \textit{CHEK2}, was also genotyped. Genotyping for the ARIC study has been performed by the ARIC Central Laboratory using Taqman® genotyping assays under direction of Dr Eric Boerwinkle.

Statistical analyses: All SNPs will be tested for significant deviation from Hardy-Weinberg equilibrium (HWE) in race-stratified samples, using an alpha=0.001 and the Exact test (Wigginton, Cutler et al. 2005). Quantitative trait distributions will be inspected and traits with skewed distribution will be natural log transformed (ACR). We will fit linear regression models in race-stratified samples (SAS 9.1) using general genetic models (2-degree of freedom test, df), adjusting for covariates as described below. All analyses will be adjusted for the effects of age, $age^2$, sex, age-by-sex interactions and study center, within each race-stratified population sample. We will also
implement models adjusting for systolic blood pressure (SBP), hypertension treatment, use of angiotensin-enzyme converting inhibitor (ACEI) or angiotensin 2 receptor blocker (ARB), type 2 diabetes, body mass index, smoking exposure and kidney function (estimated glomerular filtration rate or serum creatinine). We will also consider testing for gene-by-environment interactions for hypertension and diabetes in the ARIC sample, using an alpha=0.05, to compare the analysis to the hypertension-enriched FBPP samples.

Limitations: The cross-sectional analysis is a limitation of our study. However, our analysis is appropriate as a replication of findings from FBPP, since the FBPP study only have cross-sectional data. Therefore, we can assure comparability of the findings. Our analyses in the ARIC study will not account for population substructure, which could be an issue in African American sample. However, population stratification analysis will be feasible as the genotyping data from the ARIC genome-wide association study is available.

7.a. Will the data be used for non-CVD analysis in this manuscript? _____ Yes ___X__ No

b. If Yes, is the author aware that the file ICTDER03 must be used to exclude persons with a value RES_OTH = “CVD Research” for non-DNA analysis, and for DNA analysis RES_DNA = “CVD Research” would be used? _____ Yes ____ No

(This file ICTDER03 has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript? ___X___ Yes ___ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER03 must be used to
exclude those with value RES DNA = “No use/storage DNA”?

__X__ Yes    ____ No

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: http://www.cscc.unc.edu/ARIC/search.php

___X___ Yes  _______ No

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

ARIC manuscript proposal #1238, “DNA-damage pathway and genetic susceptibility to type 2 diabetes and insulin resistance states”. PI: Kari North

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data?     __X__ Yes    ____ No

Ancillary study #2006-12 “DNA-damage pathway and genetic susceptibility to type 2 diabetes and insulin resistance states”.

11.b. If yes, is the proposal

__X__ A. primarily the result of an ancillary study (list number*  _2006-12, aim 3)

_____ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* __________  __________ __________)

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/

12. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.


