ARIC Manuscript Proposal # 1373

PC Reviewed: 05/13/08	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: The Association of Cell Cycle *Checkpoint 2* Variants and Kidney Function: Findings from The Family Blood Pressure Study and the Atherosclerosis in Communities Study

b. Abbreviated Title (Length 26 characters): ***Chek2* and kidney function**

2. Writing Group: Nora Franceschini

Writing group members: KE North, Lynda Kao, Eric Boerwinkle, JS Pankow, D Arnett, L Baird, MF Leppert, JH Eckfeldt, CC Gu, CE Lewis, RH Myers, Steven T 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]**

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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:
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3. Timeline: 1-2 years

4. Rationale: Chronic kidney disease (CKD) is an emerging public health problem with an estimated prevalence of 9.6 % (or 19.2 million) of the adult US population (Coresh, Astor et al. 2003; Schoolwerth, Engelgau et al. 2006). Genetic and environmental factors (for example, toxins) likely contribute to CKD susceptibility. The identification of genes affecting kidney function variation may give insights into the genetic susceptibility to CKD.

Programmed cell death or apoptosis of resident kidney cells in response to injury has been described in experimental animal models and in humans (Shankland 2006). A reduced podocyte count correlates with progression to glomerulosclerosis (Kriz, Gretz et al. 1998; Steffes, Schmidt et al. 2001; White and Bilous 2004). Reactive oxygen species and DNA damage induce apoptosis, and lead 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 renal function.

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 kidney function (using estimating glomerular filtration rate equations - eGFR) in the Family Blood

Pressure Program (FBPP). One *CHEK2* SNP was associated with higher eGFR among white participants of the Hypertension Genetic Epidemiology study (HyperGEN) and 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 kidney function variation in populations. 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 1 examination of 10,933 White and 3,783 African American individuals with available genotyped data for the *CHEK2* polymorphisms. Individuals without genotyping or without available serum creatinine at Visit 1 will be excluded.

Study design: cross-sectional race-stratified analyses of the association of *CHEK2* polymorphisms with eGFR variation at ARIC Visit 1.

Exposure: Five *CHEK2* SNPs. See genotyping details below.

Outcome: eGFR will be calculated using the MDRD equation: eGFR = 186.3 x (serum creatinine)$^{-1.154}$ x (age)$^{-0.203}$ x (0.742 if female) x (1.212 if African American)(Levey, Bosch et al. 1999). GFR measures will be truncated at 200 ml/min per 1.73 m^2.

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 *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 for normal distribution. 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 and smoking exposure. 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 the 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.