ARIC Manuscript Proposal #1659

1.a. Full Title: Association of ACEI therapy and incidence of chronic kidney disease, by MYH9 status in hypertensive African Americans from the ARIC Study.

b. Abbreviated Title (Length 26 characters): ACEI, MYH9 and CKD in ARIC

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

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3. Timeline: Data analysis to start immediately. First draft of manuscript expected by July 2010.
4. **Rationale:** Renin-angiotensin system (RAS) blocking therapy, consisting of angiotensin-converting enzyme inhibitors (ACEI) and angiotensin-receptor blockers (ARBs), are routinely prescribed and recommended by evidence-based treatment guidelines as first-line anti-hypertensive and anti-proteinuric therapy for diabetic and non-diabetic chronic kidney disease\(^1\). Compared to other anti-hypertensive medications, ACEI/ARBs have been shown to achieve reduction in progression of nephropathy that results from uncontrolled hypertension and proteinuria\(^1\).

ACEIs control nephropathy through a well-studied indirect pathway and a less understood direct pathway. The indirect effect is mediated via reduction in intraglomerular blood pressure that reduces proteinuria and lowers nephropathy progression rates. Emerging biological evidence of a direct effect comes from recent studies that show existence of a local RAS within podocytes which is separate from the RAS in the juxtaglomerular apparatus involved in the pathophysiology of systemic hypertension\(^2-3\). High blood glucose levels and/or high blood pressure induce Ang II production in the local RAS which can directly damage podocyte structure and reduce the total number of functioning podocytes\(^2-3\). Podocyte loss is the first step towards proteinuria and glomerulosclerosis\(^4\). However, experimental studies have shown that ACEI can control the Ang II production in the local RAS and thus directly rescue podocytes and CKD progression via non-hemodynamic pathways\(^2-3\).

Despite strong biological evidence favoring RAS blocking therapy in control of hypertension-associated nephropathy, compared to other races, African Americans (AA), suffer from faster deterioration of renal function resulting in end stage renal disease while on ACEI/ARB regimens even while maintaining effective blood pressure control\(^5-7\). A potential source of this racial disparity was recently identified by four independent investigations of non-diabetic end stage renal disease (ESRD)\(^8-11\). These studies identified genetic variants in the MYH9 gene on Chr 22q to be associated with non-diabetic ESRD in hypertensive AA [OR 2.07-2.32 (95% C.I. 1.56 – 3.43; \(p = 3.14*10^{-5} – 4.52*10^{-12}\))], and with subclinical nephropathy in hypertensive AA [urine albumin:creatinine ratio; \(p=0.013\)]\(^8-11\). The putative at-risk MYH9 SNPs were found to be more prevalent in AA (40-60%) compared to European Americans (EA) (4%) and this large difference in allele frequencies could account for the unexplained excess risk of CKD in AA\(^8-9,12\). Additionally, these studies also found that the MYH9 gene is selectively expressed in the podocytes and affects the integrity of the structural cytoskeleton\(^8-9\).

We hypothesize that the potential mechanism leading to the progression of nephropathy in MYH9 at-risk genotype carriers involves an inherently unstable podocyte structure, which continues to deteriorate despite the effects of RAS blockade from ACEI use and is not expected to be affected by other anti-hypertensive medications. We hypothesize that therefore given MYH9 at-risk genotype, hypertensive ACEI users will show evidence of progressive CKD, compared to those who do not have the MYH9 at-risk genotype and this effect will be similar to progressive nephropathy observed with other common anti-hypertensive medications.
In contrast to our hypothesis, a recent case report from Italy of 4 European patients with established genetically-confirmed MYH9 nephropathy affecting all 4 patients, showed that RAS blockers were successful in effecting and sustaining reduced proteinuria through 1-5 yrs of follow-up among hypertensive as well as normotensive patients, independent of their anti-hypertensive action. However, these cases appear to be a Mendelian disorder and the putative alleles may be different from the alleles involved in our complex disease model.

We aim to explore the association between ACEI medication and nephropathy by MYH9 risk genotype among AA with hypertension from the Atherosclerosis Risk in Communities (ARIC) Study. The ARIC Study is a population based observational study with meticulously collected data on anti-hypertensive medication including ACEI use, blood pressure, serum creatinine, serum glucose and incident CKD over 9 years of follow up, and blood samples for genetic testing. Our study sample will consist of 4266 AA and will focus on hypertensive AA subjects - 2402 AA (56.3%) at baseline visit 1 and 125 newly diagnosed hypertensive AAs at first follow up visit 2.

There are other studies, notably the African American Study of Kidney Disease (AASK) Study, with an exclusive AA study population that might be better suited to test this interaction analysis. The AASK Study was a combined clinical trial-cohort study of ACEI versus other anti-hypertensives, in which 759 of 1094 hypertensive AA were randomized to ACEI arm and followed for CKD outcomes while on ACEI regimen. However, genotype data is not currently available in the AASK Study, and hence we chose to examine the hypothesis in the ARIC Study.

The observational design of ARIC has certain disadvantages of study populations compared to the ACEI-selected AA population in the AASK clinical trial. However, the greater sample size afforded by ARIC Study is an advantage for us to conduct our analysis with the ARIC study population. We hope that this analysis will enable better understanding of magnitude of the impact of MYH9 genotype on kidney disease and inspire replication of significant findings, if any, in larger studies such as the AASK Study after genotyping is completed, followed by a potential meta-analysis.

5. Main Hypothesis/Study Questions:
The primary goal of this proposal is to assess the association of ACEI use on incident CKD by MYH9 genotype in hypertensive African Americans.

Main interaction hypothesis:
We expect that ACEI use will be associated with lower incidence of CKD among hypertensive AA without the MYH9 genotype [MYH9(-)] and that ACEI use will not reduce incidence of CKD among hypertensive individuals with the MYH9 risk genotype [MYH9(+)].
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 study our research question in the ARIC Study population as the prospective study design with follow up visits allows us to study the change in blood pressure control and renal function over time, while on anti-hypertensive medication.

We will conduct separate analyses at visit 1 (v1) & visit 2 (v2). First, a baseline analysis at consisting only of variables assessed at visit 1 will be done and will only include those who were hypertensive at visit 1 and qualified for other eligibility criteria. The baseline analysis will consist of individuals followed for nine years until visit 4 (v4).

We expect that given only ~8% ACEI use at v1, we acknowledge that our analysis will be limited by small sample size. As ACEI use increased to ~16% at v2, we will therefore repeat our analysis using v2 as baseline analysis that only includes hypertensive individuals at v2 and follows them for six years until v4 for incident CKD. However, we realize that this v2 baseline analysis will be limited by lesser follow time for incident CKD and hence we may have fewer outcomes in comparison.

**Primary exposure:** As our study is intended to be a gene-environment interaction study, our exposures are defined as:

**i) Primary environmental exposure:** Anti-hypertensive medication, especially ACEI therapy. Medication at baseline visit might have changed over the course of follow up between visits, hence medication status will be treated as a time-varying explanatory variable. Also, as no data is available on ARB use was collected in ARIC, we will study the impact of RAS-blocking therapy from the effect of ACEI. Anti-hypertensive medication status will be treated as a categorical variable with three groups namely – ACEI therapy / Other anti-hypertensive medications / No anti-hypertensive medication. This data will be obtained by self-reported medication used to treat high BP and transcription and coding of all medication names. In case of combination medications, any combination containing ACEI will be considered in the ACEI category of antihypertensive medications.

**ii) Primary genetic exposure:** MYH9 SNP rs4821480.

A causal SNP in the MYH9 gene has not yet been identified, possibly due to high degree of linkage disequilibrium (LD). Most individual SNPs with significant association lie in the E1 haplotype block (GCCT for rs4821480, rs2032487, rs4821481, rs3752462 respectively) and, the main effect appears to come from the E1 haplotype in both AA and EA races.

Within E1 haplotype, rs4821480 is in equal near perfect correlation with the other SNPs, and is a good choice for tagging SNP to represent the E1 risk haplotype in further
Moreover, rs4821480 was one of three MYH9 SNPs and the only MYH9 E1 haplotype SNP that was found to be independently associated with non-diabetic ESRD after adjusting for age, gender and admixture. Hence, as genotyping of all ARIC subjects has been completed, we will employ rs4821480 as the tagging SNP for the MYH9 genotypes. MYH9(+) status will be assigned to those homozygous for the ‘G’ risk allele of rs4821480 as the recessive model has been shown to explain the at-risk genotype in previous studies. All others will be assigned MYH9(-) status.

**Primary outcomes:**

The primary outcome will be incidence of CKD which will be defined as individuals with baseline eGFR $\geq 60$ ml/min/1.73 m$^2$ and either eGFR $< 60$ ml/min/1.73 m$^2$ at visit 2 or visit 4. All individuals will be censored at the visit at which they either became a case or were lost-to-follow-up or administratively censored at visit 4.

eGFR$_{scr}$ will be calculated from S. Creatinine standardized 4-variable MDRD study equation with indirectly calibration of S. Creatinine to Cleveland Clinic measurement that has it has gained wide acceptance and has been shown to most laboratories estimate GFR from S. Cr calculated as follows:

$$eGFR = 175 \times (\text{standardized serum creatinine})^{-1.154} \times (\text{age})^{-0.203} \times 1.212[\text{if black}] \times 0.742[\text{if female}]$$

in which GFR is expressed as mL/min per 1.73 m$^2$ of body surface area and serum creatinine is standardized to IDMS reference values and is expressed in mg/dL. A recent study based in the ARIC study population showed that eGFR calculated by the CKD-EPI equation more accurately classified patients with respect to their true outcome using the same variables in the MDRD equation, with more frequent reclassification of younger, female and white participants. Our study solely focuses on African Americans, hence we choose to calculate eGFR using the 4 variable re-expressed MDRD study equation with standardized serum creatinine. We will perform a sensitivity analysis using eGFR calculated with the CKD-EPI equation.

**Covariates of interest:**

**Hypertension** will be defined as a dichotomous outcome and will be defined by self reported hypertension, self report of anti-hypertensive medications, medical transcription and code based identification of anti-hypertensives by study personnel, or blood pressure $> 140/90$ mm Hg at the time of visit-based physical examinations.

**Blood pressure** level will be considered as a distinct variable to assess effective blood pressure control by anti-hypertensive medications and to examine the association of
blood pressure control with eGFR_{scr}. Blood pressure will be treated as a continuous variable. Effective and adequate blood pressure control will be defined by blood pressure <130/80 mm Hg in accordance with K/DOQI guidelines in 2007\(^1\).

7. Data analysis:

1) Exclusion Criteria:
   i) Based on our exclusion criteria, we will remove all individuals who are of non-African American race, were from the Minnesota or Washington County study centers, have prevalent CKD (eGFR<60 ml/min/1.73m\(^2\) from the MDRD equation) at baseline visit v1, were lost to follow-up after visit 2, have missing genotype information or did not consent to genetic testing.
   ii) For the analysis with v1 as baseline, we will only include AA individuals who were hypertensive at visit 1.
   iii) For the analysis with v2 as baseline we will only include AA individuals who were hypertensive at visit 2.

2) Main analysis plan:
   All analysis will be performed on Stata IC version 11.

3) Steps in analysis:

   1) The steps will be repeated for both analyses - baseline visit 1 and baseline visit 2.
   2) We will estimate incidence rate of CKD by (i) ACEI use and (ii) by MYH9 at-risk genotype status, separately.
   3) We will perform exploratory data analyses to assess differences in baseline characteristics by incident CKD and by ACEI use.

   All further analyses will be stratified by MYH9 status.
   4) For the analysis using visit 1 as baseline, we will use Cox proportional hazards regression models to study the association of ACEI and risk of CKD as defined by a GFR<60 ml/min/1.73m\(^2\).
   5) We will then repeat the analyses adjusted for age, sex, study center, and diabetes.
   6) Another level of adjustment for level of blood pressure control (<130//80 mmHg) will also be done, to account for the effect of different anti-hypertensive treatments on blood pressure control that is fundamental to prevention and control of hypertension-associated nephropathy.
   7) We will repeat the analysis in the form of a time-dependent analysis to allow for medication category to change. The time-dependent analysis will not include v3 as S.Cr was not measured at visit 3 in ARIC, hence eGFR estimates cannot be calculated\(^14\).
8) For the analysis using visit 2 as baseline, we will use a logistic regression to determine the association between ACEI use at v2 and incident CKD status at v4, by MYH9 genotype.

9) Adjusted baseline visit 2 analysis will be done in the same way as detailed for baseline visit 1 analysis.

10) We will repeat our primary analyses using CKD-Epi equation for eGFR estimates in the form of a sensitivity analysis in both baseline visit 1 and baseline visit 2 analyses\(^\text{21}\). In these analyses, individuals with prevalent CKD at baseline will be excluded based on the CKD-Epi equation.

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

b. If Yes, is the author aware that the file ICTDER04 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  __X__ No

(This file ICTDER04 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 ICTDER04 must be used to exclude those with value RES_DNA = “No use/storage DNA”?  
__X__ Yes  ___ No

8.c. If yes, is the author aware that the participants with RES_DNA = ‘not for profit’ restriction must be excluded if the data are used by a for profit group?  
__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)?

None

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data?  

_x___ Yes  _____ No

11.b. If yes, is the proposal

___  A. primarily the result of an ancillary study (list number*  
2006.16 Longitudinal Study of Predictors and Consequences of Chronic Kidney Disease

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

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*ancillary studies are listed by number at http://www.csec.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.

13. References:


17) Hsu CC, Coresh J, Kao WH. Apolipoprotein E and Progression of Chronic Kidney Disease (Reply to Editor). *JAMA* 2006; 295(1) 35.


