Population Architecture using Genomics and Epidemiology (PAGE)  
Ver. 06/14/10

PAGE Manuscript Proposal Template  
Submit proposals by email to the PAGE Coordinating Center at Purn@biology.rutgers.edu

All sections must be completed; incomplete applications will be returned.  
Do not exceed 3 pages in length (not including references).

PAGE Ms. Number: __84__  Submission Date: _10/05/2015____   [Approval Date: _10/29/2015_]  

Title of Proposed Ms.: _The influence of sodium and potassium in the context of genetic variants associated with blood pressure in the Population Architecture using Genomics and Epidemiology (PAGE) study_

I. INVESTIGATOR INFORMATION:

Name of Lead Author:  Cynthia Bell  
Junior Investigator?  Y  
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Telephone Number: 713 500 7353

Name of Corresponding Author (if different):

Names, affiliations and email address of PAGE Investigators proposed as co-authors:

<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation in PAGE</th>
<th>Email</th>
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<tbody>
<tr>
<td>Eric Boerwinkle</td>
<td>CAliCo</td>
<td><a href="mailto:Eric.Boerwinkle@uth.tmc.edu">Eric.Boerwinkle@uth.tmc.edu</a></td>
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<td>Myriam Fornage</td>
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Partner studies in PAGE not collaborating in this ms. proposal:

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Names, affiliations, email address of non-PAGE investigators proposed as co-authors:
Joshua Samuels, MD, MPH, Associate Professor at UT-Houston Medical School, Division of Pediatric Nephrology, Joshua.A.Samuels@uth.tmc.edu

II. SCIENTIFIC RATIONALE (Please be specific and concise)

Worldwide more than 1 billion adults have hypertension, making it the most common modifiable risk factor for cardiovascular disease and death, accounting for more than 9 million deaths annually. Hypertension is etiologically and genetically heterogeneous. The pathophysiologic nature of hypertension is multifactorial, but primarily driven by renal regulation of urinary excretion of water and soluble ions (specifically sodium and potassium) to maintain a constant extracellular environment for proper cell function. Several of the genetic markers linked to blood pressure (BP) by GWAS in European ancestry[1-3] and African ancestry[4, 5] are functionally related to the maintenance of vascular tone and renal regulation of soluble ions[6] but causal pathways have yet to be determined for the majority of BP-related SNPs.

Treatments to reduce BP currently consist of a low-sodium diet and/or trial and error of antivolume or anti-renin medications. Recently the evidence of benefit from a low sodium diet has weakened. In response, The Institute of Medicine (IOM) raised the recommended sodium intake to <2.3 grams/day for most people but kept intake recommendations at <1.5 grams/day for African-Americans and other higher risk subgroups[7]. These new racially stratified recommendations are informed by randomized control trials showing “salt sensitivity”, a precursor of hypertension, to be more frequent in African ancestry. However, the IOM recommendations failed to discuss that this salt-sensitive hypertension is only present when dietary potassium is deficient [8-10]. Further results from a multi-racial, international cohort recently confirmed the association of high sodium and low potassium intake with higher BP at baseline but found both low and high sodium intake increased the risk of mortality over follow up [11, 12]. These recent findings indicate a paradigm shift in the field of BP that highlight the uncertainty of low sodium diet recommendations, the importance of potassium as an often ignored co-factor in BP regulation, and the racial heterogeneity of environment-influenced BP traits.

We propose to test if BP-related SNPs are also associated with sodium and potassium intake or excretion. In addition, we will test if the effects of sodium and potassium intake on BP are heterogeneous across race/ethnic groups. Lastly, we will test if sodium and potassium intake modify the existing BP-gene relationship. We have selected SNPs identified in both European-[1-3] and African-American[4, 5] cohorts to associate with studied traits and will stratify all analyses by race/ethnic group, testing all SNPs in each ancestry group. Findings from this study will elucidate the
mediation pathway of BP-related SNPs across race/ethnic groups to define a more specific hypertension phenotype and guide effective prevention of cardiovascular disease.

III. OBJECTIVES AND PLAN (Please be specific and concise)

a. Study Questions/Hypotheses.

Specific Aim 1: Test extensions of phenotype-gene associations between GWAS-established BP-related SNPs and sodium and potassium in each race/ethnic group.
   i. Determine if BP-related SNPs are related to sodium and potassium intake from dietary questionnaires.
   ii. Confirm findings of association between BP-related SNPs and sodium and potassium excretion from a subsample of PAGE studies with 24-hour urine collections.

Specific Aim 2: Estimate the heterogeneity of the effects of sodium and potassium intake on BP across race/ethnic groups.
   i. Test the extent of effect modification of potassium as a co-factor in the relationship between sodium and BP outcomes; specifically if high potassium intake can counteract the effects of high sodium intake on BP similarly in all race/ethnic groups.
   ii. Define potential sodium/potassium ratio cutoffs based on tertiles; determine if effect of sodium/potassium ratio is consistent across low, average, and high sodium intake in all race/ethnic groups.

Specific Aim 3: Test the gene-environment interaction of BP-related SNPs within sodium and potassium intake phenotypes on BP outcomes in each race/ethnic group of PAGE studies.
   i. Test phenotype effect modification of sodium and potassium intake on the association between established SNPs and BP outcomes in each race/ethnic group.

b. Study populations, study design for each

We plan to use data from PAGE studies that included blood pressure measurement and food frequency questionnaires/24-hour dietary recall used to determine sodium and potassium intake. We anticipate this phenotypic data to be available in the following studies: WHI, CHS, SOL, CARDIA, and ARIC. Validation of results using sodium and potassium excretion from 24-hour urines will be completed with available data from CARDIA and ARIC.

c. Variant/SNPs (Specify)

GWAS-established BP-related SNPs[2-5, 13] as previously genotyped by MetaboChip in PAGE studies. MEGA Array data may be added if timeline is appropriate.

d. Phenotype(s) (Specify)

Systolic Blood Pressure (mmHg)
Diastolic Blood Pressure (mmHg)
Dietary sodium (g/day from FFQ/24-hr recall)
Dietary potassium (g/day from FFQ/24-hr recall)
Urinary sodium (g/day from spot/24-hr)
Urinary potassium (g/day from spot/24-hr)
Based on PAGE I genotyped individuals available from study via dbGaP, we anticipate the following sample size from ARIC, CARDIA, and WHI available for the BP outcomes, sodium/potassium intake, and covariates (age, sex, and BMI). Additional data should be obtainable from SOL and CHS but sample size estimates were not available. Urinary sodium and potassium excretion data are available from a subset of CARDIA and ARIC subjects.

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e. **Covariates in all models**

Primary Model:
- Age
- Sex
- BMI

Secondary Model:
- Dietary protein (from FFQ/24-hr recall)
- Dietary calories (from FFQ/24-hr recall)
- Serum creatinine/eGFR
- Anti-hypertensive Medication Use/Class

f. **Main statistical analysis methods**

All analyses will be run with individual level data within each study then combined by inverse variance-weighted meta-analysis stratified by race/ethnic group. Baseline BP outcomes will be continuous systolic BP, continuous diastolic BP, and prevalent hypertension (SBP>140 or DBP>90, on anti-hypertensive medication, or ICD 9/10 code for hypertension). For patients on antihypertensive treatment 15 mmHg will be added to SBP and 10 mmHg will be added to DBP values. Upon examination of distributions of continuous variables SBP, DBP, sodium intake, and potassium intake, any extreme outliers greater >6SD will be set to the value of 6SD from the mean. Low, average, and high sodium intake will be categorized by <1.5, 1.5-3, and >3 g/day based on IOM recommendations and NHANES intake distributions [7, 14]. Based on the same sources, potassium intake will be categorized by <2, 2-3, and >3 g/day.

Linear regression models will be used to determine associations to continuous BP, sodium, and potassium outcomes after primary adjustment for age, sex, BMI. Similarly, logistic regression models will be used for prevalent hypertension. Secondary models will be fit with additional adjustment for proportion protein of total calorie intake, eGFR, and anti-hypertensive medication. We will assume an additive genetic model to assess SNP-phenotype associations. Tests of interactions by sodium and potassium categories will be made to determine effect modification.
We will adjust all significance levels to maintain experiment-wide Type I Error from multiple testing below 0.05. For Aim 1, we will test the association of 36 BP-related SNPs to 2 outcomes: sodium and potassium intake. For Aim 2, we will test the effect of 5 factors (sodium, potassium, sodium-potassium interaction, sodium/potassium ratio, and sodium-sodium/potassium ratio interaction) on 3 BP outcomes. For Aim 3, we will test the effect of 36 BP-related SNP variants and their interaction with 2 factors (sodium and potassium) on 3 BP outcomes. All analyses will be stratified within 3 ancestry groups: European, African, and Hispanics. For Aim 2, meta-analysis tests of heterogeneity ($I^2$) or random effects will be used to determine consistency of results across race/ethnic groups. In total, we will assess 682 tests using a Bonferroni corrected significance level of $<7.3 \times 10^{-5}$.

To ensure we have the power to detect a gene-environment additive effect of at 3-5 mmHg, we completed a power analysis for Aim 3 based on an additive genetic model, SBP SD 20, allele frequency range 0.2-0.4, power 0.8, and high sodium or low potassium prevalence of 0.33 using Quanto [15]. Assuming an experiment-wide Type I Error rate of $7.3 \times 10^{-5}$, sample size requirements range from n=3,188 with 0.5 allele frequency and 5 mmHg gene-environment effect, n=9,462 with 0.4 allele frequency and 3 mmHg gene-environment effect, or n=14,323 under the most constrained condition with 0.2 allele frequency and 3 mmHg gene-environment effect.

All analyses will be stratified and reported separately for race/ethnic group based on self-report or genetic ancestry estimated from an ancestry informative marker and PCs as recommended by the PAGE analysis committee and study centers. All models in the study can be extended to mixed model formation to account for admixture components of the population if necessary. For models that include data from SOL, analyses will be weighted to account for probability sampling of participants.

g. Ancestry information used? No __ Yes ___X__ How is it used in the analyses? Global genetic ancestry will be used to determine stratification of analysis by race/ethnic groups.

h. Anticipated date of draft manuscript to P&P:

i. What manuscript proposals listed on www.pagestudy.org/index.php/manuscripts/ are most related to the work proposed here? Approved PAGE ms. numbers: 7A, 7C, 31

   a. If any: Have the lead authors of these proposals been contacted for comments and/or collaboration? Yes ___X__ No __

IV. SOURCE OF DATA TO BE USED (Provide rationale for any data whose relevance to this manuscript is not obvious): Check all that apply:

Aggregate/summary data to be generated by investigators of the study(ies) mentioned:

[ ] EAGLE; [X] CALiCO; [X] MEC; [X] WHI; [ ] CC; [ ] Other:____________________

If CALiCO, specify [X] ARIC; [X] CARDIA; [X] CHS; [ ] SHS-Fam; [ ] SHS-Cohort; [X] SOL

I, CB, affirm that this proposal has been reviewed and approved by all listed investigators.
References