

## ARIC Manuscript Proposal #1868

PC Reviewed: 11/8/11  
SC Reviewed: \_\_\_\_\_

Status: A  
Status: \_\_\_\_

Priority: 2  
Priority: \_\_\_\_

### 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](mailto: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: \_\_\_\_ Submission Date : \_\_\_\_ [Approval Date: \_\_\_\_]

**Title of Proposed Ms.:** Discovery and fine mapping of blood pressure loci to African American and Hispanic individuals using the MetaboChip array: The PAGE Study

#### I. INVESTIGATOR INFORMATION:

**Name of Lead Author:** Nora Franceschini  
Email Address: [noraf@unc.edu](mailto:noraf@unc.edu)  
Telephone Number: 919/966-1305

| **Junior Investigator?** Yes

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

N, N	Affiliation in PAGE	Email
Eric Boerwinkle	CALiCo – ARIC	<a href="mailto:Eric.Boerwinkle@uth.tmc.edu">Eric.Boerwinkle@uth.tmc.edu</a>
Kari North	CALiCo – SOL	<a href="mailto:Kari_north@unc.edu">Kari_north@unc.edu</a>
Aravinda Chakravarti	CALiCo – ARIC	<a href="mailto:aravindachakravarti@gmail.com">aravindachakravarti@gmail.com</a>
Ran Tao	CALiCo	<a href="mailto:taor@live.unc.edu">taor@live.unc.edu</a>
DanYu Lin	CALiCo	<a href="mailto:lin@bios.unc.edu">lin@bios.unc.edu</a>
Lucia Hindorff	NHGRI	<a href="mailto:hindorffl@mail.nih.gov">hindorffl@mail.nih.gov</a>
Steve Buyske	CC	<a href="mailto:buyske@stat.rutgers.edu">buyske@stat.rutgers.edu</a>
Jose Luis Ambite	CC	<a href="mailto:ambite@ISI.EDU">ambite@ISI.EDU</a>
Sylvia Smoller	CALiCo - SOL	<a href="mailto:Sylvia.Smoller@einstein.yu.edu">Sylvia.Smoller@einstein.yu.edu</a>
Lifang Hou	CALiCo - SOL	<a href="mailto:l-hou@northwestern.edu">l-hou@northwestern.edu</a>
Shelley A Cole	CALiCo – Strong Heart	<a href="mailto:scole@txbiomedgenetics.org">scole@txbiomedgenetics.org</a>
Dana Crawford	EAGLE	<a href="mailto:dana.crawford@chgr.mc.vanderbilt.edu">dana.crawford@chgr.mc.vanderbilt.edu</a>
TBA	MEC	
Megan Fesinmeyer	WHI	<a href="mailto:mfesinme@WHI.org">mfesinme@WHI.org</a>
Chunyuan Wu	WHI	<a href="mailto:mfesinme@WHI.org">mfesinme@WHI.org</a>
Garnet Anderson	WHI	<a href="mailto:garnet@whi.org">garnet@whi.org</a>
Sue Mann	WHI	<a href="mailto:smann@whi.org">smann@whi.org</a>
Lisa Martin	WHI	<a href="mailto:lwmartin@mfa.gwu.edu">lwmartin@mfa.gwu.edu</a>
Chris Carlson	WHI	<a href="mailto:ccarlson@fhcrc.org">ccarlson@fhcrc.org</a>
Elizabeth Bluhm	WHI	<a href="mailto:elizabeth.c.bluhm@medstar.net">elizabeth.c.bluhm@medstar.net</a>

Fornage, Myriam	CALICo - CARDIA	Myriam.Fornage@uth.tmc.edu
-----------------	-----------------	----------------------------

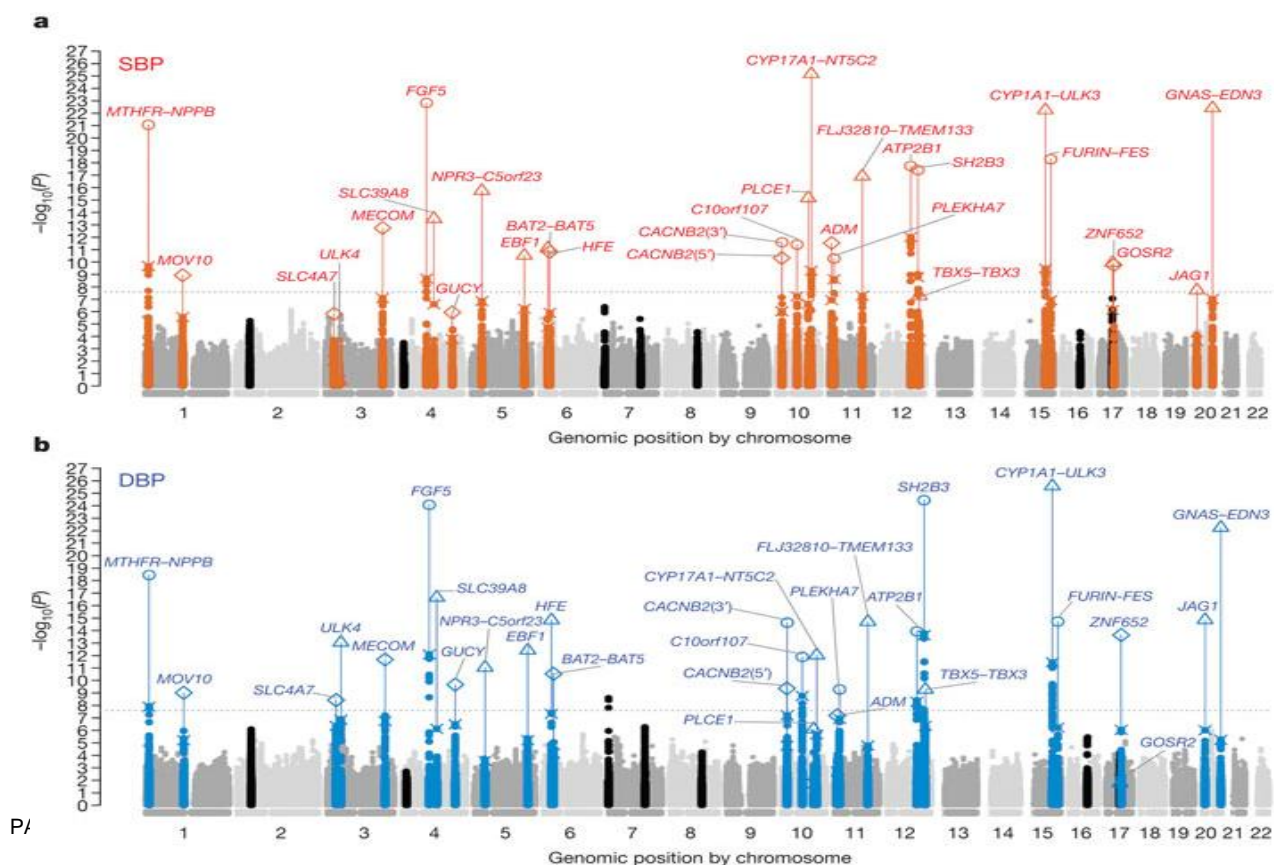
Study	Contacted? Y/N	Declined? / Other?
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
31		
32		
33		
34		
35		
36		
37		
38		
39		
40		
41		
42		
43		
44		
45		
46		
47		
48		
49		
50		
51		
52		
53		
54		
55		
56		
57		
58		
59		
60		
61		
62		
63		
64		
65		
66		
67		
68		
69		
70		
71		
72		
73		
74		
75		
76		
77		
78		
79		
80		
81		
82		
83		
84		
85		
86		
87		
88		
89		
90		
91		
92		
93		
94		
95		
96		
97		
98		
99		
100		

Names, affiliations, email address of non-PAGE investigators proposed as co-authors:  
None

## II. SCIENTIFIC RATIONALE (Please be specific and concise)

Hypertension is a leading cause of cardiovascular disease (CVD) mortality and disability worldwide, contributing to an estimated global burden of 54% of stroke and 47% of coronary heart disease in 2001 (Lewington, Clarke et al. 2002; Lawes, Vander Hoorn et al. 2008). African Americans have earlier onset and higher age-adjusted prevalence of hypertension than European Americans, Asians, and Hispanics (Burt, Whelton et al. 1995). In the Multi-ethnic Study of Atherosclerosis, Hispanic participants aged 45 years or older had a higher incidence of hypertension compared with whites (Carson, Howard et al. 2011).

Blood pressure (BP) is a complex trait, and genetic factors account for 30 to 40% of the blood pressure variation in a population (Samani 2003). Recent genome wide association studies (GWAS) have highlighted the contribution of common variants (minor allele frequency, MAF,  $\geq 5\%$ ) to blood pressure (BP) and hypertension risk in various



**Figure 1.** GWAS BP findings in individuals of European ancestry

populations, including individuals of European ancestry, East Asians and African Americans (Levy, Ehret et al. 2009; Newton-Cheh, Johnson et al. 2009; Ehret, Munroe et al. 2011; Fox, Young et al. 2011; Kato, Takeuchi et al. 2011), reviewed in (Franceschini, Reiner et al. 2011). So far, 28 loci for BP traits have been identified in GWAS of individuals of European descent (N = 200,000, International Consortium of Blood Pressure, ICBP)(Ehret, Munroe et al. 2011) (**Figure 1**), but combined joint effect of these common variants in these loci explained only 0.9% of the population variation in BP. Additional loci have been recently described in East Asians (*ST7L-CAPZA1*, *FIGN-GRB14*, *ENPEP* and *ALDH2*) and *ALDH2* showed ethnic specificity (Kato, Takeuchi et al. 2011). Most of these loci have not yet replicated in individuals of African ancestry or in Hispanics. Common variants usually have small effect sizes and explain a small proportion of the phenotypic variation. It was estimated in ICBP that there are 116 independent BP variants of small effect that collectively explain 2.2% of the phenotypic variance. Recent studies have shown that low frequency (MAF 0.5-5%) and rare variants (MAF 0.05–0.5%)(Altshuler, Gibbs et al.) in GWAS loci explain some of the missing heritability of complex traits (Sanna, Li et al. 2011). For example, a recent investigation of seven LDL-cholesterol GWAS loci in Sardinians identified new variants that in combination accounted for an increased in the LDL-cholesterol variance from 3.1% to 6.5 (Sanna, Li et al. 2011).

Therefore, we propose to interrogate variants from a range of allele frequencies (0.5 to 5% or more) in known and novel loci for BP in loci represented in the MetaboChip array. We will extend our analyses from the pilot study in African Americans to additional genotyped data in both African Americans and Hispanic individuals. Using data from minorities with diverse genomic background, we expect to identify new variants in known loci and novel loci associated with BP traits.

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

#### **a. Study Questions/Hypotheses.**

1. For loci represented in the MetaboChip array, are there novel associations with BP traits in African American and Hispanic individuals (discovery new variants from low frequency to common variants, new loci)
2. For known loci associated with BP and represented in the MetaboChip, are there one or more independent signals in African Americans and Hispanics (generalization and fine mapping).
3. For variants identified in aims 1 and 2, do the effect estimates vary by environmental factors including smoking (current, past or never), alcohol intake (current, past, never), obesity (BMI  $\geq 30$  kg/m<sup>2</sup> versus  $< 30$ ), type 2 diabetes.

#### **b. Study populations, study design for each**

All PAGE study populations with MetaboChip data and measured blood pressure.

#### **c. Variant/SNPs (Specify)**

We will evaluate all the variants in the MetaboChip that passed quality control from a range of allele frequencies, including rare alleles with MAF of 0.5-5%, for our discovery effort and fine mapping of known loci.

#### **d. Phenotype(s) (Specify)**

The phenotypes to be used for analyses are:

1. Quantitative traits of systolic (S)BP, diastolic (D)BP, mean arterial pressure and pulse pressure.
2. Secondary phenotypes are mean arterial pressure and hypertension, defined by the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7)(Chobanian, Bakris et al. 2003) of SBP>140, DBP>90 or use of anti-hypertensive medications.

#### **e. Covariates (Specify) and interaction covariates**

Continuous age, sex, study center or region (if needed), continuous body mass index and ancestry principal components. We will also adjust for local ancestry estimates if available.

Interaction variables: smoking (current, past or never), alcohol intake (current, past, never), obesity (BMI $\geq$ 30 kg/m<sup>2</sup> versus < 30), type 2 diabetes.

#### **f. Main statistical analysis methods**

Analyses will be stratified by self-reported race and within each PAGE study. We will use linear regression models for quantitative traits and logistic regression models for the qualitative trait of hypertension. We will use additive genetic models, sex-stratified (due to large sample of women in WHI) and adjusted for age, study center or region (if needed), body mass index and ancestry principal components. Study-specific association results will be combined across sex and ethnic-specific samples using an inverse variance meta-analysis approach implemented in METAL (Willer, Li et al. 2010). Because individuals were recruited using a probability sampling in the SOL study, all analyses of this study will use weights to account for identification and selection of community areas and random selection of households within those areas (Lavange, Kalsbeek et al. 2010).

For aim 1. Discovery. We will perform single variant test for all variants with minor allele frequency (MAF) >1% and use Bonferroni correction to declare a significant p-value threshold based on multiple testing.

For aim 2. Generalization and fine mapping. We will perform comprehensive analyses of all known loci for BP represented in the MetaboChip. We will use single variant test approach for variants with MAF>1% and adjust for the number of independent tests using a Monte Carlo approach that accounts for linkage disequilibrium (LD) between variants across the tested loci (Lin 2005). We will perform conditional analyses using the most significant variant as covariate if there is suggestive evidence for secondary independent signals in these regions. For low frequency variants (0.1%<MAF<5%), we will explore novel methods for collapsing variants across regions including a recent method developed by Lin et al (Lin and Tang 2011). For novel variants identified in these known loci, we will use bioinformatics tools for functional characterization of the variants.

For aim 3. we will select common variants (MAF> 1%) identified in aims 1 and 2, for gene-by-environmental interaction analyses. We will test a multiplicative interaction using interaction terms. In addition, we will perform stratified analyses and test heterogeneity across strata. P-values will be adjusted using Bonferroni correction.

In our previous analyses of BP traits in WHI-SHARe Hispanics, we identified some replication of known loci for BP. Therefore, we will use the similar approaches to study variants in Hispanic individuals but will incorporate new strategies if needed as this population has been understudied. We have established collaborations with the FBPP (HyperGEN, GenNet) for combined analyses of African Americans and we are in process of collaboration with MESA for replication of Hispanic findings.

**g. Ancestry information used? No ☐ Yes ☒ How is it used in the analyses?**

We will use estimates of global ancestry (principal components) to adjust for population stratification. Because global ancestry may not fully account for population stratification in African Americans and Hispanics, we will also adjust for local ancestry, as available.

**h. Anticipated date of draft manuscript to P&P: 6-8 months after the data are available. Analyses should be done in 1-2 months and remaining time is for replication of main findings in collaborating studies and for manuscript writing.**

**i. What manuscript proposals listed on [www.pagestudy.org/index.php/manuscripts/](http://www.pagestudy.org/index.php/manuscripts/) are most related to the work proposed here? Approved PAGE ms. numbers: Metabo 05**  
– If any: Have the lead authors of these proposals been contacted for comments and/or collaboration? Yes ☒ 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; ☒ CALiCO; ☐ MEC; ☒ WHI; ☐ CC; ☐ Other: \_\_\_\_\_  
If CALiCo, specify ☒ ARIC; ☒ CARDIA; ☒ CHS; ☐ SHS-Fam; ☐ SHS-Cohort; ☒ SOL

I, Nora Franceschini, MD, affirm that this proposal has been reviewed and approved by all listed investigators.

## **V. REFERENCES**

Altshuler, D. M., R. A. Gibbs, et al. "Integrating common and rare genetic variation in diverse human populations." Nature **467**(7311): 52-58.

- Burt, V. L., P. Whelton, et al. (1995). "Prevalence of hypertension in the US adult population. Results from the Third National Health and Nutrition Examination Survey, 1988-1991." Hypertension **25**(3): 305-313.
- Carson, A. P., G. Howard, et al. (2011). "Ethnic differences in hypertension incidence among middle-aged and older adults: the multi-ethnic study of atherosclerosis." Hypertension **57**(6): 1101-1107.
- Chobanian, A. V., G. L. Bakris, et al. (2003). "The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report." Jama **289**(19): 2560-2572.
- Ehret, G. B., P. B. Munroe, et al. (2011). "Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk." Nature.
- Fox, E. R., J. H. Young, et al. (2011). "Association of genetic variation with systolic and diastolic blood pressure among African Americans: the Candidate Gene Association Resource study." Human molecular genetics **20**(11): 2273-2284.
- Franceschini, N., A. P. Reiner, et al. (2011). "Recent findings in the genetics of blood pressure and hypertension traits." American journal of hypertension **24**(4): 392-400.
- Kato, N., F. Takeuchi, et al. (2011). "Meta-analysis of genome-wide association studies identifies common variants associated with blood pressure variation in east Asians." Nature genetics **43**(6): 531-538.
- Lavange, L. M., W. D. Kalsbeek, et al. (2010). "Sample design and cohort selection in the Hispanic Community Health Study/Study of Latinos." Annals of epidemiology **20**(8): 642-649.
- Lawes, C. M., S. Vander Hoorn, et al. (2008). "Global burden of blood-pressure-related disease, 2001." Lancet **371**(9623): 1513-1518.
- Levy, D., G. B. Ehret, et al. (2009). "Genome-wide association study of blood pressure and hypertension." Nat Genet.
- Lewington, S., R. Clarke, et al. (2002). "Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies." Lancet **360**(9349): 1903-1913.
- Lin, D. Y. (2005). "An efficient Monte Carlo approach to assessing statistical significance in genomic studies." Bioinformatics **21**(6): 781-787.
- Lin, D. Y. and Z. Z. Tang (2011). "A general framework for detecting disease associations with rare variants in sequencing studies." American journal of human genetics **89**(3): 354-367.
- Newton-Cheh, C., T. Johnson, et al. (2009). "Genome-wide association study identifies eight loci associated with blood pressure." Nat Genet.
- Samani, N. J. (2003). "Genome scans for hypertension and blood pressure regulation." Am J Hypertens **16**(2): 167-171.
- Sanna, S., B. Li, et al. (2011). "Fine mapping of five Loci associated with low-density lipoprotein cholesterol detects variants that double the explained heritability." PLoS genetics **7**(7): e1002198.
- Willer, C. J., Y. Li, et al. (2010). "METAL: fast and efficient meta-analysis of genomewide association scans." Bioinformatics **26**(17): 2190-2191.