ARIC Manuscript Proposal #1381

PC Reviewed: 06/10/08	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Genome-wide admixture mapping scans of retinal vascular caliber and retinopathy signs

b. Abbreviated Title (Length 26 characters): Admixture scans of eye traits

2. Writing Group:
Writing group members: Ching-Yu Cheng, Tien Wong, Ron Klein, Barbara Klein, Richey Sharrett, Eric Boerwinkle, David Reich, Josef Coresh, Linda Kao, and others as suggested by Publications Committee

This proposal is based on a previously approved general proposal MP#1309 "Genome-wide admixture mapping analyses of cardiovascular and related metabolic traits", in which we proposed to perform analyses on white blood cell counts, type 2 diabetes, and obesity-related traits. In the present proposal, we will be performing admixture mapping analyses for retinal vascular caliber and retinopathy signs.

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

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3. **Timeline**:

Starting Analyses: June 2008
First Draft: September 2008 for retinal vascular caliber, October for retinopathy signs
Submission for publication: November 2008

4. **Rationale**:

Admixture mapping is an efficient method to scan the genome in recent admixed populations, such as African Americans, for genomic regions which may harbor variants that not only differ in frequency but can also partially explain differences in phenotypes between genetically diverse populations, such as Europeans and Africans.[1-6] In African Americans (~6 generation since first mixture, on average), genomic segments with contiguous European or African ancestry have not had much time to break up by recombination.[4-6] Therefore admixture mapping only requires genotyping genetic markers every couple of million based pairs, which is 100-1,000 times less density required by genome-wide linkage disequilibrium (LD) association studies.[7]

Admixture mapping is particularly appropriate for traits for which there is a large difference in the phenotypic prevalence between two ancestral populations. Such phenotypes have a high a priori chance of being a difference in disease allele frequencies between ancestral populations. Although prevalence difference in the ancestral populations hundreds of years ago cannot be directly estimated, it may be roughly derived from the difference between today's racial/ethnic populations, after taking into account environment factors.

Prior studies in ARIC have shown that African Americans have a 2-fold higher prevalence of hypertensive retinopathy (including retinal hemorrhage, microaneurysm, and cotton wool spots), compared to whites, and this disparity exists even after adjusting for blood pressure and other vascular risk factors.[8] There is also increasing evidence that retinal vascular caliber differ between racial/ethnic groups. In the ARIC study, the ratios of arteriolar-to-venular caliber were significantly lower for African Americans than whites.[9] Moreover, in the Multi-Ethnic Study of Atherosclerosis, the African Americans have larger retinal arteriolar and venular caliber than the whites, despite adjustment for vascular risk factors.[10] The underlying reasons for these racial/ethnic differences are unclear. A recent study showed that retinal pigmentation could be a source of error in the measurement of retinal vascular caliber when measurements were done with a computer-based program.[11] In the ARIC study, the measurements of retinal vascular caliber were done manually by trained graders,[9] so there is less likely to be biases. Therefore, the racial/ethnic differences may possibly reflect variations in genetic factors in part. The greater risk of retinopathy signs and larger retinal vascular caliber in African Americans in comparison to whites makers these two traits ideal problems to study with the admixture mapping approach.

5. Main Hypothesis/Study Questions:

The main hypothesis of the present proposal is that some susceptibility alleles for retinopathy signs and genetic variants influencing retinal vascular calibers are present at higher frequency in West Africans than in Europeans, and that specific region in African Americans' genome contains alleles which are in admixture LD with the susceptibility alleles for retinopathy signs and the variants influencing retinal vascular caliber.

We will test this hypothesis by conducting a genome-wide admixture mapping scans using the genotypes of ~1,350 ancestry informative SNP markers in ARIC African-American participants.

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).

Inclusion criteria
All African-American participants who 1) are from either Jackson or Forsyth County, 2) give consent for use of DNA by non-profit investigators, 3) have sufficient DNA, and 4) had retinal photographs taken in ARIC visit 3.

Outcome of interest: retinal vascular caliber and retinopathy signs

Exposure: estimated percent European ancestry using ancestry informative SNPs
Covariates include, but not limited to, age, sex, study sites, hypertension (including blood pressure, and use of antihypertensive medication), diabetes, fasting glucose levels, adiposity, lipid levels, markers of kidney function (eGFR and albuminuria), smoking status, and socioeconomic status.

Data analysis
For this analysis, cases of retinopathy will be defined as if they have either one of the following: 1) retinal hemorrhages (blot, dot, and flame-shaped hemorrhages), 2) microaneurysms, and 3) cotton wool spots. We all also analyze focal arteriolar narrowing and arteriovenous nicking, which are two other retinal signs relevant to hypertensive retinopathy, particularly in its early stage.[12] Each retinopathy sign will be analyzed separately and for all signs combined. We will perform further analysis stratified by diabetes and hypertension status, because these signs are closely associated with diabetes and hypertension.

For the purpose of the analysis on retinal vascular caliber, study participants will be ranked by the residuals estimated from the linear regression models with retinal vascular caliber as dependant variables, and age, sex, study sites, blood pressure, fasting glucose levels, lipid levels and other relevant vascular risk factors as

independent variables. The top 20% of participants with the highest residuals in retinal venular caliber will be defined as cases and the bottom 20% as controls, and the bottom 20% of participants with the smallest retinal arteriolar caliber will be defined as cases and the top 20% as controls. We will also perform further analysis stratified by diabetes and hypertension status.

For each individual, we will estimate a global ancestry using ANCESTRYMAP, a Bayesian method developed by Patterson et al. In this method, a Hidden Markov Model is used to estimate ancestry states along the genome from the observed genotypes.[5] Then we will use ANCESTRYMAP[5] to search for association of case-control status to genomic regions with an increased European or African ancestry. It will produce two statistics. (1) It calculates a "locus-genome statistic" in cases only by comparing the likelihood of any locus being a disease locus (average ancestry at the locus) versus it being not related to disease (average ancestry across the genome).[5] (2) It produces a "case-control statistic", which compares mean estimates of ancestry in cases versus controls at every locus in the genome, and ensures that any deviation in ancestry from the genome-wide average only seen in cases, but not in controls.[5]

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 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?
_X_ 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

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)?**

    MP#1309 "Genome-wide admixture mapping analyses of cardiovascular and related metabolic traits"

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
_X_ A. primarily the result of an ancillary study (list number* 2004.10)
___ 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.**

References

1. Rife, D.C. (1954). Populations of hybrid origin as source material for the detection of linkage. Am. J. Hum. Genet. *6*, 26-33.

2. Chakraborty, R. and Weiss, K.M. (1988). Admixture as a tool for finding linked genes and detecting that difference from allelic association between loci. Proc. Natl. Acad. Sci. U. S. A *85*, 9119-9123.

3. McKeigue, P.M. (1997). Mapping genes underlying ethnic differences in disease risk by linkage disequilibrium in recently admixed populations. Am J Hum Genet *60*, 188-196.

4. Hoggart, C.J., Shriver, M.D., Kittles, R.A., Clayton, D.G., and McKeigue, P.M. (2004). Design and analysis of admixture mapping studies. Am J Hum Genet *74*, 965-978.

5. Patterson, N., Hattangadi, N., Lane, B., Lohmueller, K.E., Hafler, D.A., Oksenberg, J.R., Hauser, S.L., Smith, M.W., O'Brien, S.J., Altshuler, D. et al (2004). Methods for high-density admixture mapping of disease genes. Am. J. Hum. Genet. *74*, 979-1000.

6. Smith, M.W., Patterson, N., Lautenberger, J.A., Truelove, A.L., McDonald, G.J., Waliszewska, A., Kessing, B.D., Malasky, M.J., Scafe, C., Le, E. et al (2004). A high-density admixture map for disease gene discovery in african americans. Am. J. Hum. Genet. *74*, 1001-1013.

7. Carlson, C.S., Eberle, M.A., Rieder, M.J., Smith, J.D., Kruglyak, L., and Nickerson, D.A. (2003). Additional SNPs and linkage-disequilibrium analyses are necessary for whole-genome association studies in humans. Nat. Genet. *33*, 518-521.

8. Wong, T.Y., Klein, R., Duncan, B.B., Nieto, F.J., Klein, B.E., Couper, D.J., Hubbard, L.D., and Sharrett, A.R. (2003). Racial differences in the prevalence of hypertensive retinopathy. Hypertension *41*, 1086-1091.

9. Hubbard, L.D., Brothers, R.J., King, W.N., Clegg, L.X., Klein, R., Cooper, L.S., Sharrett, A.R., Davis, M.D., and Cai, J. (1999). Methods for evaluation of retinal microvascular abnormalities associated with hypertension/sclerosis in the Atherosclerosis Risk in Communities Study. Ophthalmology *106*, 2269-2280.

10. Wong, T.Y., Islam, F.M., Klein, R., Klein, B.E., Cotch, M.F., Castro, C., Sharrett, A.R., and Shahar, E. (2006). Retinal vascular caliber, cardiovascular risk factors, and inflammation: the multi-ethnic study of atherosclerosis (MESA). Invest Ophthalmol. Vis. Sci. *47*, 2341-2350.

11. Rochtchina, E., Wang, J.J., Taylor, B., Wong, T.Y., and Mitchell, P. (2008). Ethnic variability in retinal vessel caliber: a potential source of measurement error from ocular pigmentation?--the Sydney Childhood Eye Study. Invest Ophthalmol. Vis. Sci. *49*, 1362-1366.

12. Wong, T.Y. and Mitchell, P. (2004). Hypertensive retinopathy. N. Engl. J. Med. *351*, 2310-2317.