ARIC Manuscript Proposal # 1693

PC Reviewed: 9/14/10	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: *Brain-derived Neurotrophic Factor (BDNF)* Variability Mediates Smoking Persistence in African Americans

b. Abbreviated Title (Length 26 characters): BDNF and Smoking

2. Writing Group (ARIC investigators): Nora Franceschini, David Couper, Eric Boerwinkle, others welcome

I, Ajna Hamidovic, confirm that all the coauthors have given their approval for this manuscript proposal. F**irst author**: **AH**

Corresponding/senior author (if different from first author correspondence will be sent to both the first author & the corresponding author):

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3. Timeline: The paper for this manuscript is already written (it is attached as part of this proposal) and is uploaded in the Molecular Psychiatry manuscript submission system. Following the projected approval of the proposal and manuscript we ask to submit the paper by October 1st.

4. Rationale: Cigarette smoking continues to be the leading cause of preventable death in the United States due to its causative link to cancer, cardiovascular, and respiratory diseases (MMWR; 2005). The burden of lung cancer is greater in African Americans compared to all other racial groups in the United States[1, 2] with the incidence and mortality rates of lung cancer in African Americans being 82.7 per 100, 000 and 64.1 per 100,000 per year respectively, in comparison to 64.3 per 100,000 and 54.1 per 100,000 per year for Caucasians.[3]

To date, the strongest evidence for genetic association with smoking behavior occurs at the 15q25.1 locus that contains a cluster of *CHRNA5-CHRNA3-CHRNB4* genes encoding the α3, α5 and β4 subunits of the nicotinic acetylcholine receptor (nAChR). The process of identifying functional variants in this region as well as additional variants in the genome has been challenging. Initial GWAS identified a strong signal from a nonsynonymous SNP, rs16969968, located in *CHRNA5.*[4] More recent meta- analyses in Caucasians applied imputation methods to data from the first phase of the 1,000 genomes project and identified the strongest association signal from a SNP rs55853698 that may affect transcription of *CHRNA5* as well as an independent signal from SNP rs6495308 in *CHRNA3.*[5] The search for functional variants is complicated by strong linkage

disequilibrium in this region in populations of European descent. Conducting association studies in diverse populations, and in particular in African Americans who, on average, have lower levels of linkage disequilibrium, can further refine association signals and lead to the identification of new loci that would be missed through the study of European descent populations alone.

To this end, we evaluated smoking persistence in African American participants in the ARIC study to advance our understanding of tobacco addiction genetics. We sought to do this with improved phenotyping and genotyping methods: our measure of the smoking persistence phenotype (measured in pack years) incorporates information on total nicotine exposure accounting for periods of time of intermittent smoking cessation within a longer period of smoking. Our genotyping platform, ITMAT-Broad-CARe (IBC) array, provides extensive coverage of important regions hypothesized to influence smoking persistence.

5. Main Hypothesis/Study Questions: We undertook an association study of smoking persistence, as measured by pack-years of cigarette smoking, in unrelated African-American participants from The Atherosclerosis Risk in Communities (ARIC).

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 methodological limitations or challenges if present).

Study Design: Genome-wide Association study.
Inclusion Criteria: All smokers (current and former)
Outcome Variable: DERV1C1 (pack-years of smoking)
Additional Variables: RACEGRP, CENTERID, GENDER, V1AGE01, ELEVEL01, ELEVEL02.
Specific Reference to the time of the collection: Baseline (first screen)
Summary of Methods:
Our total sample consisted of 1,490 current or former smokers who were 45-65 years of age. The phenotype we investigated, smoking persistence, as measured by pack years of cigarette exposure (N=1,490) accounting for intermittency in smoking (see formula below), was available in The Atherosclerosis Risk in Communities (ARIC) cohort from the CARe project. Current Smokers: PCKYR= AVGCIGDY/20 x ((CURAGE-AGEINIT)-NONSMK)
Former Smokers: PCKYR= AVGCIGDY/20 x ((AGEQUIT-AGEINIT)-NONSMK)
(Abbreviations: PCKYR=Pack-Year; AVGCIGDY=Lifetime Average Cigarettes per Day; CURAGE=Current age; AGEINIT=Age of smoking initiation; AGEQUIT=Age Quit Smoking; NONSMOK= Intermittent non-smoking period (i.e. total period of non-smoking in the overall smoking period).
Genotyping Assay
ITMAT-Broad-CARe (IBC) SNPs (49,320 total) were chosen to densely map about 2,100 candidate gene loci deemed to be relevant to phenotypes available in the CARe Phenotype Database. All DNA samples passing initial quality checks were interrogated with the IBC chip. For detailed genotyping and QC information for the CARe project see Musunuru et al. [5].
Summary of Results:
Markers in and around *Brain-Derived Neutrophic Factor (BDNF)* on 11p14.1, including rs925946 previously found to be associated with childhood and adult obesity, were the most strongly associated with smoking persistence. Additional results included variants in

the cluster of genes encoding nicotinic acetylcholine receptor subunits (*CHRNA5-CHRNA3-CHRNB4*) on 15q25.1. Our findings indicate that the *BDNF* locus is an important mediator of smoking persistence among African Americans.

Current Limitation

Following the completion of our analysis with ARIC data we spent 6 months in an attempt to find an appropriate database to replicate our findings. Our attempt was limited for two reasons. 1. Existing databases include only cigarettes per day. We (as well as the CARe Primary paper group) found that this measure is not a sensitive phenotype in the CARe database as smoking quantities change over time. 2. If existing databases include a measure of smoking duration (pack-year), they do not include a measure of intermittent smoking. We have found that 28% of ARIC smokers reported not smoking for 1 year or longer. wE found that this information is critical for a reliable estimate of smoking persistence. It is only when we analyzed this "intermittency in smoking" component that we were able to reach a finding that passed correction for multiple comparisons. ARIC has a good measure of smoking persistence because of the question regarding intermittency in smoking period, but the majority of other studies (and none that we know which include African Americans) don't include this critical question in their questionnaires. We have only recently found an appropriate database (Lung Health Study GWAS; PI: Kathleen Barnes; Johns Hopkins University), which we will be able to use to replicate our results. However, the group is just finishing their QC and will need to complete their own analysis of smoking phenotypes before we can use their data to replicate our results. We have an agreement to contact them in 6 months. We ask the permission to publish our current results. Following projected replication with Lung Health Study GWAS, we plan to submit an additional manuscript for publication.

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 ICTDER02 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 ICTDER02 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 ICTDER02 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)? 1382 Genome-wide association study of smoking initiation, intensity, and cessation in African American and white ARIC participants, and meta-analysis of smoking within the Tobacco & Genetics (TAG) Consortium

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)
2008.04 Incorporating ARIC into the Tobacco and Genetics (TAG) Consortium: Unraveling the genetics of nicotine dependence.
2007.02 (CARe, genotyping in African Americans)

___ 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 (see manuscript for additional references)

1. Haiman CA, Stram DO, Wilkens LR, Pike MC, Kolonel LN, Henderson BE *et al.* Ethnic and racial differences in the smoking-related risk of lung cancer. *New England Journal of Medicine* 2006; **354**(4)**:** 333-342.

2. Risch N. Dissecting racial and ethnic differences. *New England Journal of Medicine* 2006; **354**(4)**:** 408-411.

3. Ries L, Melbert D, Krapcho M, Stinchcomb D, Howlader N, Horner M *et al.* SEER cancer statistics review, 1975-2005. National Cancer Institute: Bethesda, MD, 2008.

4. Bierut LJ, Madden PA, Breslau N, Johnson EO, Hatsukami D, Pomerleau OF *et al.* Novel genes identified in a high-density genome wide association study for nicotine dependence. *Human Molecular Genetics* 2007; **16**(1)**:** 24-35.

5. Musunuru K, Lettre G, Young T, Farlow DN, Pirruccello JP, Ejebe KG *et al.* Candidate gene association resource (CARe): design, methods, and proof of concept. *Circulation Cardiovascular Genetics* 2010; **3**(3)**:** 267-275.