**ARIC Manuscript Proposal #2015**

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| **PC Reviewed: 10/9/12** | **Status: A** | **Priority: 2** |
| **SC Reviewed: _____** | **Status: _____** | **Priority: ___** |

**1.a. Full Title:**

An Evaluation of Obesity-related Proteins and Genes in Migraine Participants from the Atherosclerosis Risk in Communities Study

**1.b. Abbreviated Title (26 characters):**

Obesity-related Proteins and Genes in Migraine

**2. Writing Group Members:** B. Lee Peterlin, Michelle Williams, Tobias Kurth, Kathy Rose, Linda Kao, Grace Lee, Rebecca Gottesman, Others Welcome

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. *BLP*


**First author**: **B. Lee Peterlin, DO**
Address: Meyer 6-113
Johns Hopkins University School of Medicine
600 N. Wolfe St.
Baltimore, MD 21287
Phone: (410) 550-9550
E-mail: lpeterlin@jhmi.edu


**ARIC author** to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located (this must be an ARIC investigator).


Name: **Rebecca Gottesman, M.D. Ph.D.**
Address: Meyer 6-113
Johns Hopkins University School of Medicine
600 N. Wolfe St.
Baltimore, MD 21287
Phone: 410-614-2381
FAX: 410-955-0672
E-mail: rgottesm@jhmi.edu


**3. Timeline**: Analyses to begin as soon as manuscript proposal is approved. Goal for abstract submission for International Headache Society Symposium in 2013.

**4. Rationale**

Recent data have demonstrated that obesity is associated with an increased odds of migraine. [1-3] Potential mechanisms for this association are not known. Several obesity-related, neuroinflammatory factors, including neuropeptide y (NPY) and cytokines such as interleukin-6 and tumor necrosis factor-alpha, have been linked to migraine onset and progression. [4-6]

Additionally at least two adipocytokines (cytokines secreted primarily from adipose tissue cells) have been implicated in migraine in small clinical cohorts. Specifically, elevated leptin levels have been found in women with episodic migraine; [7] while elevated levels of both total adiponectin (T-ADP) and high molecular weight adiponectin (HMW-ADP) has been demonstrated in women with chronic migraine. [8] Previous research has suggested the involvement of an insulin receptor gene in migraineurs with aura.[9] In addition, previous research has also demonstrated that female carriers of the melanocortin4 receptor allele (MC4R I103) exhibit significantly lower body weight than non-carriers. [10]

Given the association between obesity and migraine, MC4R I103 carriers may also have a lower risk of migraine. Further, other MC4R allele variations could be modulated in migraine as well. It is of note that MC4R knockout mice exhibit increased NPY and that increased cerebrospinal fluid levels of NPY have been demonstrated in migraineurs in one study. [11] Finally given the data suggesting leptin levels may be increased in episodic and chronic migraineurs, LEP gene variations may also be associated with changes in migraine disease risk.[7]

In summary, taken together these findings suggest obesity-related markers and genes may contribute mechanistically to migraine generation and progression. Thus, the aim of the proposed research is to evaluate obesity-related proteins and genes in those with migraine as compared to those without migraine.

## 5. Main Hypothesis/Study Questions

**To evaluate obesity-related plasma proteins levels in migraineurs as compared to controls and their correlation with headache frequency.**

We hypothesize the following:
Hypothesis 1.1: Plasma levels of total adiponectin, high molecular weight adiponectin and leptin will be increased in those with migraine as compared to controls, for both obese and non-obese categories.
Hypothesis 1.2: The magnitude of effects for hypotheses 1.1 will be modified by body composition (obese versus non-obese), sex (women versus men), and race (Caucasians versus African Americans).

**To investigate the effects of variation in adiponectin, leptin, and the melanocortin-4 receptor genes.**

Given the data demonstrating: 1) an epidemiological association between obesity and migraine, 2) the presence of abnormalities in adipokines in migraineurs, and 3) the genetic data suggesting an association between two obesity-related genes (a insulin receptor gene (INSR) and a dopamine-2 receptor gene (DRD2)) in those with migraine we hypothesize the following:
Hypothesis 2.1: Migraine risk is associated with variations within the adiponectin, leptin and MC4R genes. In particular those participants with ADP, LEP, MC4R gene variations which are positively associated with obesity will be associated with a greater risk of migraine than in those with mutations associated with lowered body weight, (eg. LEP A19 and MC4R V103I)
Hypothesis 2.2: The magnitude of effects for hypotheses 2.1 are modified by body composition (obese versus non-obese), sex (women versus men), race (Caucasians versus African Americans) and headache characteristics (eg. headache frequency (episodic versus chronic) and aura (yes or no). (If no differences between these subgroups are found we will combine groups for final analyses.)

**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).**

Study Design: This will be a cross-sectional analysis.

Inclusion: All ARIC Caucasian and African American participants for whom these data are available. For hypothesis 1, this includes individuals in the ARIC case-cohort diabetes analysis in whom at visit 1 adiponectin and leptin levels are available (diabetes cases n=581 and a random cohort sample, n=693) and in whom completedheadache questionnaires are available at visit 3 (n=12,830). For hypothesis 2, this includes all individuals with the leptin and melanocortin-4 receptors genotypes available (n=6042 males; n=7,363 females) and who also subsequently completed headache questionnaires at visit 3, (n=12,830).

Exclusion: For hypothesis 1: Participants missing headache questionnaire or adipokine data. For hypothesis 2: participants missing headache questionnaire or genetic data.

Primary outcome variables (adipokine levels and genotyping) for those participants fulfilling ARIC modified international classification of headache disorders (ICHD) criteria for current migraine (presence of migraine ≤12 months of the time completeing headache question) and will be compared and contrasted to those with non-active migraine history vs those without migraine and those with no lifetime history of headache (migraine or generic headache). ARIC headache questions are adequate to fulfill these criteria and define individuals as with or without migraine or headache. Additional analyses will evaluate primary outcomes in those with and without migraine stratified by: 1) age (utilizing two stratification strategies : (a) 5 year increments from 45 years of age up to 55 and (b) those <50 and ≥50 years of age) 2) race, and 3) sex. Analyses will also be completed in women participants stratified by menopausal status, (pre vs post menopause). Finally, analyses will be conducted to evalute the effect modification of aura (absence or presence) as well as headache frequency (episodic versus chronic) with respect to all primary outcome variables.

The following parameters will be evaluated:

Demographics including age, sex, race-field center, marital status, income, and education will be evaluated. Age will be categorized in 5 year increments. Education will be categorized as less than high school or vocational school, high school or vocational school completion, or more than high school or vocational school . Additionally menopausal status will be assessed in women as premenopausal, perimenopausal, postmenopausal.

Headache Assessments

All participants in ARIC were asked about the frequency, duration, quality and location of headache as well as the presence of migraine associated symptoms at the 3rd clinic visit. Migraine will be defined using the modified ICHD ARIC definition of migraine as previously described. [12,13] In brief, participants endorsing the following 4 criteria will be classified as migraine: 1) headache lasts 4 or more hours; 2) headache is throbbing, pounding, pulsating or was unilateral; 3) headache occurred with nausea, vomiting, or photo or phonophobia. In addition an affirmative response to a question about the occurrence of visual aura (ie spots, jagged lines or heat waves

in one or both eyes) will be utilized to assess visual aura. Additionally, participants will be classified as "active" if endorsing migraine within 12-months of answering headache questions and "non-active" if endorse history of migraine in past but not within the previous 12-months of answering headache questions. Finally, those participants endorsing headaches which are less than 4 hours or which last 4 hours but did not fulfilling all other ARIC criteria will be referred to as non-migraine headache; and those who do not report migraine or any headache will be classified as no headache.

**Body Composition:** Body Mass Index (BMI) will be characterized on the basis of the World Health Organization (WHO) categories: underweight (<18.5), normal weight (18.5-24.9), overweight (25-29.9), obesity grade I (30-34.9), obesity grade II (35-39.9) and obesity grade III (≥40). Waist circumference (WC) will be categorized as normal weight (<80 cm women ;<94 cm men), overweight (94-101cm men; 80-87 cm women) and obese (≥102 cm men; ≥88 cm for women). Waist to hip ratio (WHR) will be determined by dividing WC by hip circumference. The anthropometric data will be used from the ARIC visit 1 given this is the visit when blood draws for adipocytokines were drawn and visit 3, given this is when headache questionnaires were completed.

Covariates

The following factors represent potential migraine and/or obesity confounders and will be evaluated at first and third visit as follows:

1) Physical activity will be assessed using a modified version of the Baecke physical activity questionnaire as previously described by Schmitz et al.[14] The frequency of baseline overall sport and exercise-related leisure activity participation will be categorized into low (sport index <2), medium (sport index 2-2.29) and high (sport index >2.9) from the Baecke questionnaire as previously described.[15]

2) Diabetes status will be defined as a fasting glucose level ≥126 mg/dl, a non-fasting glucose level ≥200 mg/dl and/or a self-reported history of or treatment for diabetes as previously described.[16]

3) Hypertension at baseline will be defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg or antihypertensive medication use in the previous 2 weeks.

4) Cigarette smoking status will be defined as current smokers vs. former smokers and non-smokers.

Laboratory Evaluations

1) Standard Laboratories will include: total cholesterol, HDL, triglycerides, fasting and non-fasting glucose, and insulin from visit 1.

2) Adipokine Plasma Evaluations will include fasting and non-fasting total adiponectin, high molecular weight adiponectin, and fasting leptin levels taken from visit 1.

3) Allele Detection & Genotyping of the *LEP* and *MC4R* polymorphisms will be evaluated using the TaqMan assay (Applied Biosystems, Foster, CA, USA) as previously described. Finally we propose exploratory analysis to evaluate APOE and APOC associations with migraine given that previous research has demonstrated: 1) that migraineurs are more likely than controls to have

hypercholesterolemia,[17] and 2) that the E2 and E4 alleles of the apolipoprotein E (*APOE*) confer increased risk of migraine.[18]

Data Analysis:

I. Sample sizes were determined using Sample Power 2; all calculations are for two-tailed tests with 80% power and α=0.05. Sample size calculations were run for each adipokine individually based on our preliminary results as well as work from others using paired sample t-tests.[8,19,20] Based on leptin levels, the adipokine with the greatest inter-individual difference and the largest sample calculation as based on our preliminary results and a previous study evaluating leptin levels in those with chronic stable angina, acute myocardial infarction and controls [20], 30 participants per group are needed by sample t-tests to compare migraineurs to controls in order to detect statistical differences in resting levels between groups. Thus given that in Visit 1 of ARIC total adiponectin and HMW-ADP levels were evaluated in 581 diabetics and a random sample of 693 participants, and Leptin was evaluated in 471 participants for incident CDH and in 237 participants for prevalent PAD we should have sufficient power to detect statistical differences between groups.

II. Statistical analyses will be conducted using Stata 11.0 (StataCorp, 2010). Covariates will include sociodemographics (e.g., age, race, marital status) as well as physical activity, smoking status, body composition, diabetes, and cardiovascular disease. Preliminary analyses will examine descriptive statistics (e.g., mean, variance, distribution, percent of missing data, collinearity among variables, detection of outliers) and whether systematic differences exist between the two migraine (i.e., chronic and episodic) and control (i.e. no migraine) groups before proceeding to hypothesis testing. Independent t-tests (for continuous variables) and chi-square tests (for categorical variables) will determine whether statistically significant differences exist across groups for any of the demographic variables and the study variables of interest. If significant differences are found, the source will be located and appropriate covariates used in subsequent analyses. Patterns of missing data will be examined, and if it can be deemed either missing completely at random (MCAR) or missing at random (MAR), multiple imputation methods will be utilized for such missing data.

III. For **Hypothesis 1.1** (plasma levels of total adiponectin, HMW adiponectin, and leptin levels in migraineurs versus controls) linear regression models adjusted for covariates will be used to analyze the association between the dependent variable (migraine) and the independent variables (adiponectin, high molecular weight adiponectin, and leptin levels). Three different models will be run for each plasma protein level. The first will include the entire sample, irrespective of obesity status. The sample will next be stratified by obesity status, and separate models will be run for obese and non-obese participants. For **Hypothesis 1.2** (the impact of body composition (obese versus non-obese), sex (women versus men), and race (Caucasians versus African Americans) on the magnitude of effects for hypotheses 1.1), interaction terms will be created for body composition, sex, and race and each of the three plasma protein levels (for a total of 9 interaction terms). For each plasma protein level, the interaction terms will be entered into linear regression models similar to those used in Hypothesis 1.1 for the entire sample. For **Hypothesis 2.1** (the determination of migraine risk associated with the ADP, LEP and MC4R genes). Two dichotomous (Yes/No) variables will be created for the presence of ADP, LEP, and MC4R gene variations positively associated with obesity. Logistic regression models adjusted for covariates can next analyze the association between the outcome of migraine and the presence of each gene variation. Additionally, two dichotomous (Yes/No) variables will be created for the presence of LEP A19 and MC4R I103. Logistic regression models adjusted for covariates can next analyze the association between the outcome of migraine and the presence of each gene variation. If power is adequate, additional stratified analyses will be

conduct for obesity status (yes/no), race (Caucasian/African American/Asian), sex (male/female), headache characteristics (including active vs non-active migraine status, headache frequency (episodic/chronic), presence of aura (yes/no), as well as menopausal status in women (pre/post-menopausal).

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Potential Limitations: Given that the association between obesity and migraine is strongest in those of reproductive age (those <50-55 years of age), the smaller number of pre-reproductive aged individuals in ARIC may represent a limitation. In addition, the limited number of individuals with adipokine measurement may limit our conclusions.

**7.a. Will the data be used for non-CVD analysis in this manuscript? Y/N**

**8.a. Will DNA data be used in this manuscript? Y/N**

*LEP*, *MC4R,* APOE and APOC genotypes will be used to evaluate hypotheses on their associations with migraine.

**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

**Y/N**

**10. What are the most related manuscript proposals in ARIC (authors are encouraged tocontact lead authors of these proposals for comments on the new proposal or collaboration)?**


Carson AP, Rose KM, Sanford CP, Ephross SA, Stang PE, Hunt KJ, Brown CA, Szklo M. Lifetime prevalence of migraine and other headaches lasting 4 or more hours: the Atherosclerosis Risk in Communities (ARIC) study. Headache . 2004;44:20-28.

Duncan BB, Schmidt MI, Pankow JS, Bang H, Couper D, Ballantyne CM, Hoogeveen RC, Heiss G. Adiponectin and the development of type 2 diabetes: the atherosclerosis risk in communities study.Diabetes. 2004 Sep;53(9):2473-8.

Hart Sailors ML, Folsom AR, Ballantyne CM, Hoelscher DM, Jackson AS, Linda Kao WH, PankowJS, Bray MS Genetic variation and decreased risk for obesity in the Atherosclerosis Risk in Communities Study.Diabetes ObesMetab. 2007

MP399. Paton CC et al. A study of MRI-diagnosed white matter lesions and infarcts among migraineurs participating in a population-based study of cardiovascular disease: the ARIC study, 1993-94.

Schmidt MI, Duncan BB, Vigo A, Pankow JS, Couper D, Ballantyne CM, Hoogeveen RC, Heiss G; ARIC Investigators. Leptin and incident type 2 diabetes: risk or protection? Diabetologia. 2006 Sep;49(9):2086-96. Epub 2006 Jul 19.

Zhu N, Pankow JS, Ballantyne CM, Couper D, Hoogeveen RC, Pereira M, Duncan BB, Schmidt MI. High-molecular-weight adiponectin and the risk of type 2 diabetes in the ARIC study. J Clin Endocrinol Metab. 2010 Nov;95(11):5097-104. Epub 2010 Aug 18.

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

**11.b. If yes, is the proposal**

**A. Primarily the result of an ancillary study**

We will use ancillary studies which measured adipokines (adiponectin, HMW adiponectin, and leptin) at visit 1 as well as studies which evaluate the LEP and MC4R AND APO E and C genotypes from visits 1-3. For hypothesis 1, this includes individuals in the ARIC case-cohort diabetes analysis whom adiponectin and leptin levels are available at visit 1 (diabetes cases n=581 and a random sample of the cohort, n=693) and who also subsequently completed headache questionnaires at visit 3 (n=12,830). For hypothesis 2, this includes all individuals in whom the adiponectin, leptin and melanocortin-4 receptors genotypes are available (n=6042 males; n=7,363 females) and who answered headache questions at visit 3 (n=12,830).

This will include: 1995.09 Inflammatory Precursors of Diabetes (for adiponectin, HMW adiponectin, leptin) and 1995.07 Gene-Environment Interaction in CVD for above genotypes.

**B. Primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list numbers)**

*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**

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12. Carson AP, Rose KM, Sanford CP, et al. Lifetime prevalence of migraine and other headaches lasting 4 or more hours: The atherosclerosis risk in communities (ARIC) study. *Headache*. 2004;44(1):20-28.

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