1a. Full Title:

Characteristics of Intracranial Arterial Remodeling and its Risk Factor Associations in the ARIC Cohort

b. Abbreviated Title (Length 26 characters):

Intracranial Arterial Remodeling

2. Writing Group:

Writing group members:

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

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3. **Timeline:**
The manuscript will be complete within 2-3 months upon the approval of this proposal.

4. **Rationale:**

Extracranial arteries have long been known to have the capacity to remodel in response to plaque formation to maintain lumen patency [1, 2]. It is also known that the degree and direction of vascular remodeling depends on the vessel location with some segments expanding and others retracting to the same degree of plaque formation [1]. The ability of intracranial vessels to remodel has been the subject of much debate, with studies based on limited samples both supporting and refuting its occurrence [3-6]. Furthermore, ongoing studies of risk factors and clinical implications for these vascular changes have been largely inconclusive, perhaps because of the inability to study these changes noninvasively in large population studies. What we do know is primarily based on extracranial vascular studies of symptomatic lesions which are not generalizable to the highly prevalent intracranial lesions known to occur in asymptomatic individuals [7].

In ARIC NCS we acquired high-resolution MR angiography and black blood MRI (BBMRI) images of intracranial arteries that allowed us to identify intracranial atherosclerotic disease (ICAD) even when remodeling preserves luminal patency. Measurements derived from these images will enable the quantification of atherosclerotic plaque remodeling for each major intracranial arterial segment. This can provide unique insight into the development of intracranial stenosis in response to plaque formation and the dependability of angiography for identifying ICAD. The importance of understanding the nature of stenosis development in response to ICAD is highlighted by the high prevalence of low-grade stenosis in ICAD upstream from strokes, with a stenosis of $\geq 30\%$ shown in only $50\%$ of ICAD lesions in patients with fatal stroke on autopsy [8]. Preliminary data acquired by our group based on these high-resolution MRI techniques not only supports remodeling of intracranial arteries to accommodate plaque formation but demonstrates regional differences in the ability and extent of arterial segments to remodel [9]. For example, posterior circulation vessels seem to have a greater capacity for positive remodeling.

The identification of positive remodeling may have important clinical implications given evidence that it is associated with features of plaque vulnerability, specifically intraplaque hemorrhage, higher lipid content, and inflammation, in coronary arteries [10, 11]. In intracranial arteries, positive arterial remodeling has been shown to occur more frequently in symptomatic than asymptomatic MCA plaques [4, 5, 12] and microemboli were seen more frequently on transcranial Doppler in patients with MCA plaques and positive remodeling than without positive remodeling [13]. To our knowledge, there have been no reports of the clinical implications of remodeling seen in plaques other than the MCA.

There is ongoing debate regarding risk factors that might influence the occurrence of positive or negative remodeling. Intravascular ultrasound (IVUS) studies of coronary
arteries have reported cholesterol levels are associated with both positive [14, 15] and negative [16, 17] remodeling, inflammatory markers with positive remodeling [18] or are not associated with remodeling [19], and smoking with negative remodeling [14, 15, 20]. Diabetes and hypertension were not associated with coronary remodeling by IVUS [20] and pathologic specimen studies [21] though other pathologic studies have shown positive remodeling to be associated with diabetes [22, 23]. There have been no reports of risk factor associations with intracranial arterial remodeling.

In ARIC-NCS study, we have implemented vascular sequences (i.e., 3D vessel wall imaging (BBMRI) and MRA) in the brain MRI exam to identify ICAD and measure its size when present. The use of both luminal (MRA) and vessel-wall (BBMRI) imaging enables the identification of ICAD in the absence of stenosis as remodeling accommodates plaque formation, and our measurements of wall thickness and lumen area over the length of an atherosclerotic vessel segment enables the quantification of remodeling degree. These can be examined in relation to risk factors and vascular markers (contemporaneous and change from earlier baseline measures).

5. **Main Hypothesis/Study Questions:**

A. To determine the distribution of ICAD lesions with no detectable luminal narrowing (qualitative data) for each intracranial vessel segment and assess the frequency of these non-stenotic lesions by race, sex and age in ARIC participants aged 70-89 years. We will adjust for lumen area at the reference site since lumen narrowing might be negligible in large compared to small vessels for the same wall thickness. Positive remodeling based on remodeling ratios (quantitative data) will also be reported by race, sex and age.

**Hypothesis:** We expect a higher frequency of non-stenotic ICAD in the basilar and vertebral arteries compared to anterior circulation segments adjusting for wall thickness. We expect positive remodeling will be more frequent in younger age groups and will be independent of race and sex.

B. To evaluate the association between remodeling ratios for plaques with the presence of downstream infarctions.

**Hypothesis:** Positive remodeling will be associated with plaques upstream from an infarction.

C. To evaluate the associations between known risk factors and vascular markers for cardiovascular disease, measured repeatedly from middle-age beginning over 20 years ago, with remodeling patterns (e.g., positive, negative) for plaques involving each arterial segment.
D. To explore the relation between lumen area and wall thickness and to determine if a threshold for lumen stenosis (i.e., remodeling threshold) exists as wall thickness increases for each intracranial arterial segment.

**Hypothesis:** As wall thickness increases, lumen area, adjusted for age, sex, race, height, and height squared, will be maintained until a threshold is reached beyond which the lumen narrows. This threshold will differ between segments.

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:**
The study will use data from 2,000 ARIC participants who underwent brain MRI to measure intracranial atherosclerosis in visit 5. The vascular MRI protocol consisted of a 3-dimensional time-of-flight MR angiogram (TOF MRA) and a 3-dimensional high-isotropic resolution black blood MRI (BBMRI) sequence centered at the Circle of Willis. Qualitative analysis of the MRI images included plaque presence by vessel territory (RMCA, LMCA, RPCA, LPCA, ACA, BA, VA, RICA, and LICA), number of plaques, and the ordinal degree of narrowing (i.e., no detectable stenosis, <50%, 51%-70%, 71-99%, and occlusion) for the most stenotic plaque per territory.

Quantitative measurements were obtained at designated vessel segments (supraclinoid ICA, M1 of MCA, A1 of ACA, proximal and distal BA, and V4 of VA) over a fixed length for all participants and for the largest plaque identified for each vascular territory in the qualitative assessment. For each vessel segment and plaque, we recorded lumen size and stenosis, wall/plaque thickness, area, volume, and normalized wall index (wall area/outer wall area).

**Inclusion:**
Complete set of qualitative and quantitative MRI measurements. Image quality and protocol adherence scores of adequate or excellent on both MRA and BBMRI.

**Exclusion:**
Poor or failed exams

**Variables of interest:**
- Plaque presence by vessel segment (e.g., n_rmca_plaq, n_lmca_plaq, n_raca_plaq, etc) including those with no detectable stenosis
- Total number of plaques (n_plaq)
- Ordinal degree of narrowing (no detectable stenosis, <50%, 51%-70%, 71-99%, and occlusion)
- Wall thickness Segment Average (mm) – average wall thickness by vessel segment measured on BBMRI
• Wall thickness Segment Maximum (mm) – maximum wall thickness by vessel segment measured on BBMRI
• Cerebral infarct location
• Loc_vwi_wallthic_tot_max – Location of maximum wall thickness in segment
• Out Loc_vwi_wallthic_tot_max – Outer wall area at location of maximum wall thickness
• mra_lumen_area_Loc_vwi_wallthic_tot_max – Lumen area at location of maximum wall thickness
• Cardiovascular risk factors (e.g., total and HDL cholesterol, blood pressure, diabetes mellitus, smoking) measured in midlife (ages 45-64) and cumulatively since midlife to better predict ICAD presence and burden than current measurements at visit 5 after adjusting for age, sex and race.

Summary of data analysis:

Analysis of qualitative MRI variables:
The frequencies of intracranial vessel segments with only no detectable stenosis (qualitative ordinal degree of narrowing) will be determined for each vessel segment and compared with the remaining participants with ICAD with stratification by race, sex and age (Study Question A). Participants with a nonstenotic plaque identified in one or more vessel segments will be compared to the remaining participants with plaques by race, sex and age with weighting by the number of segments with nonstenotic plaques per participant. A multivariable mixed linear regression model will be used to test differences in mean and max wall thickness (WT) of vessel segments with nonstenotic plaques versus stenotic plaques. If no difference is detected we can assume differences in remodeling are not the effect of differences in WT. The frequency of participants with only nonstenotic plaques will be determined and weighted to the Visit 5 ARIC cohort (i.e., percent of participants with angiographically-occult ICAD). A multivariable mixed logistic regression model will be used to evaluate positive remodeling by remodeling ratios (vs negative and intermediate remodeling) adjusting for WT and for the effect of race, age, and sex.

For prevalence of nonstenotic ICAD lesions, we will use Stata svy commands with sampling weights and strata to account for oversampling of participants with cognitive impairment in ARIC-NCS and provide estimates referable to the overall ARIC population.

Analysis of quantitative MRI variables:

For this study, additional image processing will be supported and performed at the JH Vascular Reading Center. This will include reprocessing output files generated by LAVA software (LAVA, Leiden University Medical Center, the Netherlands) to extract measurements needed to derive remodeling ratios. These measurements include the outer wall area at a reference point that lacks wall thickening (OWA_ref), distance from
reference point to the point of maximum wall thickness (D), and slope of the iterative linear regression of segmental lumen area (S), and the location of the reference point where the wall is thinnest (ref_loc).

1. Remodeling ratios (RMR) will be derived for each vessel segment as follows:

ref_loc (reference location) = The location of the thinnest mean WT of the 5 spatial points at the proximal and distal ends of each vessel segment.

OWA_lesion = Outer wall area at location of largest value of max WT
OWA_ref = Outer wall area at point where ref_loc is defined
D = Distance from lesion site to point where ref_loc is defined
S = Slope of iterative linear regression of lumen area based on MRA. The sign of S will be positive or negative depending on whether the vessel lumen expands or tapers, respectively, from the reference location to the lesion site.
OWA_adj (Adjusted outer wall area) = OWA_ref + SxD (assumes no change in WT as lumen tapers or expands)

RMR = OWA_lesion / OWA_adj

Vessel remodeling is defined as positive (outward expansion in response to plaque formation) if RMR >1.05; intermediate if 0.95 ≤ RMR ≤1.05; and negative (vessel wall shrinkage) if RMR <0.95.

Multilevel mixed-effects linear regression models will be used to compare the differences in MRI measurements (e.g., area, thickness, vessel tapering (S), plaque burden, RMR) between anterior and posterior circulations by including random intercept terms to account for measurements of multiple arterial segments within participants (Study Question A). A multivariable logistic regression model will assess whether positive remodeling is associated with vessel segments adjusting for wall thickness.

RMR will also be reported as the average for all plaques per participant. Multivariate ordinal logistic regression will be used to assess associations between remodeling category (based on average RMR per participant) and risk factors (Study Question C). Exposure of interest will include traditional cardiovascular risk factors measured over 20 years including age, sex, race, BMI, smoking, alcohol consumption, physical activity, total cholesterol, LDL and HDL cholesterol, triglycerides, metabolic syndrome, fasting glucose/diabetes, blood pressure/hypertension, history of cardiovascular event, use of antiplatelet drugs, use of statin and use of antihypertensive medications. We will first evaluate individual risk factor in the model adjusting for age, sex, race, and enrollment center. Risk factors associated with the outcome will then be included simultaneously in the same model (with age, sex, race forced into the model regardless of their significance). Potential interactions by time since risk factor measurements, by sex and by race will also be examined by including interaction terms the models.
Cerebral infarct locations will be reviewed and matched with upstream ICAD location when present. ICAD lesions will be categorized as culprit (one lesion upstream from infarct), indeterminate (not the only ICAD lesion upstream from infarct), and indeterminate (no downstream infarct). The associations (odds ratio) of culprit lesion (non-culprit and indeterminate) with RMR and vessel tapering will be estimated using mixed effects logistic regression models (Study Question B).

2. The association of maximum wall thickness with lumen area will be assessed with linear regression after adjusting for participant age, sex, race, height, and height squared so data are comparable across the cohort (Study Question D). Data will also be weighted based on the sampling design. A linear spline regression model will be performed with the same adjustments to identify a single knot that represents the best fit. Additional knots will be identified that significantly (p<.05) improve the model fit. Model fit will be determined by calculating the coefficient of determination for multivariate analysis (R2). Additional models will be tested, including a third-order polynomial and a piecewise polynomial with knots set at the 5th, 25th, 50th, 75th, and 95th percentiles. If a knot is identified that reflects a threshold for significant lumen area reduction with incremental wall thickness increase (i.e., a threshold for remodeling), (a) the plaque area stenosis (i.e., wall area / outer wall area) will be assessed for plaques straddling the knot as a reflection of the plaque burden at the remodeling threshold, and (b) risk factor associations will be assessed for the slope of lumen area reduction (Study Question C).

7.a. Will the data be used for non-CVD analysis in this manuscript? _____ Yes  ____X____ 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? _____ Yes  ____X____ No
(This file ICTDER 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? _____ Yes  ____X____ No

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”? _____ Yes  ____X____ 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

__ Yes    ___X___ 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)?

NA.

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* 2009.27, 2009.28)

___ 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/

12a. 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.

12b. The NIH instituted a Public Access Policy in April, 2008 which ensures that the public has access to the published results of NIH funded research. It is your responsibility to upload manuscripts to PUBMED Central whenever the journal does not and be in compliance with this policy. Four files about the public access policy from http://publicaccess.nih.gov/ are posted in http://www.cscc.unc.edu/aric/index.php, under Publications, Policies & Forms. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to Pubmed central.
References


