1.a. **Full Title:** Retinal microvasculature in intracranial large-vessel atherosclerotic disease: the ARIC study
   b. **Abbreviated Title (Length 26 characters):** Retinal microvasculature in intracranial atherosclerosis

2. **Writing Group:**
   Writing group members: Michelle P. Lin (first author), Bruce A. Wasserman (senior author), Rebecca F. Gottesman, Ye Qiao, Li Liu, A. Richey Sharrett, Jennifer Deal, Ronald Klein, Barbara E. Klein, Pradeep Ramulu, Alison Abraham, others welcome.

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

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3. **Timeline:** analysis will be performed following approval of current proposal. We aim to complete manuscript within 3 months after completion of analysis.
4. **Rationale:**
Retinal and cerebral microvasculature share a common embryological origin, and abnormal retinal microvascular findings have long been associated with cerebral small vessel disease (SVD) and stroke risk.\(^1\)\(^-\)\(^3\) However, less is known about whether retinal microvascular lesions may be associated with large-vessel atherosclerotic disease, particularly intracranial atherosclerosis.

Intracranial atherosclerotic disease (ICAD) is a common cause of stroke and dementia worldwide with higher prevalence in Asian and blacks.\(^4\)\(^,\)\(^5\) Previous investigations on the relationship between abnormal retinal signs and large-vessel atherosclerotic disease have been largely based on indirect associations between retinal abnormalities and risk factors or laboratory markers for atherosclerosis (e.g., dyslipidemia, smoking, lipid levels, fibrinogen, CRP) rather than on the direct imaging of intracranial large-vessel atherosclerosis.\(^6\)\(^,\)\(^7\) The few studies that are based on imaging have used indirect measures of ICAD by transcranial doppler.\(^8\)\(^,\)\(^9\) However, this technique has a limited view of the intracranial circulation and we know that, in the ARIC cohort, as many as 11% of participants with intracranial plaque will be missed when relying on stenosis detection because of the vessel’s ability to remodel.\(^10\) High-resolution vessel wall MRI (VWMRI) of the intracranial arteries has been shown to reliably detect and measure ICAD without relying on luminal stenosis.\(^11\)

The relationship between arteriolar changes and ICAD remains poorly described, yet emerging imaging data show a high prevalence of concurrent cerebral small and large-vessel diseases independent of vascular risk factors.\(^12\)\(^,\)\(^13\) In this study, we plan to evaluate the association between abnormal retinal signs and large-vessel ICAD burden using VWMRI imaging in ARIC participants.

5. **Main Hypothesis/Study Questions:**
- Abnormal retinal signs detected at midlife (visit 3) are associated with ICAD presence in late-life (visit 5).
- Abnormal retinal signs are associated with higher ICAD burden measured as number and size of intracranial plaques and degree of intracranial vessel stenosis.
- The association between abnormal retinal signs and ICAD may be modified by the presence of white-matter hyperintensities (WMH) and inflammation (CRP). (Given the known effect of inflammation on the pathogenesis of ICAD, and the known association between abnormal retinal signs and WMH).\(^1\)\(^,\)\(^14\)

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).
Design: Prospective observational study of participants with funduscopic photography (visit 3) and intracranial vessel imaging (visit 5)

Inclusion: Participants with gradable retinal imaging and MRI brain scans.

Exclusion: Contraindication for MRI, poor imaging quality or incomplete brain MRI data, missing or ungradable fundoscopic photograph.

Outcomes: MRI variables for intracranial atherosclerosis-
- Any plaque
- Any plaque by vessel territory: ACA, MCA, ICA, PCA, basilar, vertebral
- Total number of plaques (0, 1, ≥2)
- Normalized wall index (NWI): An established measure of plaque burden
- Any stenosis
- Degree of stenosis: 0%, <50%, 50-70%, >70%, occluded

Exposures: Fundoscopic photography variables for abnormal retinal signs-
- arteriovenous nicking
- generalized narrowing
- focal narrowing
- retinal microaneurysm
- retinal hemorrhage
- exudate
- age-related macular degeneration
- central retinal artery equivalent (CRAE), central retinal vein equivalent (CRVE):
  abnormal will be defined as 1st or 4th quartile

Covariates: (visit 3)
- Demographic: age, sex, race, education
- Comorbidities: smoking, hypertension, diabetes, coronary heart disease (prevalent), peripheral artery disease (ABI<0.9), extracranial carotid atherosclerosis.
- clinical: systolic blood pressure, diastolic blood pressure, mean arterial pressure, glucose, low-density lipoprotein, lipoprotein(a), c-reactive protein, use of antihypertensive, use of statin, use of antithrombotics, and white matter hyperintensities (WMH).

Analysis Plan
The primary independent variable is abnormal retinal signs, while the outcome variable is ICAD. We will fist describe the distribution of ICAD presence (any plaque in ACA/MCA/ICA/PCA/basilar/vertebral) by abnormal retinal findings (AV nicking, generalized narrowing, focal narrowing, microaneurysm, retinal hemorrhage, exudate, age-related macular degeneration). Conversely, we’ll also describe the prevalence of abnormal retinal signs by ICAD location.
To evaluate the association between abnormal retinal signs and ICAD, we will first perform multivariable logistic regression models with any presence of ICAD and any presence of abnormal retinal signs as binary variables. Models will include adjustment for demographics (model1); demographics plus comorbidities (model 2). We will then examine the relations based on each retinal marker and ICAD location. Retinal signs will be analyzed as binary variables except for CRAE and CRVE that we will analyze as a continuous and a categorical (quartiles) variables with first quartile representing the narrowest arterioles and/or venules and the fourth quartile representing the least narrow arterioles and/or widest venules).

To test the hypothesis that abnormal retinal signs are associated with higher ICAD burden, we will stratify ICAD markers by plaque numbers (0, 1, ≥2), degree of stenosis (0%, <50%, 50-70%, >70%, occluded), and NWI in quartiles. We’ll then perform ordinal logistic regression using the same 2 models as described above.

Formal interaction terms will also be analyzed for the effect of small-vessel disease (WMH) and inflammation (CRP) on the relationship between abnormal retinal signs and ICAD.

**Limitations:** There are several study limitations. First, retinal photography was performed on one eye with a relatively narrow field of view. This may underestimate the retinal microvascular findings. Second, many retinal findings are binary leading to a loss of quantitative information on each of these finding. Third, there is a selection bias for those who undergo an intracranial MRA since participants who survived to visit 5 likely have different health states and atherosclerosis burden than those who did not survive to visit 5. We will perform IPAW.

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 _____ 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 _____ 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.csc.unc.edu/ARIC/search.php](http://www.csc.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)?**

Prevalence and risk factors for intracranial atherosclerosis in the ARIC cohort. Qiao et al.

Prevalence of Intracranial Atherosclerotic Stenosis (ICAS) and its Association with Vascular Risk Factors. Suri et al.

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data?  
___ Yes  __x__ No

11. b. If yes, is the proposal  
___ A. primarily the result of an ancillary study (list number* __________)  
___ 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.cscu.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. Understood.

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.cscu.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.

13. Per Data Use Agreement Addendum, approved manuscripts using CMS data shall be submitted by the Coordinating Center to CMS for informational purposes prior to publication. Approved manuscripts should be sent to Pingping Wu at CC, at pingping_wu@unc.edu. I will be using CMS data in my manuscript ____ Yes __x__ No.

References:


