ARIC Manuscript Proposal #4062

PC Reviewed: 6/14/22 **Status: _____** **Priority: 2**
SC Reviewed: _____ **Status: _____** **Priority: ____**

1.a. Full Title: Are midlife and late life retinal microvascular abnormalities associated with late-life brain amyloid deposition?

b. Abbreviated Title (Length 26 characters):

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. __ME___ **[please confirm with your initials electronically or in writing]**

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3. Timeline:
Manuscript prepared and submitted in ~4-5 months.

4. Rationale:

According to the amyloid cascade hypothesis, β-amyloid (Aβ) pathology accumulation is a key feature in the brain triggering the pathogenesis of Alzheimer's disease (AD) [1]. Florbetapir PET, a ligand with high affinity and specificity to Aβ, has provided the opportunity to detect Aβ in subjects with preclinical AD years before the onset of clinical symptoms [2]. However, the usage of PET in detecting Aβ pathology in the general ageing population is limited due to high cost, technical complexity, and invasiveness. Furthermore, amyloid accumulation may be preceded, likely by years, by other alterations in vascular regulation [3]. Thus, more accessible measures of these earliest alterations, such as retinal measures, may be helpful to identify persons at risk for preclinical AD, better understand AD's pathogenesis, and allow developing and testing new preventive therapies in future clinical trials.

Previous studies showed that vascular risk factors and markers of cerebral vascular disease were associated with Aβ accumulation in AD [4, 5, 6]. These findings are in line with the 2-hit vascular hypothesis of AD stating that damage to the blood vessels is attributed to effects of genetic predispositions, vascular risk factors, diabetes, or hyperlipidemia as the initial insult resulting in blood-brain barrier (BBB) dysfunction and degraded brain perfusion subsequently causing neuronal injury and Aβ accumulation in the brain [7, 8]. Unfortunately, it has so far been very challenging to directly image the brain's small vessels in vivo to determine the cerebral microvascular contributions to the AD pathology.

One way to image in vivo microvascular changes in the eyes is to employ retinal fundus photography. The retinal microvasculature is both anatomically and physiologically similar to the small vessels in the brain, and retinal microvascular markers have been associated with cerebrovascular changes, incident clinical stroke and radiological markers of cerebral small vessel disease [9, 10, 11 12]. The clinical significance of retinal vascular signs has also been demonstrated in the ARIC study showing that microaneurysms and retinal hemorrhages were independently associated with 20-year cognitive decline and dementia conversion [13, 14]. Differences in retinal microvascular network alterations have also been observed in case-control studies with AD patients. Patients with AD had narrower arteriolar and venular calibers, smaller total and arteriolar

fractal dimensions and more tortuous arterioles and venules than their healthy counterparts [15]. Individuals with lower venular fractal dimension and arteriolar tortuosity were also more likely to be diagnosed with AD after accounting for confounders such as demographic factors, smoking, hypercholesterolemia, diabetes mellitus and history of cardiovascular disease and cerebrovascular disease [15] .

Only a few studies with small sample sizes have so far examined the association between retinal microvascular measures and Aβ pathology in preclinical AD participants. In the Australian Imaging, Biomarkers and Lifestyle (AIBL) study of ageing, participants with high neocortical Aβ burden had larger venular branching asymmetry factor (AFv) and arteriolar length-to-diameter ratio (LDRa). When combined with age and APOE E4 carrier status, AFv and LDRa predicted high Aβ burden with a high classification performance (82.8% AUC) [16]. Significant differences between preclinical AD and healthy controls were also found using optical coherence tomography angiography (OCT-A) measures. Patients with PET and CSF amyloid positive status had larger foveal avascular zone (FAZ) area compared to individuals with biomarker-negative findings. FAZ also predicted amyloid positive status with a high classification rate (*AUC*= 0.801) [17]. More cross-sectional and longitudinal studies with larger sample sizes are needed to test whether consistent associations between retinal microvascular signs and amyloid pathology exist in the population.

In this study, we propose testing the association between retinal microvasculature signs in midlife and amyloid accumulation in late life. Evidence of a significant association would potentially allow for a low cost, non-invasive method for identifying persons at risk for brain amyloid deposition in the general population. We furthermore propose to test the cross-sectional association between retinal microvascular signs and amyloid accumulation in late life. This would be the first study with a large sample size to determine the retinal microvascular associations with amyloid in late life and would allow us to evaluate if retinal health in midlife is associated with late life amyloid more strongly than is late-life retinal health, as is the case with other vascular risk factors. Finally, we propose to test whether the association between changes in retinal microvasculature between midlife and late life is associated with amyloid pathology. Significant associations may have clinical implications as repeated sampling of the retinal measures between mid and late life could identify individuals at risk for the amyloid progression in late life.

5. Main Hypothesis/Study Questions:

Main hypothesis:
Microvascular retinal signs measured in midlife are associated with elevated global cortical brain amyloid by PET in late life in the ARIC-PET cohort.

Secondary hypothesis:
Microvascular retinal signs in late life are associated with elevated global cortical brain amyloid by PET in late life in the ARIC-PET cohort.

Explorative hypothesis:
Change in microvascular retinal signs between midlife and late life are associated with brain amyloid by PET in late life in the ARIC-PET cohort.

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: cross-sectional & longitudinal

Participant inclusion: All ARIC-PET participants without dementia will be eligible for inclusion. 1 ARIC-PET participant was recruited who ultimately was given a research diagnosis of dementia. This participant will be excluded from the analysis.

Outcome: Florbetapir PET global cortical SUVR. SUVR will be treated continuous and dichotomized at the study median of 1.2 as is standard in ARIC-PET analyses.

Exposures: Retinal photographs collected for the first time in ARIC at Visit 3 (1993-95) and at Visit 5 will be used in this study. 4 most observed microvascular retinal signs by photography will be included in the analysis: retinopathy (and its components, including hemorrhages and microaneurysms), focal arteriolar narrowing, arteriovenous (AV) nicking, and generalized arteriolar narrowing (CRAE).

Covariates: Demographic variables such as age and vascular risk factors such as smoking status, drinking status, BMI, prevalent coronary heart disease, prevalent stroke, diabetes, and

hypertension will be used as covariates from visit 3 and 5. The time-invariant variables educational status and APOE4 gene status will also be included as covariates.

Classification variable: Participants' cognitive status was classified by an adjudication committee as cognitively normal, mild cognitive impairment or dementia based on a comprehensive battery of neurocognitive examinations.

Data analysis: The association between retinal microvascular signs and global SUVR will be tested using a logistic and linear regression model both in the cross-sectional and longitudinal analysis. The statistical assumptions underlying the regression models will be met. In case that the statistical assumptions are violated for the linear regression model, a robust linear regression model with bootstrapping or permutation will be employed. In the longitudinal analysis, a sensitivity analysis will also be employed restricting to those without diabetes in midlife and adjusting for diabetes in late life. This will test whether the associations between midlife retinal microvascular markers and amyloid accumulation in late life are not explained by diabetes.

We will use a two-step model building process for adjustment. Covariates in model 1 will include demographic variables, ApoE4, as well as race variable. In model 2 risk factors such as smoking status, drinking status, BMI, prevalent coronary heart disease, prevalent stroke, diabetes and hypertension will be entered as additional covariates. To test whether a model containing microvascular retinal measures and traditional vascular risk factors (model 2) significantly better captures the data than a model with traditional vascular risk factors only, a likelihood ratio test (LRT) will be employed. The areas under the curve (AUCs) showing the accuracy of the models' prediction will also be computed.

To determine the accuracy of prediction when only using the mid- vs. late-life retinal measures, receiver operating curves (ROC) will be computed and the models' AUCs will be compared. Using random forest models, the relative importance of the 4 retinal microvascular measures predicting amyloid accumulation will be determined.

A subgroup analysis will additionally be conducted testing the association between retinal microvascular measures and amyloid accumulation in those individuals with normal cognitive status at visit 3.

Changes in the continuous retinal microvascular variables will be determined and participants' slopes will be estimated using growth models. If the statistical assumptions of the growth model are not satisfied, the difference in retinal measures between visit 3 and visit 5 will be computed. The association between change in retinal microvascular signs and global SUVR will be tested using a logistic regression and a linear regression model adjusted by the covariates at visit 3. Again, if the statistical assumptions of the linear regression model are violated, a robust linear regression model with bootstrapping or permutation will be employed here.

7.a. Will the data be used for non-ARIC analysis or by a for-profit organization in this manuscript? ____ Yes _x__ No

b. If Yes, is the author aware that the current derived consent file ICTDER05 must be used to exclude persons with a value RES_OTH and/or RES_DNA = "ARIC only" and/or "Not for Profit" ? ____ Yes ____ No
(The 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? ___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 current derived consent file ICTDER05 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/aricproposals/dtSearch.html

___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 # 2822. Gottesman et al., Subclinical cerebrovascular disease and brain amyloid deposition: The ARIC-PET study

MP #2169 Deal et al. Association of retinal microvascular abnormalities with 23-year cognitive decline: The Atherosclerosis Risk in Communities Study

MP #2797 Deal et al. Retinal signs and incident dementia in the Atherosclerosis Risk in Communities Neurocognitive Study (ARIC NCS)

MP #3068 Lee et al. Association of Retinal Microvascular Abnormalities and Cognitive Status: The Atherosclerosis Risk in Communities Neurocognitive Study

MP #2565 Retinal Microvascular Abnormalities and Subsequent MRI Cerebrovascular and Neurodegenerative Signs: the Atherosclerosis Risk in Communities Neurocognitive Study (ARIC NCS)

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* __2009.29_____)
___ 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 https://sites.cscc.unc.edu/aric/approved-ancillary-studies

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.

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