

ARIC Manuscript Proposal #3911

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1.a. Full Title: Olfactory impairment and relations to microstructural integrity of the brain in the Atherosclerosis Risk in Communities Study

b. Abbreviated Title (Length 26 characters): Olfactory impairment and microstructural integrity of the brain

2. Writing Group:

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

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3. Timeline: Analysis will start immediately following proposal approval

4. Rationale:

Olfactory impairment (OI) can result from gradual or sudden damage to peripheral (e.g., olfactory epithelium) or central (e.g., olfactory bulb, entorhinal cortex) olfactory structures. Some known risk factors of OI include respiratory illnesses, head injury, and exposures to toxicants. OI can also be an early manifestation of neurodegenerative diseases including Alzheimer's disease (AD) and Parkinson's disease (PD) [1, 2]. Olfactory structures have been suggested to be one of the earliest brain structures to develop AD and PD-related neuropathology, decades before the onset of clinical symptoms.

With accumulating evidence on associations of OI with Alzheimer's dementia and PD risk [3-6], OI has been suggested to have potential to serve as a non-invasive early marker of these health outcomes [1, 2]. A few cross-sectional studies have also linked OI with adverse neuropathological outcomes including diminished primary and secondary olfactory cortices and elevated brain amyloid accumulation in cognitively normal individuals [7-9], although inconsistently [10, 11]. However, prospective studies are currently inadequate, and additional clarity on where OI lies in the pathophysiological continuum of dementia is warranted. We also lack adequate evidence of the association of OI with brain effects other than those known to be targeted by Alzheimer's pathogenesis.

Diffusion tensor imaging (DTI), a magnetic resonance imaging (MRI) technique that provides measures of tissue microstructure [12, 13], has been widely used for studying white matter (WM) of the brain, although less often for gray matter (GM) microstructure [14]. Relevantly, DTI based measures of WM microstructural integrity loss have been shown to antecede WM hyper-intensities/lesions [15, 16] and have been linked with future cognitive decline [17, 18], suggesting that DTI-based assessment may inform about neurodegeneration much earlier, prior to emergence of more conspicuous pathology. Research on DTI measures of the brain and olfaction, both subclinical early manifestations of neurodegeneration, can provide further clarity on the pathophysiology of Alzheimer's dementia and PD. However, to our knowledge, apart from a few cross-sectional clinical studies comparing DTI measures and OI between cognitively normal individuals and clinical PD and AD cases [19, 20] or a correlational study in non-demented adults [21] (all with sample sizes < 100), there are no well-powered epidemiologic studies on DTI measures and olfaction.

Some cerebral substrates involved in olfactory processing are also associated with neurocognitive functions including memory and learning. So, neurodegenerative changes in those common brain regions may partially underlie the observed association between OI and neurocognitive decline, although other underlying pathophysiological processes may also contribute. Studies on DTI based measures of neuronal integrity may inform about possible contribution of earliest neurodegenerative changes in the association between OI and cognitive decline. However, such studies are lacking.

The Atherosclerosis Risk in Communities (ARIC) Study is a bi-racial prospective cohort study of community-based US adults (enrolled 1987-1989). The ARIC study collected comprehensive information on socio-demographics, cardiovascular, and inflammatory risk profiles at enrollment and all follow-up surveys from study participants; the study measured olfaction at visit 5 (2011-2013) and visit 6 (2015-2016) and obtained MRI of the brain including DTI at visit 5. Here, to address prior research gaps, we propose the following analyses:

- (i) to examine associations of DTI measures of WM and GM microstructures with visit 5 olfaction and decline in olfaction from Visit 5 to Visit 6 (Aim 1);

- (ii) to evaluate the patterns of the DTI signs related to OI to determine whether they suggest the specificity of OI as a marker of Alzheimer's pathology, and whether this marker is effective in identifying AD associated pathology prior to the onset of cognitive impairment (Aim 2);
- (iii) to determine if DTI measures will explain associations between olfaction and cognition (Aim 3). This aim will be an extension of two previous ARIC investigations that examined association between olfaction and cognition [22] and DTI based measures and cognition [17].

5. Main Hypothesis/Study Questions:

Aims 1 and 2:

a: To examine cross-sectional associations between visit 5 olfaction and visit 5 DTI measures of WM and GM microstructures

Hypothesis: We hypothesize that poor DTI measures, specifically in brain regions involved in olfactory processing (i.e., orbitofrontal, temporal, and fusiform regions, amygdala, entorhinal area, hippocampus, and thalamus) and related pathways, will be associated with poor olfaction.

Alternative Hypothesis: Poor olfaction will be associated only with those regions which are known targets of early Alzheimer's pathogenesis: e.g. entorhinal area, hippocampus, and others.

Given that ability to identify smell also depends on neurocognitive ability, and dementia and PD are progressive in nature, we may detect associations between poor olfaction and DTI measures in brain regions other than those implicated in olfaction.

b: To examine if visit 5 DTI measures of WM and GM microstructures in brain regions involved in olfaction (namely, orbitofrontal, temporal, and fusiform regions, amygdala, entorhinal area, hippocampus, and thalamus) are associated with decline in olfaction.

Hypothesis: We hypothesize that longitudinal olfactory decline will show stronger associations with DTI measures in olfactory-eloquent regions.

Note: the findings from the current evaluation, in conjunction with those from prior investigations, will help evaluate whether the patterns of the DTI signs related to OI suggest the specificity of OI as a marker of Alzheimer's pathology, and whether this marker is effective in identifying AD associated pathology prior to the onset of cognitive impairment.

Aim 3: To determine if visit 5 DTI measures found to be related to olfaction explain the association between visit 5 olfaction and cognition

Hypothesis: We hypothesize that DTI measures will partially explain the association between olfaction and cognition.

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: Cross-sectional and prospective

Diffusion Tensor Imaging (DTI) measures: We will use fractional anisotropy (FA) and mean diffusivity (MD) as measures of WM microstructural integrity, and MD as a measure of GM microstructural integrity. The ARIC study has employed two separate atlases to estimate MD and FA for brain regions: (i) the Lobar 22 atlas based on the STAND400 (created at Mayo Clinic) that delineates the brain into twenty two lobar and deep WM regions [23]; and (ii) the Johns Hopkins University (JHU) single subject atlas that provides more detailed, hemisphere-specific WM tracts and GM structures [24]. We will use ROI MD and FA estimates from both atlases. Although not all brain regions are involved in olfactory processing, we will consider exploring all the regions delineated by the atlases for the Aim 1a (i.e., cross-sectional association between olfaction and DTI measures). Given that ability to identify smell also depends on neurocognitive ability, and that dementia and PD are progressive in nature, we expect to detect associations with DTI measures in brain regions other than those implicated in olfaction. For Aim 1b (i.e., DTI measures and decline in olfaction), we will focus on DTI measures of the brain regions involved in olfaction (identified *a priori* from the literature or from Aim 1a).

Measures of olfaction: The 12-item Sniffin' Sticks screening test was used to measure olfaction at visits 5 and 6. We will use smell identification test score as a continuous olfaction variable; additionally, we will create a categorical outcome anosmia (defined using a conventional cut-off of score ≤ 6)[25].

Other covariates: Age, sex, race, field center, smoking status, body mass index, heart disease, hypertension, diabetes, PD status, dementia status, head injury, *APOE* $\epsilon 4$, visit 5 cognitive status, total intracranial volume, WM hyper-intensity volume, hippocampal volume

Statistical analysis:

Aims 1 and 2: DTI measures (FA and MD) as predictors of olfaction/ Evaluation of the patterns of the DTI signs related to OI to determine whether they suggest the specificity of OI as a marker of Alzheimer's pathology

The analyses will be restricted to visit 5 participants who underwent MRI (including those with mild cognitive impairment) and with complete data on olfaction and covariates of interest. We will also perform sensitivity analyses (i) excluding participants with dementia and PD and (ii) restricting to cognitively normal individuals to evaluate whether the DTI association with olfaction occurs before cognitive impairment.

a: Visit 5 Olfaction measures in relation to visit 5 DTI

We will use multi-level linear regression models to examine cross-sectional associations of visit 5 olfaction test scores and anosmia prevalence with DTI measures accounting for correlation within brain regions (as delineated by the Lobar 22 or JHU atlases) and adjusting for potential confounders. We will consider age, sex, race-site, smoking status, prevalent heart disease, hypertension, diabetes, head injury, APOE $\epsilon 4$, cognitive status, intracranial volume, and WM hyperintensity volume as potential confounders (all assessed at visit 5). We will incorporate sampling weights to account for sampling approach used to select participants for MRI. We will also consider Bonferroni correction and Benjamini-Hochberg false discovery rate adjustment to deal with multiple comparisons.

b: Visit 5 DTI in relation to olfaction score change through visit 6

We will focus on the brain regions that are associated with olfaction (identified *a priori* from the literature and from Aims 1/2a). We will use linear mixed models with random slopes and random intercepts to examine the associations between visit 5 DTI measures and changes in continuous olfaction test scores over time adjusting for potential confounders (confounders same as above). We will examine distributional assumptions and, when they are not met, we will consider other appropriate distribution/family in the generalized linear mixed model framework. Further, we will use mixed-effects logistic regression to examine longitudinal change in anosmia. We will incorporate sampling weights to account for sampling approach used to select participants for MRI. We will perform sensitivity analysis using shared parameter models to examine potential bias due to cohort attrition [26].

Aim 3: To determine if visit 5 DTI measures explain the association between olfaction and cognition

This aim will focus on DTI measures of the brain regions involved in olfaction. We will use visit 5 data to examine how much of the association between olfaction and cognition is explained by DTI measures using two linear regression models. With cognition being the outcome, the first model will include olfaction as a predictor while the second model will include both DTI measures and olfaction, both adjusting for potential confounders. We will assess whether the association between olfaction and cognition is attenuated when adjusted for DTI measures and will estimate how much of the association is explained by DTI measures. We will also use Generalized Structural Equation Modeling (GSEM) techniques to identify ‘mediation’ pathways and construct direct and indirect effect estimates. We may also consider longitudinal analysis using data on olfaction and cognition from later visits.

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”? 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/mantrack/maintain/search/dtSearch.html>

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)?

- Dong, J., et al., The prevalence of anosmia and associated factors among U.S. black and white older adults. J Gerontol A Biol Sci Med Sci, 2017. 72(8): p. 1080-1086.
- Palta, P., et al., Olfactory function and neurocognitive outcomes in old age: The Atherosclerosis Risk in Communities Neurocognitive Study. Alzheimers Dement, 2018. 14(8): p. 1015-1021.
- ARIC Manuscript Proposal #2841 Mid-life biomarkers in relation to anosmia late in life
- ARIC Manuscript Proposal #3423: Neural correlates of anosmia among persons with and without mild cognitive impairment: A voxel-based morphometry (VBM) study (Kamath; Schneider)

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

11.b. If yes, is the proposal

A. primarily the result of an ancillary study (list number* 2008.06, 2010.17, 2020.01)

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.csc.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.csec.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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