ARIC Manuscript Proposal #3423

PC Reviewed: 6/18/19	**Status: _____**	**Priority:2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1a. Full Title: Neural correlates of anosmia among persons with and without mild cognitive impairment: A voxel-based morphometry (VBM) study

b. Abbreviated Title (Length 26 characters): Neural correlates of olfaction in aging

2. Writing Group:
Writing group members (in alphabetical order): Rebecca F. Gottesman, Clifford Jack, Vidyulata Kamath, David Knopman, Priya Palta, Andrea L.C. Schneider, A. Richey Sharrett, Matthew L. Senjem, others welcome

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

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3. Timeline: Data are currently available. Analyses and manuscript preparation will be performed over the next 6-12 months.

4. Rationale: Olfaction has emerged as a promising yet relatively underutilized sensory pathway for understanding neurodegenerative disease risk. The olfactory system is centrally located in the medial forebrain and the olfactory nerve is the only cranial nerve with direct exposure to the environment, making it especially vulnerable to illness, injury, and environmental pathogens. Age-related olfactory decline is hypothesized to result from structural and functional changes in the nose, olfactory epithelium, and cortical brain regions involved in smell perception. The pathologic processes associated with Alzheimer's disease (AD) also affect olfaction, and the olfactory areas affected in AD may differ from the olfactory areas affected by other processes.

The olfactory neuroepithelium becomes increasingly compromised as we age, resulting in reduced clearance of bacteria and other agents, fewer olfactory receptor cells and regression of the olfactory vasculature. The cumulative effects of exposure to air-borne environmental agents, including air pollution, cigarette smoke, viruses, and bacteria, leads to damage of the olfactory epithelium, and is posited to have greater functional consequences over the lifespan. In addition, age-related changes observed in olfactory bulb size and in brain regions important for olfactory processing have been attributed to diminished afferent neural activity transmitted by damaged olfactory receptor cells. Brain regions important for olfactory processing include the primary olfactory areas (piriform cortex, entorhinal cortex (ERC), amygdala, and anterior olfactory nucleus) and the secondary olfactory areas (insula, orbitofrontal cortex, anterior cingulate, hippocampus, and thalamus).

Postmortem studies indicate that the olfactory system is one of the earliest sites of tau deposition in AD and α-synuclein pathology in Parkinson's disease (PD), even in the absence of overt dementia (Kovács, Cairns, & Lantos, 2001; Ohm & Braak, 1987). In rodent models, the early emergence of olfactory deficits is associated with high levels of soluble amyloid beta (Aβ) in the olfactory cortex, a primary input to the lateral ERC (Xu, Fitzgerald, Nixon, Levy, & Wilson, 2015). These findings correspond with cross-sectional and longitudinal human studies, which demonstrate a relationship between olfactory impairment and thinner entorhinal cortices in cognitively normal elderly (Albers et al., 2016). Similarly, Bitter et al. (2010) used voxel-based morphometry (VBM) to conduct whole-brain comparisons of grey and white matter density associated with olfactory loss. Persons with anosmia (n = 17) showed reduced grey matter volume in the medial orbitofrontal cortex (mOFC), the right piriform cortex, and the dorsolateral prefrontal cortex. Similarly, Peng et al. (2013) found that anosmia was associated with reductions in secondary olfactory regions compared to primary olfactory regions. In contrast, Segura et al. (2013) found that odor identification ability was associated with bilateral perirhinal and entorhinal gray matter volume and right amygdala volume in 30 cognitively normal individuals. Findings from the Mayo Clinic Study of Aging (MCSA) provide one of the largest examinations of olfactory and volumetric MRI measures to date (Vassilaki et al., 2017). The authors examined the association between odor identification impairment and neuroimaging indices supportive of AD, including amyloid accumulation on ^{11}C-PiB PET and an AD-signature of abnormal cortical thickness (calculated from the average cortical thickness of the entorhinal, inferior temporal, middle temporal, and fusiform cortices). These neuroimaging markers were significantly associated with increased odds of anosmia in a cohort of 829 cognitively normal older adults.

Olfactory deficits are also prominent in probable AD and in otherwise cognitively normal individuals who ultimately transition to mild cognitive impairment (MCI) or Alzheimer's dementia at follow-up (Devanand et al., 2015; Schubert et al., 2008; Wilson et al., 2007; Yaffe et al., 2017). Several studies have established a relationship between olfactory dysfunction and reduced volumes in primary olfactory brain regions affected in early AD. Murphy et al. (2003) found a relationship between odor identification impairment and reduced left hippocampal volumes in a small sample of probable AD patients, a finding later replicated in a mixed group of amnestic MCI and AD patients (Kjelvik et al., 2014). Vasavada et al. (2015) characterized primary olfactory cortex volumes in MCI and AD patients and controls. Of note, reduced olfactory cortex volume was associated with behavioral olfactory impairment in the MCI and AD groups. In a pilot study of 17 amnestic MCI patients, Marigliano et al. (2014) compared the utility of olfactory testing to hippocampal volume loss in predicting conversion to Alzheimer's

dementia at one-year follow-up. The authors found that both biomarkers had comparable sensitivities at 92.3%; however, olfactory testing had higher specificity (75% vs. 60%) when compared to hippocampal volume loss. There was no association between olfactory scores and hippocampal volumes.

Collectively, prior work has demonstrated an association between olfactory measures and reduced volumes in brain regions affected in early AD. However, these studies are limited by their small sample sizes and/or predominantly white cohorts. Population-based studies examining the relationship between olfaction and structural MRI in multi-racial populations are needed to replicate and extend these findings. The ARIC cohort provides a unique opportunity to contribute to the existing literature by examining these associations in a well-characterized biracial sample of older adults with data on olfaction, multidimensional cognition, structural neuroimaging, and adjudicated MCI/dementia outcomes. We propose to use voxel-based morphometry to examine brain regions associated with anosmia in the presence of mild cognitive impairment vs. regions associated with anosmia in the absence of cognitive impairment. In this study, anosmia is determined by scores on the 12-item Sniffin' Sticks' screening test and cognitive impairment is classified as adjudicated MCI versus no MCI. Particular attention will be given to possible differences in these associations by race and gender subgroups. Based on prior work, we anticipate that areas in the medial orbitofrontal cortex, amygdala, entorhinal cortex, and piriform cortex will be associated with anosmia in cognitively normal individuals. In individuals with MCI, we hypothesize that anosmia will show greater associations with volume loss in AD-signature regions (entorhinal, inferior temporal, middle temporal, and fusiform cortices).

5. Main Hypothesis/Study Questions:

Aim 1: Voxel-based morphometry (VBM) measures structural neuroimaging differences (i.e., tissue volumes) between groups on a voxel-by-voxel basis without limitation to one specific brain region. VBM will be used to conduct whole brain comparisons of grey matter density associated with olfactory loss in cohorts with and without mild cognitive impairment. Participants will be stratified into four groups in a 2x2 design (see below). VBM will be used to compare group A versus C and group B versus group D.

Hypothesis 1: We hypothesize that VBM maps in anosmic cognitively normal individuals (Group A vs. C) will show smaller grey matter volumes in the medial orbitofrontal cortex, amygdala, entorhinal cortex, piriform cortex and hippocampus. We hypothesize that VBM maps in anosmic MCI patients (Group B vs. D) will show smaller grey matter volumes predominantly in AD-signature regions, including the entorhinal, inferior temporal, middle temporal, and fusiform cortices.

	No MCI/Dementia	MCI
Anosmia	**A** Anosmia, No MCI/Dementia	**B** Anosmia and MCI
Normosmia	**C** Normosmia, No MCI/Dementia	**D** Normosmia and MCI

Aim 2: Next, we will use a region of interest (ROI)-based approach to examine anatomical differences. The ROI analyses will focus on measuring brain regions previously reported to be reduced in individuals with anosmia. These include the primary olfactory areas (piriform cortex, entorhinal cortex, amygdala, and anterior olfactory nucleus) and the secondary olfactory areas (insula, orbitofrontal cortex, anterior cingulate, hippocampus, and thalamus). We will also examine any ROIs that may be suggested by VBM analysis.

Hypothesis 2: We hypothesize gray matter volumes in the medial orbitofrontal cortex, amygdala, entorhinal cortex, piriform cortex and hippocampus ROIs will be smaller among those with anosmia versus normosmia in cognitively normal individuals. Among persons with MCI or lower cognitive scores, anosmia versus normosmia will be associated more specifically with smaller gray matter volumes in AD-signature regions, including the entorhinal, inferior temporal, middle temporal, and fusiform cortices.

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 analysis using olfaction, MRI data, and adjudicated MCI/cognitive test scores from ARIC visit 5 (2011-2013).

Inclusion/Exclusion Criteria: Participants self-identified as non-white or non-black race, black participants from the Minnesota and Maryland sites, and participants with dementia will be excluded from this analysis.

Exposure: Olfaction was assessed using the 12-item Sniffin' Sticks[1] at ARIC visit 5. Participants were asked to smell 12 common odorants (orange, leather, cinnamon, peppermint, banana, lemon, licorice, coffee, cloves, pineapple, rose, and fish) and then were asked to identify each odorant using multiple-choice format. Each correctly identified odorant was assigned one point, with a total possible score of 12. A score of ≤6 will be used to define impaired olfaction (anosmia).

Outcome: Brain MRI scans at ARIC visit 5 were performed using four 3-Tesla scanners (Maryland: Siemens Verio; North Carolina: Siemens Skyra; Minnesota: Siemens Trio; Mississippi: Siemens Skyra). Each participant's T1 weighted MRI scan will be segmented into gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) probability map images using SPM12 unified segmentation[3] with the Mayo Clinic Adult Lifespan Template (MCALT; https://www.nitrc.org/projects/mcalt/) tissue priors[4] and population-optimized segmentation settings[5]. For VBM analyses, the GM images will be spatially normalized to the MCALT space, modulated and smoothed with an 8-mm full width at half maximum (FWHM) Gaussian kernel. The smoothed, modulated normalized GM images will then be used in the SPM12 general linear model framework to estimate models of associations between the olfaction/MCI groups and GM volume on a voxel-wise basis and results will be displayed in voxel-based morphometry maps. In these analyses, correction for multiple comparisons will be performed using false discovery rate with $p<0.05$.

For the region of interest level analyses, an atlas consisting of 122 ROI labels, derived from the automated anatomic labeling (AAL) atlas[6], will be propagated from the MCALT space to each participant's MRI native space using Advanced Normalization Tools (ANTs) software[7]. The participant space atlas labels will then be used to parcellate each participant's GM images into regions of interest and the GM volume for each region of interest will be computed by summing up the GM probabilities within each region of interest and multiplying by the voxel volume. To obtain total intracranial volume the GM, WM and CSF probabilities will be summed and thresholded. Hole filling and morphological operations will be performed to remove any spurious disconnected regions.

Covariates: Covariates were assessed at ARIC Visit 5, unless otherwise specified. Age, sex, education (assessed at ARIC Visit 1, <high school; high school, GED or vocational school; college, graduate, or professional school), race (white; black), study center (suburbs of Minneapolis, MN; Washington County, MD; Forsyth County, NC; Jackson, MS), cigarette smoking status (ever; never), diabetes mellitus (DM; defined as fasting glucose ≥ 126 mg/dL, HbA1c ≥ 6.5%, self-reported history of DM diagnosis, or use of DM medication); hypertension (defined as systolic blood pressure ≥ 140 mm HG, diastolic blood pressure ≥ 90 mm HG, or use of blood pressure-lowering medication); apolipoprotein E ε4 genotype (APOE-ε4; 0 versus 1 or 2 alleles); and history of head injury.

Statistical Analyses: Descriptive sample characteristics will be compared across olfaction/MCI groups using t-tests for continuous variables and $\chi 2$ tests for categorical variables. Olfaction and MCI will be analyzed as a dichotomous variable in our primary analysis. We will consider sensitivity analyses using the 12-item Sniffin' Sticks score as a continuous variable and using continuous variables to assess cognition using the ten-test factor score administered at ARIC Visit 5 (Rawlings et al., 2016).

For the voxel-based analyses, SPM12 general linear model framework will be used to estimate models of associations between the olfaction/MCI groups and GM volume on a voxel-wise basis and results will be displayed in voxel-based morphometry maps. VBM will be used to compare group A versus C and group B versus group D (see groups described above). In these analyses, correction for multiple comparisons will be performed using false discovery rate with $p<0.05$.

For ROI analyses, we will use adjusted linear regression models to examine the associations of olfactory impairment with *a priori* hypothesized group of ROI volumes. We will also conduct exploratory analyses across individually hypothesized ROIs, and in exploratory analysis, other ROI's as well. Age, sex, race, smoking status and number of ApoE ε4 alleles will be included as covariates. Additional models will be stratified by race-center and ApoE status to evaluate for interactions in the association of olfaction with ROI volumes by gender, race or ApoE status.

In our analyses, we will compare the associations of anosmia with ROI volumes in persons with MCI versus those without MCI by including an interaction term for MCI status in the associations of olfaction with ROI volumes.

Categorical Model:
ROI volume = anosmia, MCI, anosmia X MCI, age, sex, center, smoking, ApoE status

Anosmia = score ≤6 will be used to define impaired olfaction (anosmia) from Sniffin Sticks
MCI = adjudicated ARIC outcome (and in sensitivity analyses: presence or absence based on ten-test factor score)

Continuous Model:
ROI = olfaction score, cognition score, olfaction score X cognition score, age sex, center, smoking, ApoE status

Olfaction score = 12-item Sniffin Sticks (continuous)
Cognition score = 10-test factor score (continuous)

To examine potential bias from attrition or missing MRI data on our findings, we will conduct sensitivity analyses to account for missing data as appropriate using inverse probability weighting. Prior work has demonstrated that smell loss is associated with amnestic MCI, obesity, Parkinson's disease and head trauma. Therefore, if sample size permits, further sensitivity analyses will examine the association of anosmia and MCI type (e.g., amnestic MCI), BMI, history of head injury (variable derived by Andrea Schneider), and Parkinson's disease.

7a. 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.)

8a. Will the DNA data be used in this manuscript? __ Yes _X_ No

8b. 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.cscc.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)?

#2445: Prevalence and associated factors of anosmia (Honglei Chen)

#2586: Neural correlates of prior domain-specific cognitive decline: a voxel-based morphometry study (Andrea Schneider)

#2872: Olfactory function and neurocognitive outcomes in old age: The Atherosclerosis Risk in Communities Neurocognitive Study (Priya Palta)

#2957: Interrelationships of Olfaction, Brain Amyloid, and Cognition: the ARIC-PET Study (Kimystian Harrison)

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

11b. If yes, is the proposal
_X_ A. primarily the result of an ancillary study (list number* ARIC NCS 2008.06)
__ 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

Albers, A., Asafu-Adjei, J., K. Delaney, M., E. Kelly, K., Gomez-Isla, T., Blacker, D., … Albers, M. (2016). Episodic Memory of Odors Stratifies Alzheimer Biomarkers in Normal Elderly: POEM: odor memory biomarker in normal elderly. *Annals of Neurology*, *80*. https://doi.org/10.1002/ana.24792

Bitter, T., Gudziol, H., Burmeister, H. P., Mentzel, H.-J., Guntinas-Lichius, O., & Gaser, C. (2010). Anosmia leads to a loss of gray matter in cortical brain areas. *Chemical Senses*, *35*(5), 407–415. https://doi.org/10.1093/chemse/bjq028

Devanand, D. P., Lee, S., Manly, J., Andrews, H., Schupf, N., Doty, R. L., … Mayeux, R. (2015). Olfactory deficits predict cognitive decline and Alzheimer dementia in an urban community. *Neurology*, *84*(2), 182–189. https://doi.org/10.1212/WNL.0000000000001132

Kjelvik, G., Saltvedt, I., White, L. R., Stenumgård, P., Sletvold, O., Engedal, K., … Håberg, A. K. (2014). The brain structural and cognitive basis of odor identification deficits in mild

cognitive impairment and Alzheimer's disease. *BMC Neurology*, *14*(1), 168. https://doi.org/10.1186/s12883-014-0168-1

Kovács, T., Cairns, N. J., & Lantos, P. L. (2001). Olfactory centres in Alzheimer's disease: olfactory bulb is involved in early Braak's stages: *Neuroreport*, *12*(2), 285–288. https://doi.org/10.1097/00001756-200102120-00021

Marigliano, V., Gualdi, G., Servello, A., Marigliano, B., Volpe, L. D., Fioretti, A., … Fusetti, M. (2014). Olfactory deficit and hippocampal volume loss for rarly diagnosis of Alzheimer disease: A pilot study. *Alzheimer Disease & Associated Disorders*, *28*(2), 194–197. https://doi.org/10.1097/WAD.0b013e31827bdb9f

Murphy, C., Jernigan, T. L., & Fennema-Notestine, C. (2003). Left hippocampal volume loss in Alzheimer's disease is reflected in performance on odor identification: A structural MRI study. *Journal of the International Neuropsychological Society*, *9*(03). https://doi.org/10.1017/S1355617703930116

Ohm, T. G., & Braak, H. (1987). Olfactory bulb changes in Alzheimer's disease. *Acta Neuropathologica*, *73*(4), 365–369. https://doi.org/10.1007/BF00688261

Peng, P., Gu, H., Xiao, W., Si, L. F., Wang, J. F., Wang, S. K., … Wei, Y. X. (2013). A voxel-based morphometry study of anosmic patients. *The British Journal of Radiology*, *86*(1032), 20130207. https://doi.org/10.1259/bjr.20130207

Rawlings, A. M., Bandeen-Roche, K., Gross, A. L., Gottesman, R. F., Coker, L. H., Penman, A. D., … Mosley, T. H. (2016). Factor structure of the ARIC-NCS Neuropsychological Battery: An evaluation of invariance across vascular factors and demographic characteristics. *Psychological Assessment*, *28*(12), 1674–1683. https://doi.org/10.1037/pas0000293

Schubert, C. R., Carmichael, L. L., Murphy, C., Klein, B. E. K., Klein, R., & Cruickshanks, K. J. (2008). Olfaction and the 5-year incidence of cognitive impairment in an epidemiological study of older adults. *Journal of the American Geriatrics Society*, *56*(8), 1517–1521. https://doi.org/10.1111/j.1532-5415.2008.01826.x

Vasavada, M. M., Wang, J., Eslinger, P. J., Gill, D. J., Sun, X., Karunanayaka, P., & Yang, Q. X. (2015). Olfactory Cortex Degeneration in Alzheimer's Disease and Mild Cognitive Impairment. *Journal of Alzheimer's Disease*, *45*(3), 947–958. https://doi.org/10.3233/JAD-141947

Vassilaki, M., Christianson, T. J., Mielke, M. M., Geda, Y. E., Kremers, W. K., Machulda, M. M., … Roberts, R. O. (2017). Neuroimaging biomarkers and impaired olfaction in cognitively normal individuals: Biomarkers and Olfaction. *Annals of Neurology*, *81*(6), 871–882. https://doi.org/10.1002/ana.24960

Wilson, R. S., Schneider, J. A., Arnold, S. E., Tang, Y., Boyle, P. A., & Bennett, D. A. (2007). Olfactory identification and incidence of mild cognitive impairment in older age. *Archives of General Psychiatry*, *64*(7), 802–808. https://doi.org/10.1001/archpsyc.64.7.802

Xu, W., Fitzgerald, S., Nixon, R. A., Levy, E., & Wilson, D. A. (2015). Early hyperactivity in lateral entorhinal cortex is associated with elevated levels of AβPP metabolites in the Tg2576 mouse model of Alzheimer's disease. *Experimental Neurology*, *264*, 82–91. https://doi.org/10.1016/j.expneurol.2014.12.008

Yaffe, K., Freimer, D., Chen, H., Asao, K., Rosso, A., Rubin, S., … Simonsick, E. (2017). Olfaction and risk of dementia in a biracial cohort of older adults. *Neurology*, *88*(5), 456–462. https://doi.org/10.1212/WNL.0000000000003558