ARIC Manuscript Proposal #2841

1.a.  **Full Title**: Mid-life biomarkers in relation to anosmia late in life

b.  **Abbreviated Title (Length 26 characters)**: biomarkers and anosmia

2.  **Writing Group**:

Writing group members: Honglei Chen (NIEHS, PI), Bojing Liu (Karolinska student of Drs. Wirdefeldt and Chen), Thomas Mosley, Alvaro Alonso, Xuemei Huang, Fang Fang (Karolinska Institute), and Karin Wirdefeldt (Karolinska Institute)

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

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3.  **Timeline**: Manuscript submitted by July 2017

4.  **Rationale**:  
The human sense of smell declines with age, and poor sense of smell may affect up to 25% of the older US adults¹. Olfactory impairment jeopardizes mental and physical health, diminishes quality of life, and is an independently predictor for higher mortality².
Further, olfactory impairment is a prodromal symptom for Parkinson’s disease (PD) and it is associated with a higher risk of cognitive decline and Alzheimer’s disease (AD). Therefore research on olfaction impairment among older adults may also inform early detection and the etiology of major neurodegenerative diseases, PD in particular.

There is limited research on potential risk factors for olfaction impairment in older adults. A few cross-sectional studies have reported that the sense of smell decreased with age and was poor in men than in women; data on other factors such as smoking and head injury are inconsistent. To the best of our knowledge, there are only two prospective studies where exposures were assessed prior to the sense of smell test. We therefore propose to evaluate several biomarkers assessed in earlier ARIC clinical visits (V1-V4) in relation to the sense of smell at visit 5.

We choose to focus on plasma/serum levels of pro-inflammatory markers, urate, and cholesterols, mainly because of their potential roles in PD development. These biomarkers have been associated with the risk for PD in prospective studies, including ARIC. There are also biologically plausible hypotheses for these associations, for example, hCpCRP and IL-6 as biomarkers of chronic inflammation, urate as an antioxidant, and cholesterol in maintaining neuronal integrity. However, as PD may take decades to develop; there are suspicions that these biomarkers may be the results or byproducts of ongoing neurodegeneration. Therefore, investigations on early symptoms of neurodegeneration such as olfactory impairment may help understand roles of these biomarkers and relevant biological mechanisms in neurodegeneration.

It is also important to study these biomarkers in relation to olfactory impairment in their own right. We will use proinflammatory biomarkers as an example. Experimental studies suggest that a single intravenous injection of LPS induced increased expression of proinflammatory cytokines such as TNF-α and IL-6 in the olfactory bulb, and staphylococcus aureus administration compromised the olfactory epithelia cell and olfactory bulb and induced expression of proinflammatory cytokines. To the best of our knowledge, only two small cross-sectional and one prospective studies have evaluated peripheral biomarkers of inflammation and olfactory impairment. One study reported significantly higher levels of IL-6 in plasma, saliva and nasal mucus in patients of anosmia than in controls. In another study of patients with end stage renal disease, higher CRP level was also associated with poor sense of smell. The prospective study (n=1,611) reported a similar but non-significant association of anosmia with CRP, but not with IL-6 or TNF-a. Compared with these studies, the ARIC study evaluated the sense of smell of 6,078 participants, and determined several inflammatory biomarkers at various time points 15-25 years prior to the assessment of the sense of smell. We therefore are in an excellent position to evaluate the role of inflammation in mid to late adulthood in relation to anosmia. Similar rationales could also be made for plasma urate and cholesterols.

5. **Main Hypothesis/Study Questions:**
Hypothesis: Higher plasma levels of pro-inflammatory biomarkers (hs-CRP, white blood cell count, and fibrinogen) and lower levels of plasma urate and total/LDL-cholesterols are associated with poor sense of smell and higher prevalence of anosmia later in life.


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: Examine plasma/serum levels of CRP, fibrinogen, urate, and lipids at ARIC clinical visits 1-4 (1987-1998) in relation to the sense of smell and anosmia at visit 5 (2011-2013). Main analytic variables are listed in the table below.

<table>
<thead>
<tr>
<th>Table Main outcome and exposure variables and collection time</th>
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<tbody>
<tr>
<td><strong>Outcome</strong></td>
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<tr>
<td>Sense of smell / Anosmia</td>
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<tr>
<td><strong>Clinical</strong></td>
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<tr>
<td>Parkinson’s disease</td>
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<tr>
<td>Dementia/MCI</td>
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<tr>
<td><strong>Biomarkers</strong></td>
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<tr>
<td>hsCRP</td>
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<tr>
<td>Fibrinogen</td>
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<td>White blood cell count</td>
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<tr>
<td>Cognitive function</td>
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<tr>
<td>Cholesterol (total/LDL/HDL)</td>
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<tr>
<td>Triglyceride</td>
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<tr>
<td>Apolipoprotein A1</td>
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<td>Apolipoprotein B</td>
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<tr>
<td>Urate</td>
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<tr>
<td><strong>Other exposures / confounders</strong></td>
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<tr>
<td>Smoking</td>
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<tr>
<td>Coffee drinking</td>
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<td>Head injury</td>
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<td>Body mass index</td>
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<td>Education</td>
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Inclusion and exclusion criteria: The analytic population includes all ARIC participants with a valid sense of smell test at visit 5 and non-missing biomarker data. The actual analytic sample size may however differ by analysis, mainly depending on when the specific biomarker was evaluated. Exclusion rules of ARIC on non-genetic and non-cardiovascular research will also apply.

Outcome – the sense of smell and anosmia: The ARIC study used the 12-item Sniffin’ Sticks screening test (SS, Burghart, Wedel, Germany) to evaluate the sense of smell. This is a brief screening test for olfactory deficit that have been widely used in clinical
and epidemiological studies.\textsuperscript{12,13} The test asks participants to smell 12 common odorants, one at a time, and to identify the correct odorant from four possible answers in a forced multiple-choice format. One point was given for each correct answer with a total score ranging from 0 to 12. Although we also will analyze the sense of smell as a continuous variable, our primary outcome will be anosmia or loss of the sense of smell. Anosmia will be defined as a smell identification score ≤ 6, a threshold that has been validated using SS with clinically confirmed anosmia patients.\textsuperscript{12} Our preliminary cross-sectional analyses showed that 14\% of the ARIC participants had anosmia, and in our cross-sectional analysis, a higher prevalence was associated with older age, male gender, black race, poor cognitive status, PD, ApoE e4 allele, and a few other factors.

Data analyses: The primary outcome is anosmia. We will evaluate associations of biomarker with anosmia using logistic regression models and report odds ratios and 95\% confidence intervals. Biomarkers will be defined as quartiles and P for linear trend will be tested using the median of each quartile as a continuous variable. All statistical models will adjusted for age, sex, race throughout the analyses. Other potential covariates such as smoking, education, and coffee drinking will be included in the model if they change risk estimates 10\% or more. The primary analyses will include all eligible participants, and we will also conduct sex and race specific analyses. In addition to the primary analyses, we also plan to conduct parallel analyses using the sense of smell total score as a continuous variable. Appropriate transformation will be performed to account for the distribution of the sense of smell score.

Potential method limitations and challenges: The analyses will be limited to participants who survived and participated in the 5\textsuperscript{th} clinical visit and the NCS. We plan to conduct additional analysis using inverse probability weighting.

7.a. Will the data be used for non-CVD analysis in this manuscript? \textbf{X} Yes \hspace{1cm} \underline{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? \textbf{X} Yes \hspace{1cm} \underline{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? \textbf{X} Yes \hspace{1cm} \underline{No}

8.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”? \textbf{X} Yes \hspace{1cm} \underline{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)?

This project will be conducted by the PD working group of ARIC. We have four recent publications: 1) HRV and PD on *Annals of Neurology*; 2) cholesterol and PD on *Movement Disorders*; 3) vitamin D and PD on *Movement Disorders*; and 4) GWAS analyses of the sense of smell on *Medicine*. To the best of our knowledge, this proposal has no overlap with any other proposals.

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 2010.17)

___ ___ 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. 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.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. Understood.

References:
