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TITLE: Airborne Pollutants as Triggers of Parkinson's Disease via the Olfactory System

PRINCIPAL INVESTIGATOR: Dr. Honglei Chen, Professor

CONTRACTING ORGANIZATION: Michigan State University, East Lansing, MI

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14. ABSTRACT: In this multidisciplinary project, we proposed to examine the central hypothesis that ambient air pollutants contribute to Parkinson's disease (PD) development by initiating and/or exacerbating alpha-synuclein pathology at olfactory structures via inflammation. In the epidemiologic arm, we plan to investigate 1) the effect of long-term exposure to air pollutants on olfactory impairment (OI); 2) whether early PD pathogenesis is exacerbated by ambient air pollutants; and 3) whether lifetime use of ibuprofen modifies potential adverse effects of air pollutants on OI. The project will leverage ten years of extensive data collection on environmental exposures, medical history, and biospecimen from the well-established Sister Study of the National Institute of Environmental Health Sciences (NIEHS). Importantly, we proposed to objectively evaluate the sense of smell of approximately 3,400 Sister Study participants, using the brief smell identification test, efficiently administered by mail. We completed field data collection in March 2019. Of the 4,020 eligible participants, 3,535 (87.9%) have provided some data, and 3,431 (85.3%) returned the smell test kit. To date, we have received non-genetic data from our field team at NIEHS. The genotyping was completed in 2020 by our collaborators at the National Institute on Aging, and we are working with them to resolve a few data issues. We are currently analyzing the non-genetic data to define and characterize the outcome of OI in our study population, and to examine the associations of OI with air pollutants (Aim 5) and the use of nonsteroidal anti-inflammatory drugs (Aim 7).

15. SUBJECT TERMS
Parkinson's Disease, Olfaction, Sense of Smell, Air Pollutants, Prodromal, Inflammation, Risk Factor

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1. INTRODUCTION

Olfactory impairment (OI) is an under-appreciated and under-studied health problem among older adults. Importantly, OI is an early warning for several major neurodegenerative diseases such as Parkinson's disease (PD) and dementia. However, the causes of age-related OI and how it may contribute to neurodegenerative diseases are largely unknown. Therefore, we proposed a case-control study to investigate risk factors for age-related OI to better understand PD prodromal development. This project aims to define the role of ambient air pollutants in OI and explore its relevance to PD development. Specifically, we aim to 1) assess the effect of long-term exposure to air pollutants on OI (specific aim 5); 2) investigate whether early PD pathogenesis is exacerbated by ambient air pollutants (specific aim 6); and 3) examine whether lifetime use of non-steroidal anti-inflammatory drugs (NSAIDs), ibuprofen, in particular, modifies potential adverse effects of air pollutants on OI (specific aim 7). The project will leverage ten years of extensive data collection on environmental exposures, medical history, and biospecimen from the well-established Sister Study of the National Institute of Environmental Health Sciences (NIEHS). Specifically, we have objectively evaluated the sense of smell of more than 3,400 participants from the Sister Study, using a validated and self-administered brief smell identification test (B-SIT). Participants further completed a short survey on medical history relevant to their senses of smell and taste. Besides, we performed genotyping to quantify their genetic risk for PD. We will analyze these data together with the tremendous exposure data that the Sister Study has already collected. We expect this project will significantly improve our understanding of risk factors for OI and provide novel insights into the prodromal development of PD and related neurodegenerative diseases.

2. KEY WORDS: Parkinson's Disease, Olfaction, Sense of Smell, Air Pollutants, Prodromal, Inflammation, Risk Factors

3. ACCOMPLISHMENTS

3.A. What were the major goals of this project?

By objectively assessing the sense of smell of selected participants from the NIEHS Sister study and leveraging the study's extensive environmental data collection, we aim to examine the role of ambient air pollutants in olfactory impairment (OI) and explore its relevance to Parkinson's disease (PD). Specifically, we aim to 1) assess the effect of long-term exposure to air pollutants on OI; 2) investigate whether early PD pathogenesis is exacerbated by ambient air pollutants; and 3) examine whether the use of NSAIDs, ibuprofen, in particular, modifies potential adverse effects of air pollutants on OI. We initially proposed to collect data from 2,713 Sister study participants; later, with a cost-share agreement established with the Parkinson's Foundation ($151,399), we were able to collect the sense of smell data from more than 3400 participants.

3.B. What was accomplished under these goals?

We started data collection (B-SIT test and questionnaire survey) in March 2018, which was completed on Feb. 28, 2019. Of the 4,020 Sister participants we selected, 3,535 (87.9%) responded and 3,431 (85.3%) returned the B-SIT kit. The response rate was slightly higher than what we had ambitiously projected (85.0%). In May 2019, a total of 3,696 DNA samples (including duplicates for quality control /QC) were shipped to the National Institute on Aging (NIA) for genotyping. Genotyping was completed in 2020 at our collaborator's lab at NIA, and data were delivered in early 2021. We found several issues with the data, and we are working with our collaborators to resolve these issues. We are currently analyzing the non-genetic data to characterize the study outcome and examine the associations of OI with air pollutants (Aim 5) and NSAID use (Aim 7). We have just submitted two manuscripts supported by this grant, and two more manuscripts are in preparation (see 6.A. for details). The data analyses have taken longer than expected because we had to explore multiple analytic strategies to account for the specific study design.

Study participation

1. *"Obtain IRB approval or exemption from DOD and relevant study sites" by month 4.*
   Progress: The study involves multiple sites. We obtained standalone IRB approvals from MSU (IRB# 17-1208) on November 20, 2017, and the DoD (#A-20425) on January 10, 2018. Besides, NIEHS/SSS (Sister study contractor of NIEHS) and the University of Washington approved relevant study activities by amending their existing protocols. The Sister Study team carried out all field data collection activities. After initial approval, we made multiple minor revisions that MSU IRB swiftly approved. All modifications did not affect the risk and benefit of study participants.

2. *"Select participants and design survey/study materials" by month 4*
   Progress: In January 2018, we selected 2,820 eligible Sister Study participants, ages 50-79 and alive, who reported a poor sense of smell at a recent survey and a random sample of 1,200 participants who did not. Study materials were ready by January 2018.

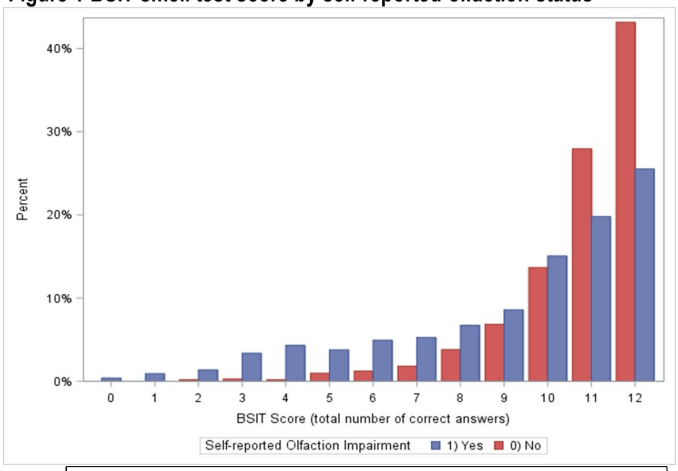
3. *"Obtain survey data from the Sister study" by month 6*
   Progress: We obtained the cohort survey data from the Sister Study first on June 1, 2018, which were updated a couple of times afterward to meet analytic needs.

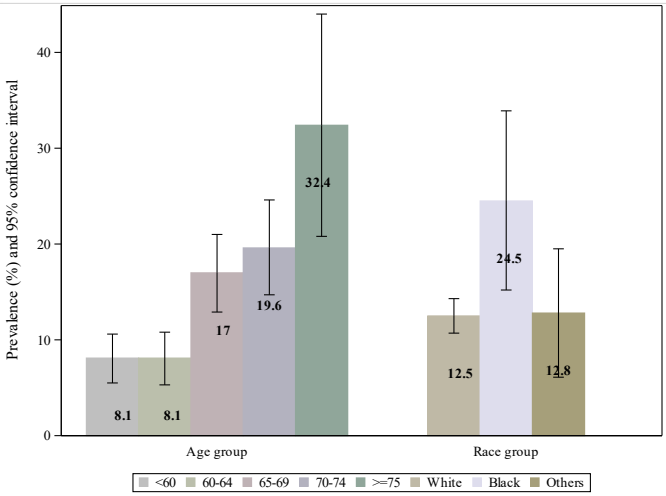
Participant recruitment and data collection

1. *"Mail/receive test kit and questionnaire" by month 18*
   Progress: We completed data collection in Feb. 2019. Of the 4,020 Sister participants selected, 3,535 (87.9%) responded, and 3,431 (85.3%) returned the sense of smell test kit. The participation rate was slightly higher than what we had projected (85.0%). Further, it was comparable between participants who self-reported a poor sense of smell and those who did not, and was little affected by age or education level.

2. *"Data QC, entry, and delivery" by month 21*

Figure 1 BSIT smell test score by self-reported olfaction status





Progress: The field team at NIEHS entered and cleaned data, which were delivered to MSU in August 2019. We have confirmed that the data are of high quality. Of those who returned the B-SIT test kit, 94.8% completed all 12 items, 4.1% missing on 1 or 2 items, and only 1.1% missing on 3 or more. The score ranges from 0 to 12 (**Figure 1**). Participants with self-reported OI overall scored lower than those who reported a normal sense of smell. Based on the distribution of these data in our study sample, we chose to use 9 as the cutoff for poor olfaction. With this cutoff, we estimated a 13.3 % (95%CI: 11.5-15.0) of the Sister Study participants, ages 50-80, have OI, and the prevalence increases with age (**Figure 2**). We also found an interesting racial disparity – the African Americans were twice likely to have poor olfaction than white participants, but they were less likely to recognize this symptom. In both populations, the awareness of this sensory deficit and reporting accuracy was low (**Table 1**), which we found was affected by a few demographic and health factors. For

example, among participants who reported poor olfaction, older age, black race, low education, underweight, fair-to-poor health,

Table 1: Self-reported vs. Objectively Tested Poor Olfaction

	Sensitivity	Specificity	PPV	NPV
Non-Hispanic Whites	24.4 (20.8,28.0)	94.5 (94.3,94.6)	38.5 (37.6,39.3)	89.8 (87.8,91.7)
Non-Hispanic Blacks	12.4 (7.0,17.8)	96.0 (94.9,97.2)	50.3 (46.5,54.0)	77.1 (67.2,87.1)
Other races	19.0 (8.3,29.7)	94.8 (93.5,96.0)	34.7 (31.1,38.3)	88.9 (81.7,96.1)
Overall	22.6 (19.6,25.6)	94.6 (94.4,94.7)	38.9 (38.0,39.7)	88.9 (87.0,90.8)

All numbers are %; PPV/NPV: positive/negative predictive value

and PD were associated with higher odds of being true vs. false positives. Among those with objectively tested poor olfaction, the black race was associated with lower odds of being true positives vs. false negatives. Given the potentially broad implications of poor olfaction on the health of older adults (including but not limited to PD and dementia), we expect our findings will generate interest to promote the awareness of this sensory deficit in older adults. The manuscript is currently in review at JAMA Otolaryngology.

Specific Aim 5

1. *"Air pollutant assessment and data delivery to MSU" by month 12*

   Progress: The aforementioned Sister Study data delivery included air pollution estimates based on participants' baseline residential addresses, the longest-lived addresses, and childhood addresses. Our collaborators at the University of Washington are updating air pollutant data by incorporating primary residential addresses after study enrollment.

2."Data analysis and manuscript preparation/submission" (months 21-48)

Progress: We have analyzed the ambient concentrations of $PM_{2.5}$ and NO_2 in relation to poor olfaction, using the primary residence at enrollment. This analysis took longer than we had expected because the conventional case-control analysis cannot account for our study design which analyzes a secondary outcome (B-SIT-tested olfaction) using case-control sampling based on the primary outcome (self-reported sense of smell). We have compared multiple statistical strategies to account for the study design and, at the same time, to retain statistical efficiency. We are still working on this methodology development. But based on the best approach so far, we found that $PM_{2.5}$ seems to be positively associated with the odds of having OI, whereas data on NO_2 point in the other direction. However, in neither case, the association was statistically significant. We also conducted multiple sensitivity analyses 1) limited the analysis to study participants who had a stable residential history before and after the enrollment 2) using updated exposure data in 2011/2012 or the cumulative exposure data between baseline and 2011/2012, we found similar results. The associations were not meaningfully different by age and race, although the ORs were larger for NonHispanic Blacks and other races than Whites. In stratified analyses by smoking, we found that both $PM_{2.5}$ and NO_2 were associated with statistically higher odds of having poor olfaction among current smokers ($OR_{per\ IQR}$=3.5, 95%CI (1.2-10.0) for $PM_{2.5}$, and 4.1 95%CI (1.3-13.5) for NO_2). These analyses, however, were based on small sample sizes. We are preparing the manuscript based on this updated analysis and plan to send it to coauthors to review in the next couple of months. If we develop a more efficient statistical method for this type of analysis, we plan to update the analysis and publish the results.

Table 1 Ambient air pollutants in relation to OI

Exposure level	Multivariable OR and 95%CI	
	PM2.5 (2006)	NO2 (2006)
Quartile 1	Reference	Reference
Quartile 2	1.28 (0.81,2.01)	0.99 (0.58,1.70)
Quartile 3	1.26 (0.77,2.06)	0.84 (0.45,1.58)
Quartile 4	1.53 (0.93,2.52)	0.82 (0.41,1.64)
Per IQR	1.22 (0.94,1.59)	0.98 (0.74,1.30)

OR: odds ratio; CI: confidence interval
IQR: inter-quartile range. Data weighted back to all eligible participants in the main cohort, adjusted for baseline age, race, education, BMI, health status, smoking status, census region, and residential area type

Specific Aim 6

1. *"DNA extracted and shipped to NIA" by month 24*
   Progress: DNA extraction was completed by March 2019. In May 2019, we shipped DNA samples to NIA for genotyping.

2. *Genotyping by NIA by month 44*
   Progress: Genotyping, QC, and imputation were completed in 2020 by our collaborators at the NIA neurogenetic lab. The data was delivered to us at MSU in March 2021, we however have identified several issues with the data, and we are actively reaching out to our collaborators at NIA to resolve the issues. This has caused a delay in the Aim 6 analysis. We will work on the analysis once receiving the correct data.

3. *Data analysis and manuscript preparation/submission by month 60:* See above, we will begin the analysis once receiving the correct data..

Specific Aim7

Data analysis and manuscript submission by month 60

Progress: Data analyses in progress.

3.C. What opportunities for training and professional development has the project provided?

Although this project has no training component, it supports Mr. Zichun Cao, who leads the analysis on air pollutants and poor olfaction.

3.D. How were the results disseminated to communities of interest?

Two manuscripts supported by this grant are currently in review

1) "Self-reported sense of smell, objective testing, and associated factors in women", submitted to *JAMA Otolaryngology – Head & Neck Surgery*.

2) "Environmental triggers of Parkinson's disease – implications of the Braak and dual-hit hypotheses", an invited review submitted to *Neurobiology of disease*.

3.E. What do you plan to do during the next reporting period to accomplish the goals?

We expect to submit findings from Aim 5 (air pollution and OI) in the next few months. We also expect to finish analysis on Aim 7 (NSAIDs and OI) in 6-9 months. For Aim 6 (PD genetic and OI), we are actively reaching out to our NIA collaborator to obtain the updated genetic data. We hope we will get the corrected data in 1-3 months and then finish analyses and manuscript submission by the end of the next performance period.

4. IMPACT:

4.A. What was the impact on the development of the principal discipline(s) of the project?

The human sense of smell decreases with age, affecting 15-25% of older US adults. Although most do not even realize they have it, OI adversely affects human functioning such as detecting environmental hazards, nutrition, mood and behavior, sexuality, emotional and physical well-being, and quality of life. Further, OI independently predicts both short-term and long-term mortality in older adults.

Most importantly, converging evidence suggests OI is one of the earliest and most important prodromal symptoms for PD. OI research may therefore represent an unprecedented opportunity to understand the early stages of PD development. Late-onset PD takes years, if not decades, to develop, and by the time of diagnosis, is generally too advanced to decelerate, stop, or reverse. Research on OI may help in the war against PD in two ways: 1) characterize at-risk populations which may eventually facilitate early diagnosis and treatment, and 2) elucidate disease etiology. Current research, including ours, has focused on how OI predicts the

risk of PD. We, however, also see OI research as an excellent opportunity to open the etiological "black-box" of the disease. A major challenge in such research is the current lack of understanding of the decades of PD prodromal development, during which many factors may come into play to initiate pathology or modify progression. By using OI as an easily measured and noninvasive intermediate marker of PD, we expect to bring new insights into this "black-box" by identifying factors that contribute to OI and factors that modify its progression to PD, fundamentally improving understanding of the poorly understood etiology of PD.

4.B. What was the impact on other disciplines?

OI or hyposmia is also an early marker for several other neurodegenerative diseases such as Alzheimer's disease. Interestingly, our recent findings suggest that poor olfaction has more to tell about the health of older adults beyond its association with neurodegenerative diseases. Specifically, we recently reported that PD and dementia combined only explained ~22% of the excess mortality among older adults with poor olfaction (*Annals of Internal Medicine 2019*), and that poor olfaction was associated with a higher risk for pneumonia hospitalization (*Lancet Healthy Longevity 2021*) and a faster decline in physical functioning (*Journal Gerontology Series A: Medical Sciences, 2021*). Therefore, data collected for this project will have much broader impacts than the proposed, and may lead to research that eventually establishes poor olfaction as a facile and easily assessable marker for multiple age-related adverse health outcomes, including but not limited to PD and dementia.

4.C. What was the impact on technology transfer?

Nothing to report

4.D. What was the impact on society beyond science and technology?

Up to a quarter of older US adults have a poor sense of smell, a sensory impairment of which most are unaware. However, poor olfaction may have broad ramifications on the health of older adults, most notable on PD and dementia. We compared the self-reported vs. objectively tested poor olfaction intending to raise the awareness, by both the general public and medical communities, of this common and important sensory deficit in older adults. As mentioned above, the manuscript is currently under review.

5. CHANGES/PROBLEMS:

5.A. Changes in approach and reasons for change

Nothing to report.

5.B. Actual or anticipated problems or delays and actions or plans to resolve them

We encountered a statistical challenge to account for the study design that analyzes a secondary outcome (B-SIT tested olfaction) based on the primary outcome (self-reported sense of smell) sampling framework. We have compared multiple statistical strategies and chose one that works the best for the current analysis. Nevertheless, we are still working on this methodology development and will update our analyses if needed.

Another challenge was the delayed genetic data delivery. We received the neuro-array genetic data from our NIA collaborator in March 2021; we found a couple of data issues that we are working with our collaborator to resolve. We will start the analysis once we receive the correct genetic data.

5.C. Changes that had a significant impact on expenditures

Nothing for this reporting period. We requested a 1-year no-cost extension, which was approved by DoD.

5.D. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report.

6. PRODUCTS:

6.A. Publications, conference papers, and presentations

Journal publications:

Two manuscripts supported by this grant are currently in review

1) "Self-reported sense of smell, objective testing, and associated factors in women", submitted to *JAMA Otolaryngology – Head & Neck Surgery*.

2) "Environmental triggers of Parkinson's disease – implications of the Braak and dual-hit hypotheses", an invited review submitted to *Neurobiology of disease*.

Two manuscripts are in preparation on the associations of poor olfaction with air pollution (Aim 5) and NSAID use (Aim 7)

Books or other non-periodical, one-time publications:
Nothing to report

Other publications, conference papers, and presentations:
Nothing to report for this reporting period.

6.B. Website(s) or other Internet site(s)
Nothing to report

6.C Technologies or techniques
Nothing to report

6.D Inventions, patent applications, and/or licenses
Nothing to report

6.E. Other Products
Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

7.A What individuals have worked on the project?

Name:	Honglei Chen
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0003-3446-7779
Nearest person month worked:	2
Contribution to Project:	Oversaw all activities of the study, including study design, and material development and purchase, IRB approvals, filed data collection, and DNA extractions, and data management and analysis.
Funding Support:	

Name:	Chenxi Li
Project Role:	Biostatistician
Researcher Identifier (e.g. ORCID ID):	0000-0003-0701-0757
Nearest person month worked:	1.2
Contribution to Project:	Develop and refine analytic strategies and oversee the statistical analysis conducted by data analyst and student
Funding Support:	

Name:	Aiwen Yang
Project Role:	Data analyst
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	8.5
Contribution to Project:	Under Dr. Chen's supervision, conducted data analyses.
Funding Support:	

Name:	Zichun Cao
Project Role:	Graduate Student functions as data analyst and research assistant
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	6
Contribution to Project:	Under Dr. Chen's supervision, conducted data analyses.
Funding Support:	

7.B. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

None since the last reporting period; please see below for the other supports of the PI.

Title: Prodromal symptoms in the Sister Study
Time Commitments: Chen H, PI, in-kind (0% effort)
Supporting Agency: Parkinson's Foundation - PF-IMP-1825
Address: 1359 Broadway Suite 1509; New York, NY 10018
Performance Period: 06/01/2018 – 05/31/2019
Level of funding: total
Project Goals: Supplemental funding to expand data collection in the above-referenced DoD study to a larger sample which enables more comprehensive analyses of risk factors for olfactory impairment and their relevance to Parkinson's development as detailed in 3.A. & 5.A.

Title: Determinants of depression in Parkinson's disease
Time Commitments: Chen H, co-I, in-kind (0% effort)
Supporting Agency: Michigan State University
Address: Office of Research, A209 East Fee Hall, 965 Wilson Road, East Lansing, Michigan 48824-1316
Performance Period: 07/01/2018 – 06/30/2021

Level of funding: total
Project Goals: To determine role of appendectomy in depression among Parkinson's patients
Specific Aims: To evaluate appendectomy in relation to depression in Parkinson's disease using data from the Swedish Patient Registry. No scientific or budgetary overlap with this project.

Title: R01ES029227-01A1 Pesticides, Olfaction, and Neurodegeneration Among US Farmers
Time Commitment: Chen H, PI, 1.35 AY months & 1 Sum month
Address: Office of Research, A209 East Fee Hall, 965 Wilson Road, East Lansing, Michigan 48824-1316
Performance Period: 02/01/19 – 01/31/24
Level of funding: total
Project Goals: To examine the connections among pesticides, olfactory impairment, and prodromal neurodegeneration.
Specific Aims: To investigate roles of pesticides in olfactory impairment among farmers and their relevance to prodromal development of neurodegenerative diseases such as dementia and Parkinson's. No scientific or budgetary overlap with this project.

7.C. What other organizations were involved as partners?

Organization Name: The Social & Scientific Systems, Inc.
Location of Organization: Durham, North Carolina
Partner's contribution to the project: collaboration

Organization Name: National Institute of Environmental Health Sciences
Location of Organization: Durham, North Carolina
Partner's contribution to the project: collaboration

Organization Name: ReproCell, Inc. (previously called Bioserve)
Location of Organization: Beltsville, MD
Partner's contribution to the project: collaboration

Organization Name: University of Washington
Location of Organization: Seattle, WA
Partner's contribution to the project: collaboration

Organization Name: Chicago University
Location of Organization: Chicago, IL
Partner's contribution to the project: collaboration

Organization Name: National Institute on Aging
Location of Organization: Bethesda, MD
Partner's contribution to the project: collabroation

Organization Name: Parkinson's Foundation
Location of Organization: New York, NY
Partner's contribution to the project: Supplemental financial support as explained above

8. SPECIAL REPORTING REQUIREMENTS

8.A. COLLABORATIVE AWARDS: Other co-PI will submit their own reports
8.B. QUAD CHARTS: attached.

9. APPENDICES: Nothing to report