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

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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/SSS. The genotyping was completed in early 2020 at our collaborator's lab at the NIA, and a polygenic risk score for PD was created over the summer. We are currently analyzing the nongenetic data to examine the prevalence and correlated factors of OI in our study population, and the relationships between air pollutants of $PM_{2.5}$ and NO_2 and OI.

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 (PD) and Alzheimer's (AD) diseases. However, the causes of age-related OI and how it may contribute to neurodegenerative diseases are largely unknown. We, therefore, proposed a case-control study to investigate risk factors for age-related OI in order to better understand PD prodromal development. The goal of this project is to define the role of ambient air pollutants in OI and to explore its relevance to PD development. 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 lifetime use of non-steroidal anti-inflammatory drugs (NSAIDs), ibuprofen in particular, 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). 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 (BSIT). 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 to 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 lifetime 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 in March 2018 and completed data collection on Feb. 28, 2019. Of the 4,020 Sister participants we selected, 3,535 (87.9%) responded and 3,431 (85.3%) returned the sense of smell test kit. The response rate was slightly higher than what we had ambitiously projected (85.0%). In May, a total of 3,696 DNA samples (including duplicates for quality control /QC) were shipped to NIA for genotyping. Genotyping was completed in early 2020 at our collaborator's lab at the NIA, and a polygenic risk score for PD was created over the summer. We expect to receive the genetic data in the fall of 2020. To date, we have mostly completed two analyses: 1) to examine the prevalence and correlated factors of OI in our study population, and 2) to examine air pollutants of $PM_{2.5}$ and NO_2 in relation to OI. The data analyses have taken longer than expected because we had to explore multiple analytic strategies to account for the specific study design. Please see below our proposed major tasks and milestones for the first three years of the project as listed in the SOW and our cumulative progress report.

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 Nov 20, 2017 and the DoD (#A-20425) on Jan 10th, 2018. Besides, NIEHS/SSS (Sister study contractor of NIEHS) and the University of Washington approved relevant study activities by amending their existing protocols. All field data collection has been carried out by the Sister Study team at NIEHS/SSS. After initial approval, we made multiple minor revisions that were swiftly approved by MSU IRB. 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.

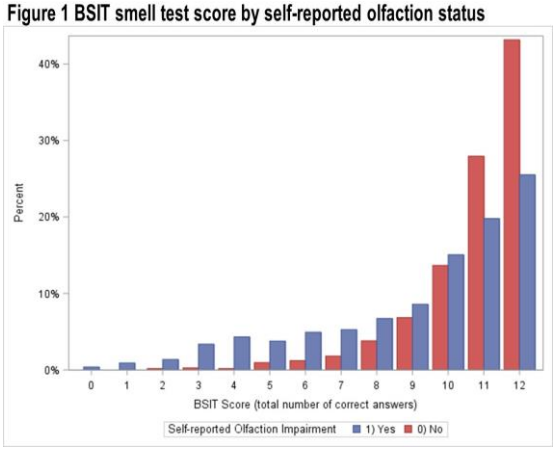
3. *"Obtain survey data from the Sister study" by month 6*
   Progress: We obtained relevant survey data from the Sister Study on June 1st, 2018. Some data (e.g., NSAID) need further updates and are in progress.

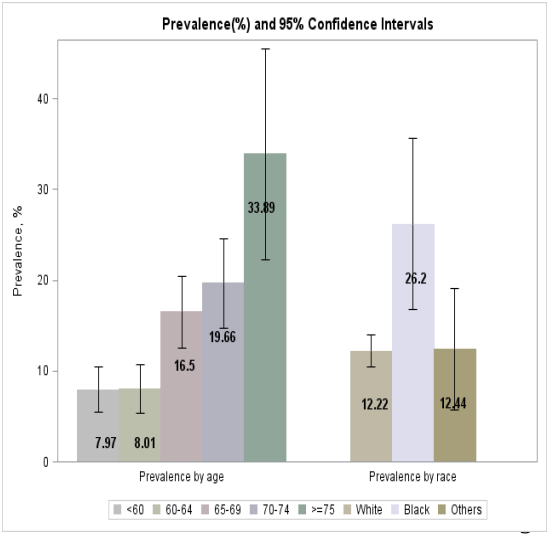
Participant recruitment and data collection

1. *"Mail/receive test kit and questionnaire" by month 18*
   Progress: We completed data collection by Feb 2019. Of the 4,020 Sister participants, 3,535 (87.9%) responded, and 3,431 (85.3%) returned the sense of smell test kit. The response rate was slightly higher than what we had ambitiously projected (85.0%) and 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: Data were entered and cleaned by the field team at NIEHS/SSS and were delivered to MSU in August 2019. We have confirmed that the data are of exceptional quality. Of those who returned the BSIT 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, we chose to use 9 as the cutoff for poor olfaction. With this cutoff, we estimated a 13.1 % (95%CI: 11.4-14.9) of the Sister Study participants, ages 50-80, have poor olfaction. The prevalence increases with age and is higher in blacks than whites or other races (**Figure 2**). We also found the following factors are associated with poor olfaction in the Sister Study population, including age, race, general health status, constipation, slowing walking, and difficulty in moving. We are currently preparing a manuscript to report these findings.

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 currently updating air pollutant data by incorporating primary residential addresses after study enrollment.

2."Data analysis and manuscript preparation/submission" (months 21-36)

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 (BSIT-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 to the other direction. However, in neither case the association was statistically significant. We are currently preparing a manuscript based on this analysis. In the case that we develop a more efficient statistical method for this type of analysis, we plan to update the analysis and publish it.

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	1.27 (0.82-1.98)	0.99 (0.64-1.55)
Quartile 3	1.02 (0.65-1.62)	0.79 (0.50-1.25)
Quartile 4	1.33 (0.85-2.07)	0.82 (0.52-1.28)

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, expecting completion late in 2019 or early in 2020.

2. Genotyping by NIA by Month 33
   Progress: Genotyping, QC, and imputation were completed early in 2020 by our collaborators at the NIA neurogenetic lab. The lab has also generated the PD polygenic risk score, and we are in discussion about data delivery, which will happen this fall.

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

Nothing to report – this project has no training component.

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

Nothing to report.

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

As described above, we have achieved major goals proposed so far. In the next annual reporting period, we expect to submit the two manuscripts mentioned above, and conduct analyses for Aims 6 (PD genetics and olfaction) and 7 (NSAIDs and olfaction), and prepare manuscripts accordingly.

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. Therefore, this project may eventually help understand a common pathway that leads to neurodegeneration.

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

Nothing to report

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

Nothing to report during this period, but eventually data from this project will raise public awareness of the importance of sense of smell in aging, especially brain aging.

5. CHANGES/PROBLEMS:

5.A. Changes in approach and reasons for change

Nothing for this reporting period.

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

No major problems to report. We did encounter a statistical challenge that we need to account for the study design that analyzes a secondary outcome (BSIT-tested olfaction) based on the primary outcome (self-reported sense of smell) sampling framework. We have compared multiple statistical strategies and choose one to work for the current analysis. Nevertheless, we are still working on this methodology development. For this reason, we have replaced Dr. Joseph Gardiner (5% effort), the previous project statistician, with Dr. Chenxi Li (10% effort). Dr. Li is a highly-experienced statistician, and will have more time to devote to this project. This, however, does not affect the budget.

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

Nothing for this reporting period.

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

Nothing to report.

Two manuscripts are in preparation, which we plan to submit in the coming year.

1) Prevalence of poor olfaction in women and associated factors.

2) Air pollutants in relation to poor olfaction in women.

Journal publications:

No publications yet directly from this project, but we acknowledged DoD support in the following publications during the 2019-2020 report period.

Liu R, Umbach DM, Tröster AI, Huang X, **Chen H**. Non-motor symptoms and striatal dopamine transporter binding in early Parkinson's disease. *Parkinsonism Relat Disord*. 2020 Feb 11;72:23-30. doi: 10.1016/j.parkreldis.2020.02.001. [Epub ahead of print] PubMed PMID: 32092703.

Purdy F, Luo Z, Gardiner JC, Pinto JM, Shiroma EJ, Simonsick EM, Harris TB, **Chen H**. Olfaction and changes in body composition in a large cohort of older US adults. *J Gerontol A Biol Sci Med Sci.* 2020 Apr 8. pii: glaa085. doi:10.1093/gerona/glaa085. [Epub ahead of print] PubMed PMID: 32267924.

Cao Z, Luo Z, Huang X, Pinto JM, Simonsick EM, Shiroma EJ, **Chen H.** Self-Reported Versus Objectively Assessed Olfaction and Parkinson's Disease Risk. *J Parkinsons Dis.* 2020 Sep 9. doi: 10.3233/JPD-202164. Online ahead of print. PMID: 32925101

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, material development and purchase, IRB approvals, filed data collection, and DNA extractions, and data management and analysis.
Funding Support:	

Name:	Frank Purdy
Project Role:	Graduate Student function as the project manager
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	2.5
Contribution to Project:	Helped Dr. Chen manage various aspects of study activities, received and managed Sister Study data, conducted preliminary data analyses.
Funding Support:	

Name:	Aiwen Yang
Project Role:	Data analyst
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	12
Contribution to Project:	Under Dr. Chen's supervision, conducted preliminary data analyses.
Funding Support:	

Name:	Zichun Cao
Project Role:	Graduate Student function as data analyst and research assistant
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	3.5
Contribution to Project:	Under Dr. Chen's supervision, conducted preliminary 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: $151,399 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: $299,975 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: $4,999,267 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