Study Title: Smell, voice and nasal swabs as markers for neurodegenerative disorders

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List of Abbreviations

AD:	Alzheimer's Disease
AE:	Adverse Event
CBD:	Corticobasal degeneration
CNS:	Central Nervous System
CSF:	Cerebrospinal fluid
DAT:	Dopamine Transporter
DLB:	Dementia with Lewy Bodies
ENT	Ear, Nose, and Throat
FTLD:	Fronto-temporal dementia
IHC:	Immunohistochemistry
IL-1:	Interleukin 1
MCI:	Mild Cognitive Impairment
MoCA:	Montreal cognitive assessment
mRNA:	Messenger ribonucleic acid
MSA:	Multiple systems atrophy
OM:	Olfactory Mucosa
PD:	Parkinson's disease
PDD:	Parkinson's Disease with Dementia
PSP:	Progressive supranuclear palsy
UPDRS:	Unified Parkinson's Disease Rating Scale
UPSIT:	University of Pennsylvania Smell Identification Test

Background and Rationale

Degenerative dementias including Alzheimer's Disease (AD), Parkinson's Disease with Dementia (PDD), Dementia with Lewy Bodies (DLB), Frontotemporal Dementias (FTLD), Corticobasal Degeneration (CBD) and Progressive Supranuclear Palsy (PSP) constitute a significant, and growing burden with an estimated cost to the US healthcare system for 2016 of $236 Billion (1). Definitive diagnosis of these dementias is based on pathological criterion upon autopsy, which presents a significant challenge to establish diagnosis in living patients. Although clinical diagnostic criteria have been developed for several of these disorders, including for AD by the NINCDS-ADRDA (2,3), PD by the United Kingdom Parkinson Disease Brain Bank Diagnostic Criteria (UKPDBB) diagnostic criteria for Parkinson Disease(4) and others, the currently available tests, including the use of imaging markers and CSF biological markers do not provide a definite diagnosis since this requires the observation of characteristic neuropathological changes in specific regions of the brain. As an example, the presence of senile plaques and neurofibrillary tangles in mesial temporal structures is required for the diagnosis of AD, loss of nigral dopaminergic neurons with presence of intracellular Lewy Bodies being the hallmark of PD.

This difficulty with ante-mortem definitive diagnosis for degenerative neurological disorders leads to several challenges pertaining to patient care and counseling, as well as recruiting the appropriate population of subjects for disease modifying therapies. Functional neuroimaging markers are being evaluated for improving the diagnostic certainty, including imaging amyloid, and phosphorylated tau (p-tau), as well as DATScan (r), but these add cost to the diagnostic process, and access is limited due to the need for nuclear imaging facilities and technical expertise.

The inability to establish a diagnosis is even more relevant in early stages of the disease, when specificity of establishing a diagnosis using the clinical diagnostic criteria is lower. As evidence, 16% of patients identified by PD experts as having PD for a longitudinal cohort study funded by the Michael J Fox Foundation for development of biomarkers for PD did not have a deficit in dopamine transporter (DAT) binding, suggesting that they did not indeed have PD (5). Similarly, in a large neuropathology study of patients diagnosed with mild to moderate AD at AD centers funded by the National Institute for Aging, 12.5% met neuropathological criteria for non-AD dementias (6). There is a clear need to develop diagnostic markers, yet blood, peripheral tissue and CSF markers have all lacked specificity to be of practical use in the clinic.

A common, early marker of several degenerative dementias is impaired smell sensation and changes in voice. Indeed, olfactory impairment has been reported in PD (7), FTLD (8), and AD (9). Voice changes appear early in many parkinsonian disorders (10), and language changes have been assessed as a marker of disease progression in

Alzheimer's Disease (11). Pathological involvement of the olfactory bulb has also been demonstrated with disease specific pathological changes (12). These changes are hypothesized to appear early in the pathological process, and olfactory neurons provide us a unique opportunity to evaluate disease specific pathological changes representative of dysfunction within the CNS in a non-invasive fashion, since these can be sampled using simple nasal brushings or swabs. Voice changes appear early in may parkinsonian disorders, and language changes have been assessed as a marker of disease progression in Alzheimer's Disease.

Techniques for nasal swabs for evaluating of rapidly progressive dementia in prion diseases have been published previously. Indeed, for prion diseases, olfactory mucosal brushings or swabs provide a very high sensitivity and specificity of diagnosis (13). Previous evaluation of the nasal epithelium have shown increased levels of A-beta and p-tau in AD compared to controls, and a correlation between A-beta plaque frequency and p-tau in olfactory epithelium and brain in post-mortem examinations (although the correlation coefficient was 0.37) (14).

In spite of the low risk, relative simplicity of sampling, and the ability to test for characteristic pathological changes on CNS tissue directly, thus far, nasal mucosal swab or cytobrush techniques have not been used broadly for diagnosis of degenerative dementias (except for rapidly progressive dementias).

We propose this pilot study for evaluation of olfactory and vocal cord function, and nasal mucosal swabs of patient volunteers with neurodegenerative disorders or at risk of neurodegenerative disorders (identified as either having changes of presbylarynx on examination of laryngeal function, or with single or multi-domain mild cognitive impairment (MCI)) on standard neuropsychometric evaluations at the Psychiatric Research Institute for evaluation of cognitive symptoms. Neuropsychometric evaluations and cranial imaging (with or without olfactory evaluation) form the existing standard of care for patients considered at risk for neurodegenerative disorders.

We will obtain buccal and nasal mucosal swabs which will be used for standard immunohistochemistry (IHC) evaluations for neuronal, disease-specific and inflammatory markers. We will correlate olfactory dysfunction (measured by the University of Pennsylvania Smell Identification Test (UPSIT), performance on neuropsychometric testing including evaluation of anxiety and depression and quality of life measures, and cranial imaging results with the nasal mucosal cells obtained through swabs. We will also evaluate the functioning of the vocal folds during voice production (phonation) and closure of vocal folds, as well as any structural abnormalities in the vocal folds, hypopharynx, pharynx (throat) and larynx (voice box), as well as measuring the quality of sound produced, including breath control during speech, intelligibility of voice and its pitch and loudness through specific speech measures.

Specific Aims

The specific aims of the study are:

- Aim 1: Determine if smell, voice or vocal cord dysfunction serves as a reliable markers to distinguish between neurodegenerative disorders or among subjects with and those without neurodegenerative disorders.
- Aim 2: Determine if nasal swabs can provide an adequate sample for evaluation using cytology and immunohistochemistry for alpha-synuclein, A-beta and p-tau staining.
- Aim 3: Measure cellular levels of IL-1 a/b protein and mRNA levels and genotyping of apo-E4 alleles on the nasal mucosal swab samples.
- Aim 4: Evaluate if IHC and neuro-inflammatory marker levels correlate with severity of impairment in various cognitive or motor domains based on neuropsychological and motor scores, or olfactory impairment based on UPSIT scores.

Study Population

This is a prospective cross-sectional study of subjects with, or at risk of neurodegenerative disorders. Initial recruitment goal is for 60 subjects:

- Twenty people with Parkinson's Disease requiring evaluation of voice dysfunction by an Ear, Nose, and Throat (ENT) doctor
- Twenty people with other neurodegenerative disorders requiring evaluation of voice dysfunction by an ENT doctor.
- Twenty people with voice tremor and/or presbylarynx, but no evidence of Parkinson's other neurodegenerative disease, requiring evaluation of voice dysfunction by an ENT doctor.

Subjects will be recruited from the population of patients with neurodegenerative disorders (seen in the UAMS Neurology Clinic) or presbylarynx and/or voice tremor patients scheduled to undergo routine voice and laryngeal assessments as part of required clinical care for problems with voice and swallowing in the UAMS ENT Clinic.

Inclusion Criteria

- Diagnosed with idiopathic Parkinson's disease, progressive supranuclear palsy, Alzheimer's disease or Mild Cognitive Impairment based on consensus criteria, or suspicion of presbylarynx based on clinical evaluation.
- Require evaluation of voice dysfunction by an ENT doctor given symptoms of impaired voice volume or quality

- Age ≥ 18 years-old to ≤ 90-years old.
- Ability to understand and the willingness to sign a written informed consent.

Exclusion Criteria

- Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
- Active nose bleeds, or abnormal anatomy of the nose that prevent safe nasal swabs, or active oropharyngeal disease that prevents laryngoscopy or voice assessments.

Study Design and Procedures

Screening Procedures

Informed Consent

Informed consent will be obtained by one of the investigators after discussing the rationale, study procedures and the risks and benefits of participation in the study with potential subjects. The consent process will take place in a dedicated research space at Translational Research Institute (TRI). The voluntary nature of participation and subjects' ability to (and process of) withdraw from the study will also be discussed during the informed consent discussion. Consent to participate will be documented by the person obtaining informed consent according to required procedures.

Some of the participants with neurodegenerative disorders will, by definition, have at least mild impairment of cognitive function (e.g., subjects with Alzheimer's Disease). One of the neurologists on staff (Drs. Dhall, Lotia or Virmani) will determine capacity to consent (determine if subject is able to understand the information relevant to participation in the study, and the completely voluntary nature of study participation, as well as appreciate the reasonably foreseeable consequences of participation, including the risks of participation) using unstructured subject and caregiver interview at the time of obtaining informed consent.

Additionally, a structured neurological examination including a Montreal Cognitive Assessment (MoCA) will be performed as a part of the study before the laryngoscopy/stroboscopy and sampling of olfactory mucosa for subjects with neurodegenerative disorders (including Parkinson's Disease). The (MoCA) will take place in a dedicated research space at Translational Research Institute (TRI). If the MoCA score is less than 21/30, or neurological examination shows moderate language or executive impairments, subject assent in addition to informed consent by a legally

authorized representative will be required for continued study participation. Since subjects with voice tremor and presbylarynx are expected to be cognitively normal, for this comparison group, neurological examination and MoCA can be performed after their ENT and speech therapy assessments (see below).

Demographics

Routine demographics, e.g., age, gender, race, ethnicity, will be collected.

Medical History

A routine medical and surgical history will be collected with a recent history of infections or nose and/or throat-related problems. Previous and concomitant medications will also be reviewed and recorded.

Review Eligibility Criteria

Documentation of subjects having an established diagnosis of Parkinson's Disease, other neurodegenerative disorders, or a voice tremor or presbylarynx will be reviewed at the time of screening.

In order to stratify subjects with appropriate diagnostic certainty of the underlying diagnosis of neurodegenerative disorder, subjects will undergo comprehensive neurological assessments (described under the study procedures section below) and review of previous assessments to ensure that they meet the current consensus criteria for neurodegenerative disorders (e.g., United Kingdom Parkinson Disease Brain Bank Diagnostic Criteria for Parkinson Disease, NINCDS-ADRDA diagnostic criteria for Alzheimer's Disease) after screening is completed.

This review will be done during the study-related neurological assessments, and will include review of imaging, neuropsychological data (if available), neurological history and examination performed during their study-related visit with the neurologist.

Study Procedures

Logistically, this study involves a routine otolaryngology clinic visit and a research-related neurology visit that are likely to occur over separate days. The research-related neurology visit and consent will take place in a dedicated research space at Translational Research Institute (TRI). The study visits will occur within 1 year of the providing informed consent, otherwise the subject will be deemed lost to follow up.

Otolaryngology Elements

Physical Exam (routine clinical care)

A general physical examination will be performed as part of routine care.

Laryngeal evaluation (routine clinical care)

An examination of the oropharynx and nose will be performed as part of routine care by the laryngologist.

Once nasal swabs have been obtained, the fiberoptic laryngoscope will be advanced through the already anesthetized nasal passage and threaded to the throat. This is to allow direct visualization of the larynx (voice box) and pharynx and hypopharynx (throat) region to assess its structure and function. A built in strobe light that allows for controlled, high speed flashes of light (stroboscopy) will allow the physician to see the voice box activation with the patient producing sound, with assessment of vocal fold vibration and vocal fold closure. Laryngoscopy and stroboscopy are routine assessments performed by the laryngologist in the office for evaluation of voice and swallowing function, which may be affected in the aging of the larynx (presbylarynx) and in degenerative disorders like Parkinson Disease and other dementias. The procedure is generally safe and carries very few side effects, other than soreness of throat or soreness of the nose.

Speech Assessment (routine clinical care)

A trained speech and language pathologist will perform assessments of speech function including measuring the quality of sound produced, including breath control during speech, intelligibility of voice and its pitch and loudness through specific speech measures.

Smell Testing (only for research purposes)

We will use the smell identification Test [UPSIT] for evaluation of smell sensation. UPSIT is a 40 item test of smell function with established age based normative data. The test is a simple scratch and sniff test which comprises of four booklets each containing 10 micro-encapsulated odors printed onto a section of the page. Forced-choice responses accompany test item on the same page of the booklet as the scratch and sniff component. The test will be administered by the patient's under guidance of the investigator or coordinator. It usually takes about 10 to 15 minutes to self-administer the test. To evaluate if there is asymmetry of smell identification when using left and right nostril, a subset of participants will be administered the test twice, at the beginning and then at the end of their physical and neurological exam, and instructed to use one and then the other nostril while occluding the opposite nostril with their finger when inhaling. The smell test will take place in a dedicated research space at Translational Research Institute (TRI).

Buccal Swabs (only for research purposes)

Sample Collection. Oral mucosa samples from subjects will be obtained using two cotton swabs, and a board certified physician (RD or OTU) will obtain the swabs by

scraping the inside of the cheek of the subject firmly with the swab, with the swab being placed in saline solution in two test tubes labeled with subject ID.

Nasal Swabs (only for research purposes)

Sample Collection. Olfactory mucosa samples from subjects will be obtained using a flocked swab. An absorbent disposable face mask will be used to cover the patient's mouth to minimize dispersion of oral secretions in case of sneezing during the olfactory mucosal (OM) sample collection. Mucosal swabs will be obtained by a board-certified otolaryngologist (OTU). Subjects will not require sedation for the swab procedure. After administration of a local vasoconstrictor (4% pseudoephedrine and 2% Tetracaine local anesthetic) with the use of a nasal pledget, a rigid fiberoptic laryngoscope will be inserted into the nasal cavity of the subject for inspection, and to locate the olfactory mucosa lining the nasal vault (at or above the level of the middle turbinate). A sterile, disposable cytoswab will be inserted alongside the laryngoscope and will be rolled over the mucosal surface gently, then withdrawn and immersed in saline solution in a 15-ml conical centrifuge tube. The procedure will be repeated for a total of 3-4 swabs. Before removal of the scope, nasal mucosa will be inspected for any signs of trauma or irritation.

Sample Processing. Cellular material will be dissociated from the swab by means of vortexing. The brush will be withdrawn and particulate matter will be pelleted for 20 minutes at 2000xg at 4°C and frozen at -80°C until assayed. We will perform cytological and immune-cytochemical analyses to evaluate the quality of the OM sampling and the relative amount of olfactory neurons collected. For western blot analysis, the OM cells will be lysed using a lysis buffer, and proteins separated in X% sodium dodecyl sulfate – polyacrylamide gel electrophoresis. Appropriate antibody and secondary antibody probes will be utilized for detection of proteins of interest. We will measure cellular levels of IL-1 a/b protein and mRNA levels and genotyping of apo-E4 alleles, and evaluate the amount of aggregated alpha-synuclein, phospho-tau and beta amyloid through proteomic assays.

Neurological Elements

Neurological Evaluation (only for research purposes)

A comprehensive neurological history and neurological examination, Unified Parkinson's Disease Rating Scale (UPDRS), Schwab and England Activities of Daily Living scale, and the Montreal Cognitive Assessment (MoCA) as well as depression and anxiety scales (Hospital Anxiety and Depression Scale), quality of life scales (EQ-5D and Vision Function Questionnaire) as well as freezing of gait questionnaire (FOG-Q), will be performed by the neurologist for ensuring appropriate stratification based on the consensus criteria for various neurodegenerative disorders, or to ensure that subjects do not have incidental findings concerning for neurodegenerative disorders (if they are

being recruited to the voice tremor/presbylarynx group). If there are any concerns for neurodegenerative disorders based on examination, the neurologist completing the examination will discuss this with the subject and may provide a referral for clinical assessment.

Any prior neuropsychological evaluation results and imaging results (including those of functional imaging studies like Dopamine Transporter imaging (DATScan®) or PET Scans will be abstracted from the subjects' medical records as part of the study procedures during the subjects' neurology visits.

Gaze Tracking While Reading (only for research purposes)

We will use infrared oculography at 150- Hz to assess eye movements while the subject read aloud an English reading passages (Arial 36 point font, 5-8 words per line, 3-8 lines per page, 8 pages total) at 8th grade reading level, which will be displayed on a desktop computer monitor.

Risks and Benefits

Risks associated with the research-related procedures include significant time burden associated with participation, with the neurological examination, smell identification test, and OM sampling which in total are expected to take 4 hours of subject's time.

There is risk of discomfort and irritation of the nose associated with sampling of the OM.

A potential risk to study participants exists in the potential for loss of confidentiality. Measures to protect the confidentiality of study participants will be implemented as described in the Data Handling and Recordkeeping section below.

This study also measures cellular levels of IL-1 a/b protein and mRNA levels and genotyping of apo-E4 alleles from the nasal mucosal swab samples. The presence of apo-E4 alleles inform the risk of developing Alzheimer's disease, and possibly other neurodegenerative diseases. A good proportion carries this genotype, so people can not be identified based on this alone. The mRNA expression varies based on several factors and cannot identify subjects either. So loss of de-identification should not be a concern.

There will be no direct benefits to the study participants; however, knowledge gained from the study could potentially benefit patients in the future.

Data Handling and Recordkeeping

The Principal Investigator (PI) will carefully monitor study procedures to protect the safety of research subjects, the quality of the data and the integrity of the study. All study subject material will be assigned a unique identifying code or number. The key to

the code will be kept in a locked file in the principal investigator's office. Only the individuals listed on the title page of this document will have access to the code and information that identifies the subject in this study.

Paper data will be stored in a locked file cabinet in the PI's office behind a locked door in a restricted access space of the in the Jackson T. Stephens Spine & Neurosciences Institute. Digitized data be stored on a secure computer located in PI's office with access permitted only to the PI, co-Investigators and other members of the research team after IRB approval.

At the conclusion of the study, the paper and digitized data will be permanently de-identified by the destruction of the key associating the unique study code with the subjects' identity.

Olfactory Mucosal (OM) samples will be similarly de-identified and stored in a-40°C or lower temperature freezer in Dr. Sue Griffin's lab in the Donald W. Reynolds Institute on Aging.

De-identified OM samples and linked accompanying study data (including diagnostic certainty, motor and cognitive scores as well as smell testing data from study related procedures) will be stored for future research, and may be shared, de-identified, with other researchers at or outside UAMS, provided the subjects have given their written informed consent. Regarding the genotyping of apo-E4, results will not be shared with the study subjects. There exist several uncertainties about the reliability of the small sample and we do not intend to validate the results against a traditional method since this is a proof of concept pilot study.

Subjects can withdraw their consent and require destruction of their samples/data to prevent their use in future research by sending a written request to Drs. Dhall or Tulunay-Ugur.

Data Analysis

As a first pass, frequency distributions and cross tabulations will be obtained to look at the data. Counts of missing data will be obtained. The distributions will be looked at for heaping, skewness and general amenability to analysis. We will compare the individuals with Parkinson's disease to those with presbylarynx or other degenerative disorders to look for any demographic differences. Cross tabulations will be done between "independent" (diagnosis of Parkinson's disease versus presbylarynx or other degenerative disorders) and "dependent" variables including abnormalities on laryngoscopy or stroboscopy, performance on smell identification test as both a continuous measure, and when classified into hyposmia and anosmia based on age and gender expected norms.

Additionally, we will look at the presence of histopathological markers for inflammation and misfolded protein deposition by potential confounding variables like age, gender, duration of disease, treatment characteristics at baseline and performance on smell testing etc. If needed, analyses will be repeated using stratification to investigate the effect modification by age, gender and time since diagnosis of Parkinson Disease, etc. Since these are preliminary analyses to determine effect size estimates to help power future studies, a power sizes cannot be estimated at this time.

Ethical Considerations

This study will be conducted in accordance with all applicable government regulations and University of Arkansas for Medical Sciences research policies and procedures. This protocol and any amendments will be submitted and approved by the UAMS Institutional Review Board (IRB) to conduct the study.

The formal consent of each subject, using the IRB-approved consent form, will be obtained before that subject is submitted to any study procedure. All subjects for this study will be provided a consent form describing this study and providing sufficient information in language suitable for subjects to make an informed decision about their participation in this study.

The person obtaining consent will thoroughly explain each element of the document and outline the risks and benefits, alternate treatment(s), and requirements of the study. The consent process will take place in a quiet and private room, and subjects may take as much time as needed to make a decision about their participation. Participation privacy will be maintained and questions regarding participation will be answered. No coercion or undue influence will be used in the consent process.

This consent form must be signed by the subject or legally authorized representative (LAR) and the individual obtaining the consent. If the subject is determined to lack capacity to consent and a LAR provides consent, assent will be sought from the subject, and dissent will be honored. A copy of the signed consent will be given to the participant, and the informed consent process will be documented in each subject's research record.

Dissemination of Data

Results of this study may be used for presentations, posters, or publications. The publications will not contain any identifiable information that could be linked to a participant.

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