A Pilot Study Measuring The Proteome of Nasal and Oral Breath in Alzheimer's Disease

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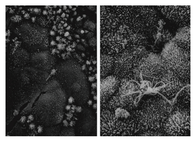
Abstract

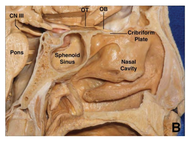
A wide body of work has established hyperphosphorylated tau and plaques of β-amyloid (Aβ) in the brain as key biomarkers for Alzheimer's Disease (AD). Aβ and tau are involved in complex signaling pathways, and AD pathology is complex, including associations with higher order systems (Tong et al. 2018, Stampher 2006, Jung et al. 2019). In line with this, large-scale proteomic analyses of human brain tissue and Cerebrospinal Fluid (CSF) have revealed numerous proteins associated with AD (Olsson et al. 2016, Johnson et al. 2020), suggesting that numerous novel AD biomarkers remain to be discovered.

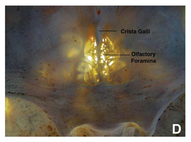
Given that brain tissue and CSF sampling are invasive, there remains a need for development of new, cost-effective, minimally-invasive biomarkers that could be used for screening in the general population and in community settings. Identification of novel biomarkers meeting these criteria could lead to both earlier diagnosis and potential insights into the mechanisms of disease progression.

Our lab has developed and patented a new device for collecting exhaled breath condensate (EBC) from clinical populations. We are using this device to study changes in the protein composition of nasal exhaled breath in aged and AD populations. EBC is typically studied in the context of respiratory illnesses, and is collected during oral, not nasal, breathing. We hypothesized that nasal EBC contains different proteins compared to oral EBC, potentially due to volatilization of proteins from the olfactory epithelium during nasal breathing. Furthermore, CSF, which is rich in AD biomarkers, may drain through the cribriform plate into the olfactory epithelium (Johnston et al. 2004, Kida et al. 1993, Melin et al. 2020). Since olfactory deficits are commonly identified early in the course of AD, this novel sample type may hold promise for exhibiting changes in novel and unexplored proteins associated with the presence of AD at early stages.

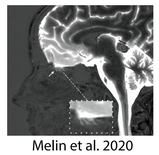


Morrison and Costanzo, 1990





Lopez-Elizalde et al 2018



Melin et al. 2020

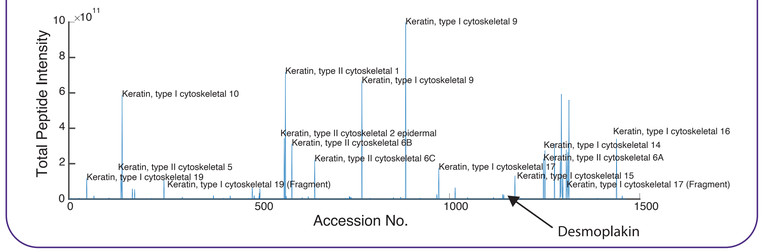
Methods

In this proof-of-concept preliminary study, we collected exhaled breath condensate samples from three different groups: 10 healthy young participants, 10 healthy aged participants and 10 patients with Alzheimer's disease. Each participant provided a nasal and oral sample. N=30, 60 samples total.

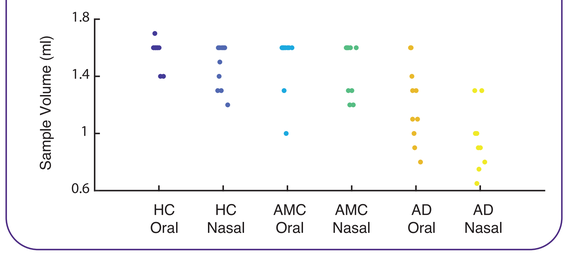
Specimens were analyzed using mass spectrometry (Liquid Chromatography-Tandem Mass Spectrometry). Proteomics allowed us to conduct an initial exploratory analysis to identify proteins that were present in breatn of the different groups and to identify potential targets for future analyses using more sensitive methods.

Results

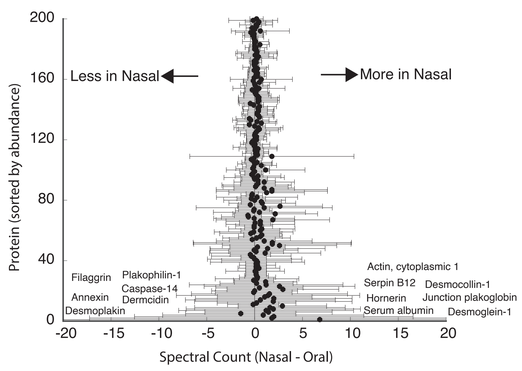
Keratin Proteins are Abundant in EBC



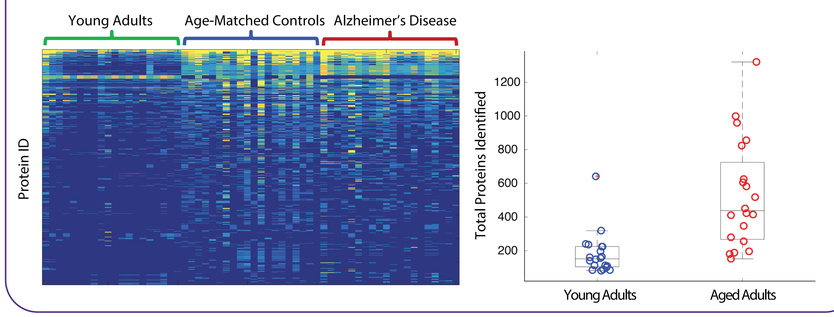
AD Patients Produce Lower EBC Sample Volumes



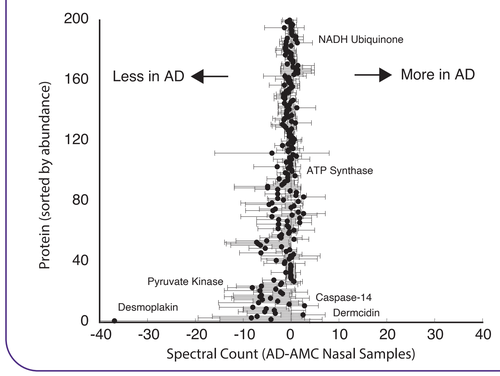
Differences in Proteome of Nasal and Oral EBC Samples



Total Protein Abundance is Increased in EBC Collected From Older People

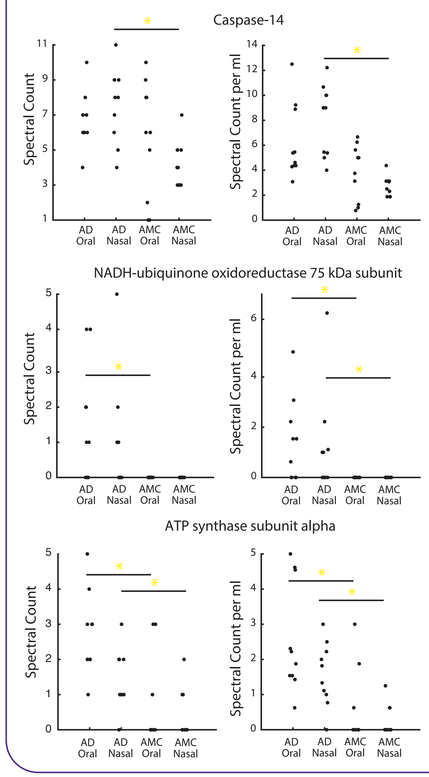


Differences in Proteome of EBC collected from AD Patients and Age-Matched Controls

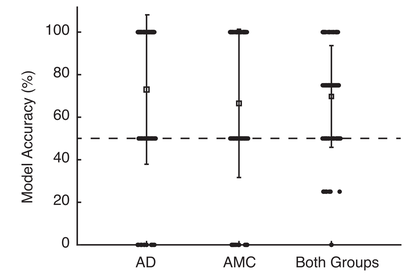


Protein	Cell Location	Implicated in AD?
ATP Synthase	Mitochondria	Yes
NADH Ubiquinone	Mitochondria	Yes
Caspase	Cytosol	Yes
Dermcidin	Secreted	Yes
Hornerin	Mitochondria	No
Suprabasin	Secreted	No
Pyruvate Kinase	Cytosol	Yes

Potential Biomarkers of AD



Machine Learning Predicts AD Based on Protein Abundance



Random Forests models predicting AD using protein abundance in nasal EBC using 3 out of the 10 most abundant proteins in 100 permutations

Future Directions

Collect more data (Nasal and Oral)

Include Technical Control for Keratins

Run targeted analyses such as ELISA assays on proteins of interest.

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