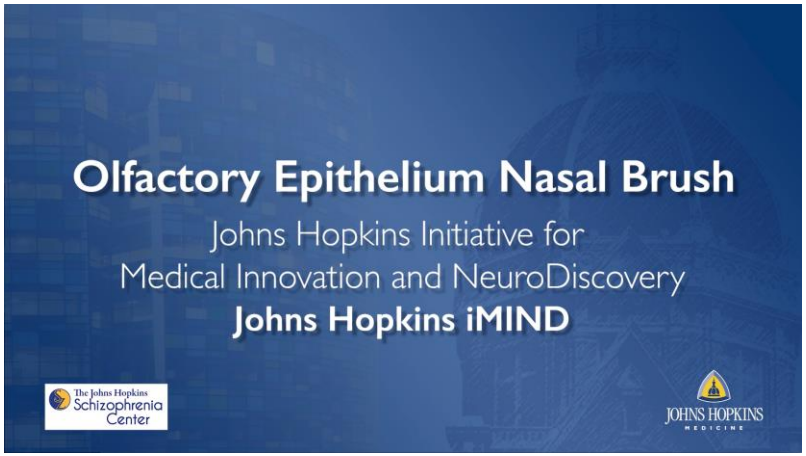
Exploration of Brain Disorder Biomarker Using Non-invasively Collected Olfactory Neurons

Johns Hopkins Schizophrenia Center (JHSZC)

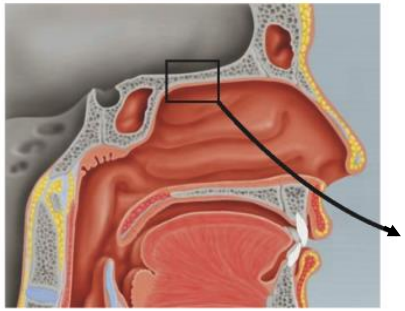
Johns Hopkins Initiative for Medical Innovation and NeuroDiscovery (iMIND)

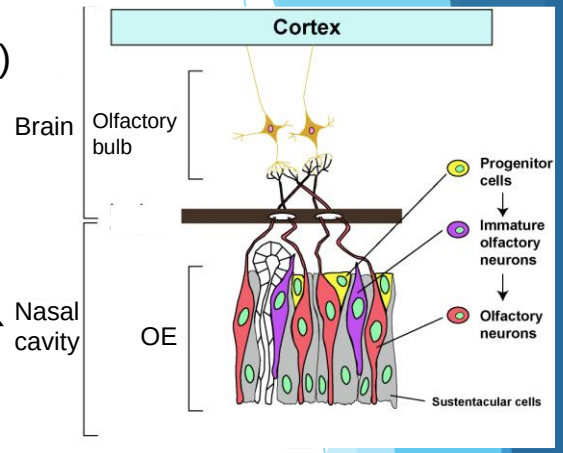
Non-invasive collection of olfactory neurons using nasal brush



(video available upon request)

Olfactory epithelium (OE)





- Nasal swab can be repeated without impairing olfactory function since olfactory sensory neurons can quickly regenerate.
- 0.5-2 million cells are collected from one procedure (4-6 brush swabs) among which 10-30 % cells are olfactory sensory neurons
- U.S. Patent Application No.16/107,246 (pending)

OE-derived molecules as non-invasive biomarkers for brain disorders

On going project:

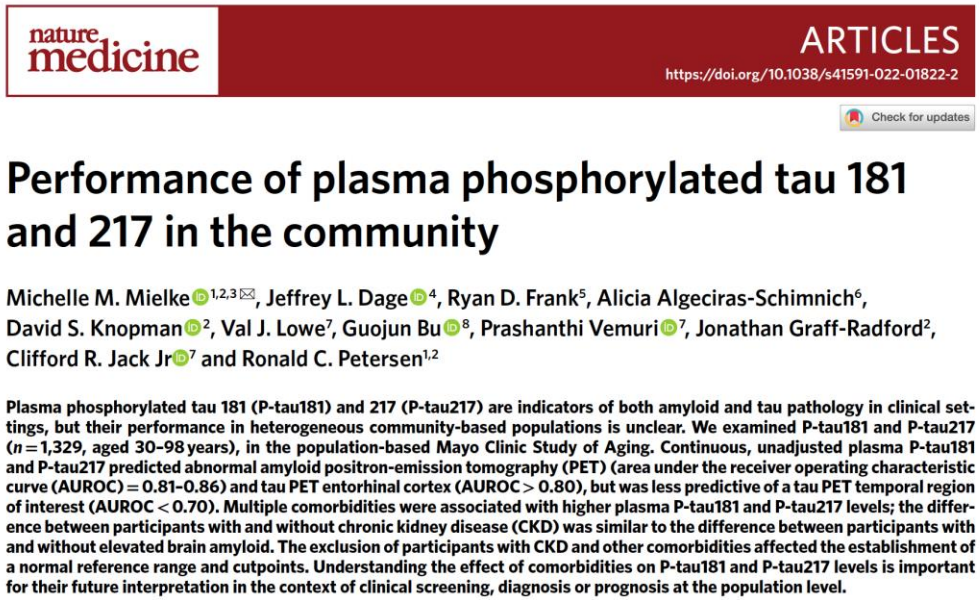
- Alzheimer's disease biomarker discovery (pT181-tau)

  (NIA/NIH grant #R21AG070754)

Future perspectives:

- Parkinson's disease (alpha-synuclein)
- Psychotic disorders (pDISC1)

Why neurons are better than liquid biopsy for biomarker detection in Alzheimer's disease?



nature medicine

ARTICLES

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Performance of plasma phosphorylated tau 181 and 217 in the community

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Plasma phosphorylated tau 181 (P-tau181) and 217 (P-tau217) are indicators of both amyloid and tau pathology in clinical settings, but their performance in heterogeneous community-based populations is unclear. We examined P-tau181 and P-tau217 ($n = 1,329$, aged 30–98 years), in the population-based Mayo Clinic Study of Aging. Continuous, unadjusted plasma P-tau181 and P-tau217 predicted abnormal amyloid positron-emission tomography (PET) (area under the receiver operating characteristic curve (AUROC) = 0.81–0.86) and tau PET entorhinal cortex (AUROC > 0.80), but was less predictive of a tau PET temporal region of interest (AUROC < 0.70). Multiple comorbidities were associated with higher plasma P-tau181 and P-tau217 levels; the difference between participants with and without chronic kidney disease (CKD) was similar to the difference between participants with and without elevated brain amyloid. The exclusion of participants with CKD and other comorbidities affected the establishment of a normal reference range and cutpoints. Understanding the effect of comorbidities on P-tau181 and P-tau217 levels is important for their future interpretation in the context of clinical screening, diagnosis or prognosis at the population level.

New research (*Mielke et al, Nat Med, 2022*) shows concerns about a simple blood test to diagnose Alzheimer's disease since biomarker levels in blood were significantly impacted by comorbidities, such as chronic kidney disease or history of stroke, which can potentially give false positive results.

Association between AD status and pT181-tau level in olfactory sensory neurons

pT181-tau levels were measured in olfactory neurons by single-cell Western Blotting (WB)

Clinically diagnosed patients with AD and cognitively normal controls were recruited, and cells were collected by nasal brush swab. Single-cell WB was conducted with a pT181-tau antibody and an OMP (marker for olfactory mature neurons) antibody. pT181-tau levels were measured only in OMP-positive olfactory neurons at the single-cell resolution. Interestingly, the pT181-tau levels in patients with AD were significantly higher than those in controls. The pT181-tau levels were also associated with their cognitive test scores.

(NIA R21AG070754)

Publication plan for current nasal brush studies

- Proof of concept paper: 2023
  - Characterization of nasal brush cells
  - AD biomarker study data (cross-sectional association study)
- Longitudinal study of AD biomarker paper: 2025
  - Prediction of AD

Future technical directions

Current single-cell strategy

Low throughput

- Manual single-cell preparation from nasal swab cells
- Single-cell WB
- Median value of pT181-tau levels from ~100 single olfactory neurons

Future bulk cell strategy

High throughput

- Single-cell preparation from nasal swab cells
- Purify olfactory neurons
- Regular ELISA for pT181-tau
- pT181-tau levels normalized by olfactory neuron marker protein levels

Future technical directions

Current single-cell strategy

Low throughput

Manual single-cell preparation from nasal swab cells

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Single-cell WB

↓

Median value of pT181-tau levels from ~100 single olfactory neurons

Future single-cell strategy

High throughput

Single-cell preparation from nasal swab cells

↓

Multiplex single-cell WB

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Median value of pT181-tau levels from ~100 single olfactory neurons

Merit of working with JHU

I. Our resources/experiences
   - Human sample collection with multimodal clinical data

II. Potential outcomes
   - Product co-branding® with JHU
   - Joint publication

Expectations to the industry partner

I. Funding i.e. sponsored research

II. Technology licensing

III. Technology improvement

Expectations to the industry partner

III. Technology improvement

- Sample collection
  - Quick single-cell preparation
  - Cell fixation/freezing/preservation
  - Direct purification of neurons on the bench
- ELISA plates pre-coated with pT181-tau and neuronal marker antibodies

JHU key personnel

Name	Position	Role
Akira Sawa, M.D.	Director, JHSZC, iMIND Professor, SOM, SPH, Hospital	PI Inventor
Koko Ishizuka, M.D., Ph.D.	Assistant professor, JHSZC, iMIND SOM	Co-PI Inventor
Yukiko Lema	Research program manager, JHSZC, iMIND SOM	Program/Project support Clinical trial support Clinical sample logistics

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