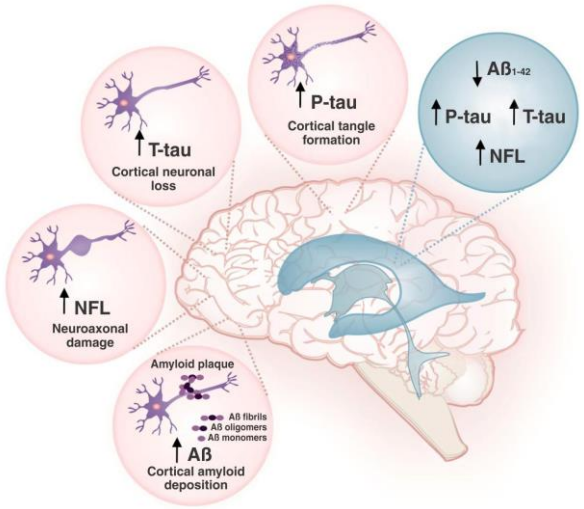

Generalizability of Fluid Biomarkers

May 6, 2024

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Disclosures: Suzanne Schindler, MD, PhD

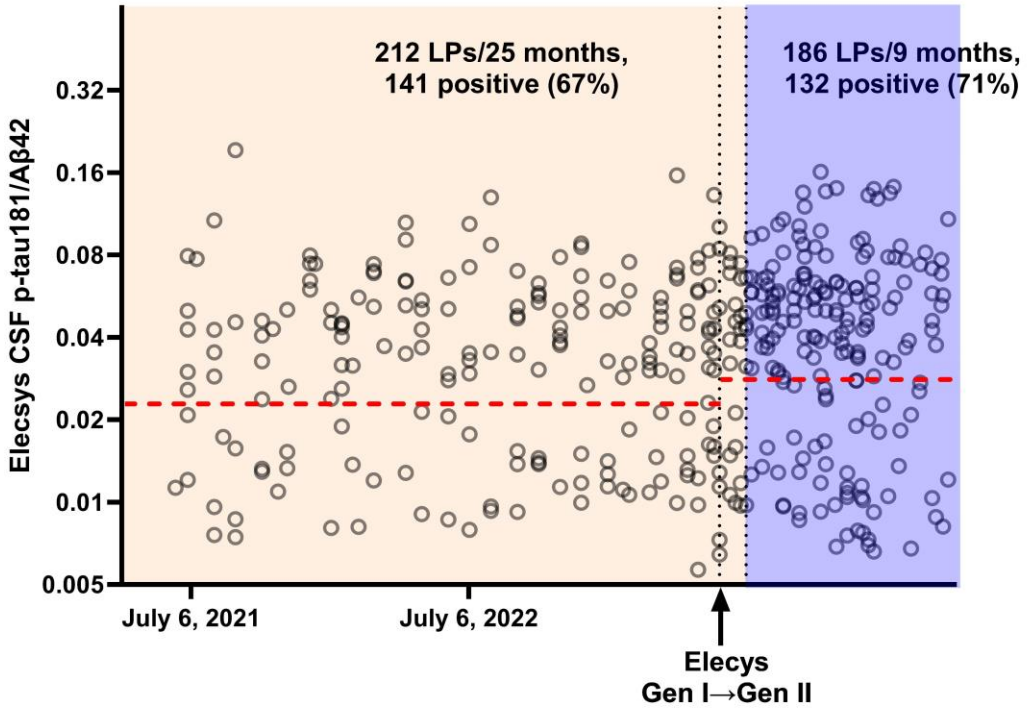
- Research support/grants: Salary and research support is primarily from R01AG070941 (PI Schindler); P30AG066444, P01AG003991 and P01AG026276 (PI Morris)
- **Dr. Schindler has previously analyzed biomarker data provided to Washington University by C2N Diagnostics and Roche Diagnostics; no financial incentives or research funding were provided to Dr. Schindler in return.**
- Stock/Equity: None
- **Consulting/Employment: Dr. Schindler has been compensated by Eisai and Medscape for providing guidance and educational content on biomarker testing. Dr. Schindler has not received any personal compensation or direct research funding from any diagnostics companies.**
- Speakers Bureau/Honoraria: Dr. Schindler receives honoraria as a member of the biorepository review committee for the non-profit National Centralized Repository for Alzheimer's Disease (NCRAD); she has received honoraria for participating in expert panels and reviewing grants from non-profit organizations
- Other: Dr. Schindler previously served as a sub-PI for the A4, DIAN-TU, and ENGAGE trials. Dr. Schindler participated in the IDEAS trial.

Why is generalizability of fluid biomarkers important?

- We are increasingly using fluid biomarkers in research, clinical trials and clinical diagnosis
- We are increasingly making treatment decisions based on the results of fluid biomarkers

- **The utility of a biomarker test depends on its ability to provide an accurate result for all individuals who undergo the test**

Clinical CSF testing at Washington University



Unpublished data

How do we assess the generalizability of a fluid biomarker test?

- Biomarkers are useful when levels strongly **reflect key biological and/or clinical factors** related to a disease of interest

Signal

- Biomarker levels may additionally be affected by **factors not directly related to the disease of interest** that partially obscure the signal

Noise

- Generalizability refers to **how consistently biomarkers reflect key biological and/or clinical factors across all individuals**

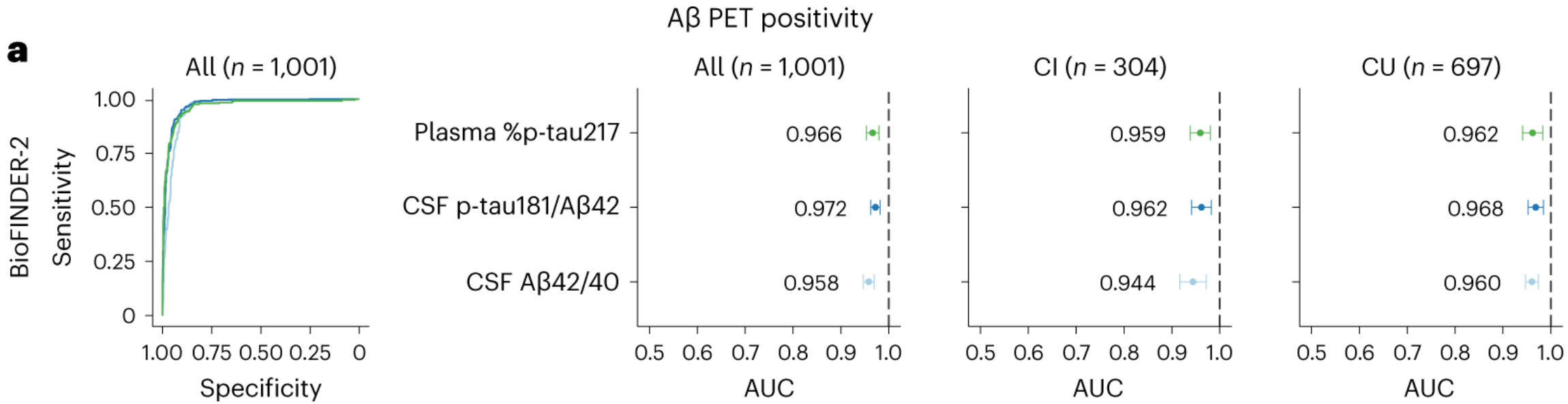
Signal:Noise

Low Signal:Noise **High Signal:Noise**

High generalizability requires a high signal:noise to clearly "see" the underlying biological/clinical condition, regardless of individual factors

Evaluating the signal: classification accuracy

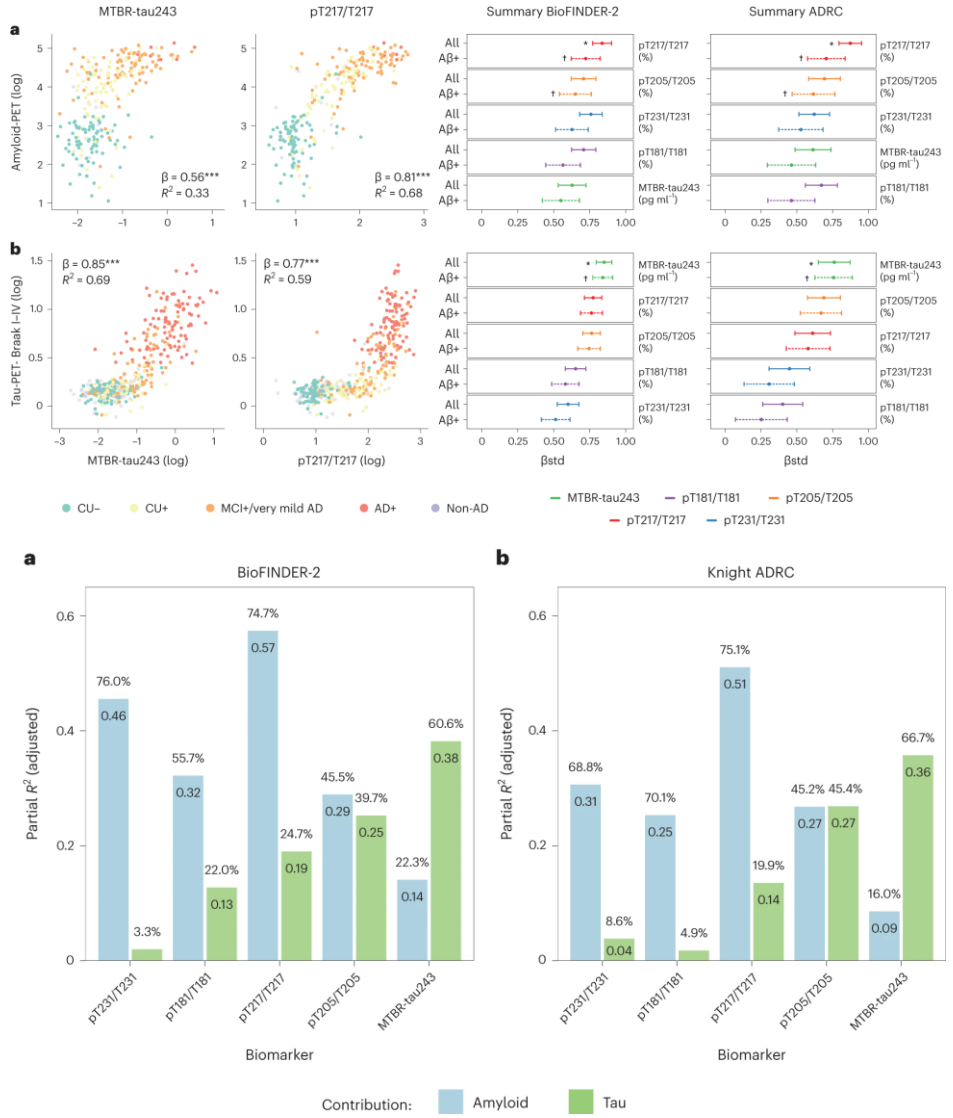
- An excellent “gold standard” for key biological and/or clinical factors is essential
- High associations between fluid biomarkers and the reference standard demonstrates a strong signal and high signal:noise



Barthelemy, Salvado, *et al. Nature Medicine* 2024

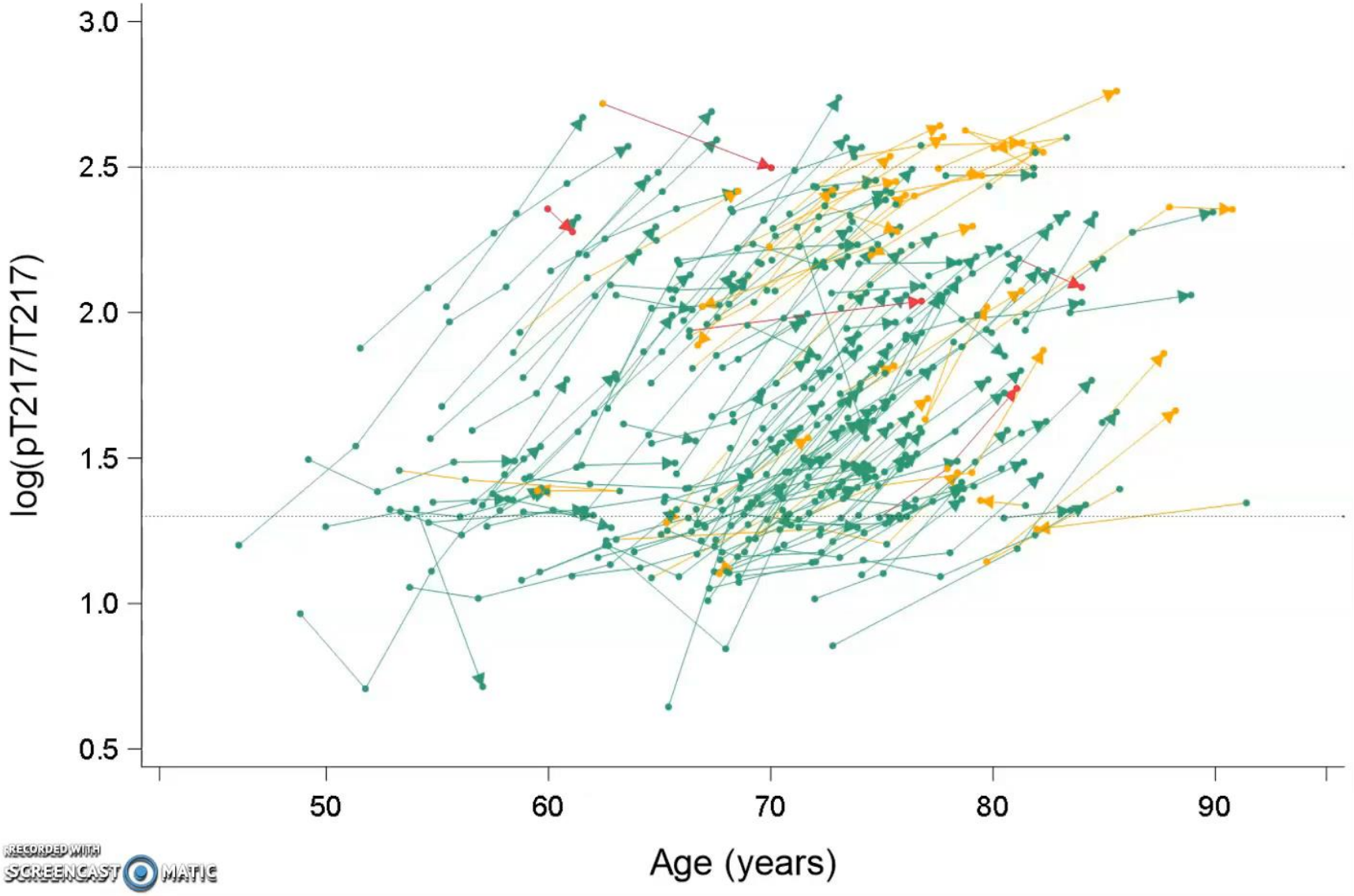
Evaluating the signal: continuous relationships

- Modeling can be performed to assess the strength of the association between continuous values for fluid biomarkers and reference standards
- Non-linear relationships are common, with stronger relationships during specific phases of disease
- Models can evaluate associations of fluid biomarkers with multiple pathologies simultaneously (e.g., amyloid and tau)



Horie, *et al. Nature Medicine* 2023

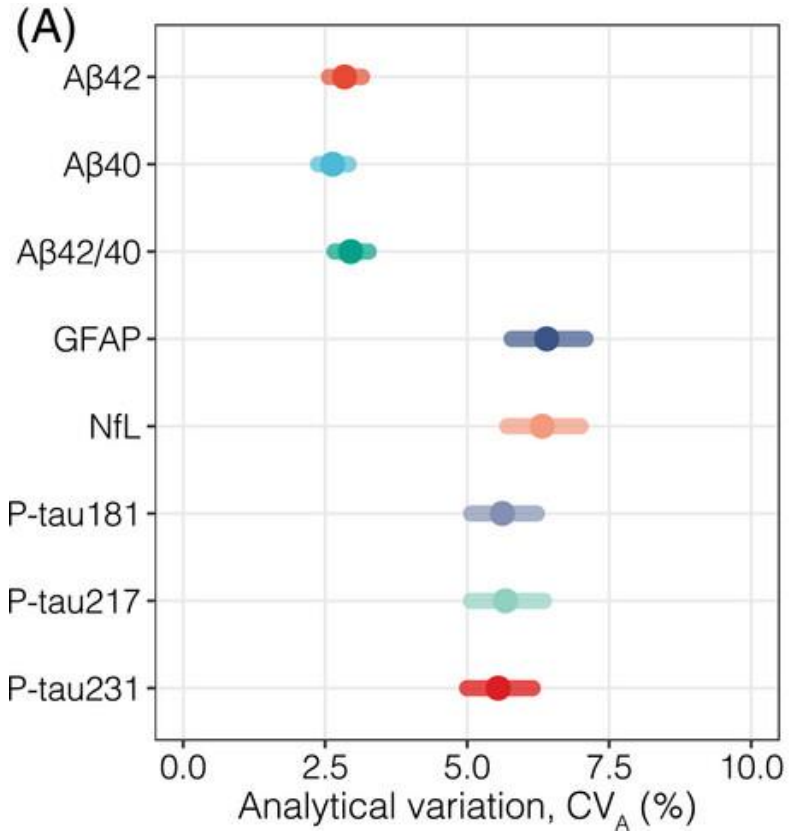
Evaluating the signal: longitudinal relationships



Evaluating the noise: cross-sectional and longitudinal variance

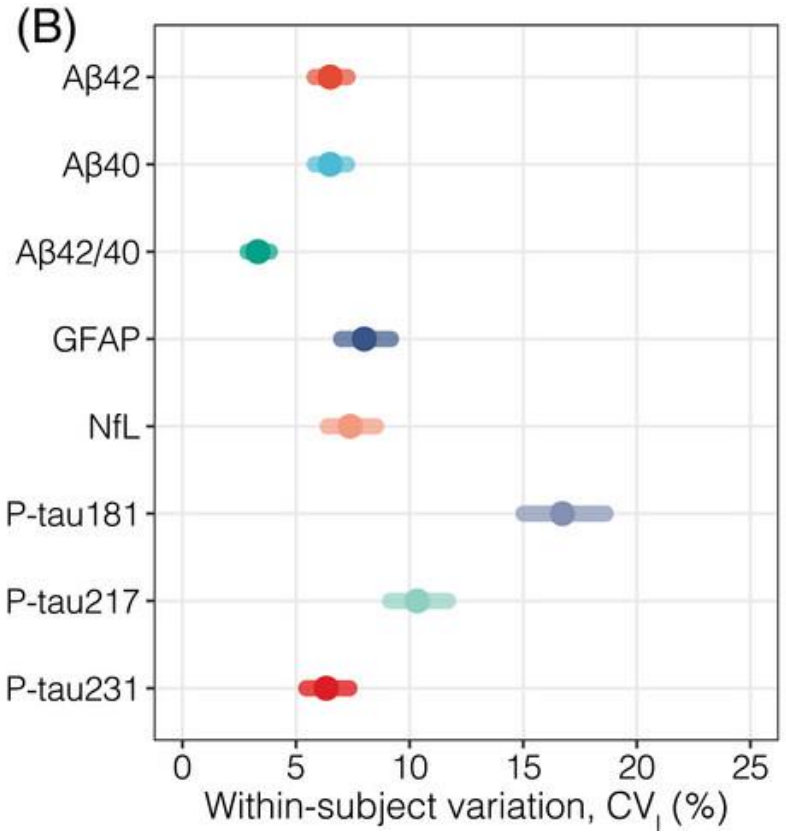
Noise

Analytical variation



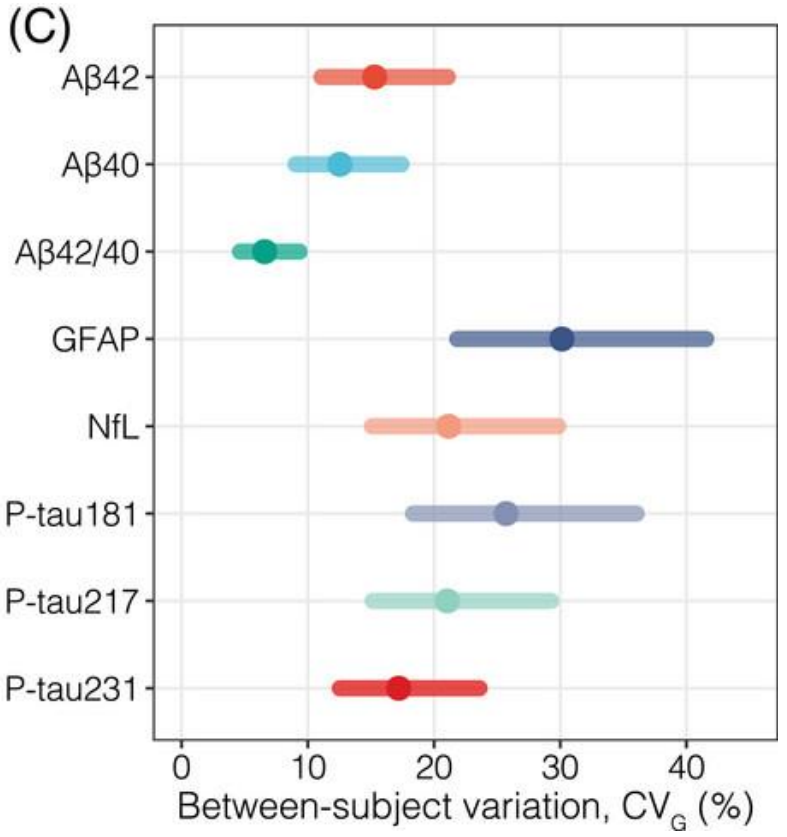
Noise

Intra-individual variation



Signal + Noise?

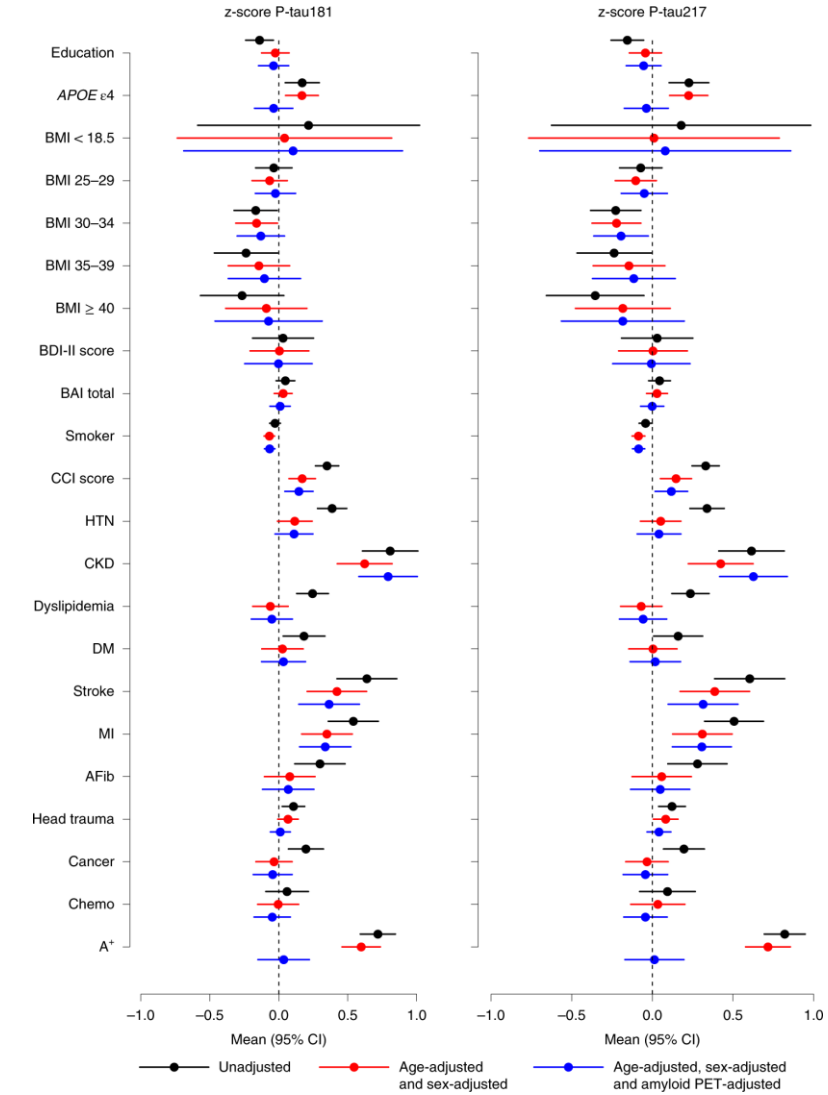
Related + un-related to disease



Brum, *et al. Alzheimer's and Dementia* 2023

Evaluating the noise: continuous relationships

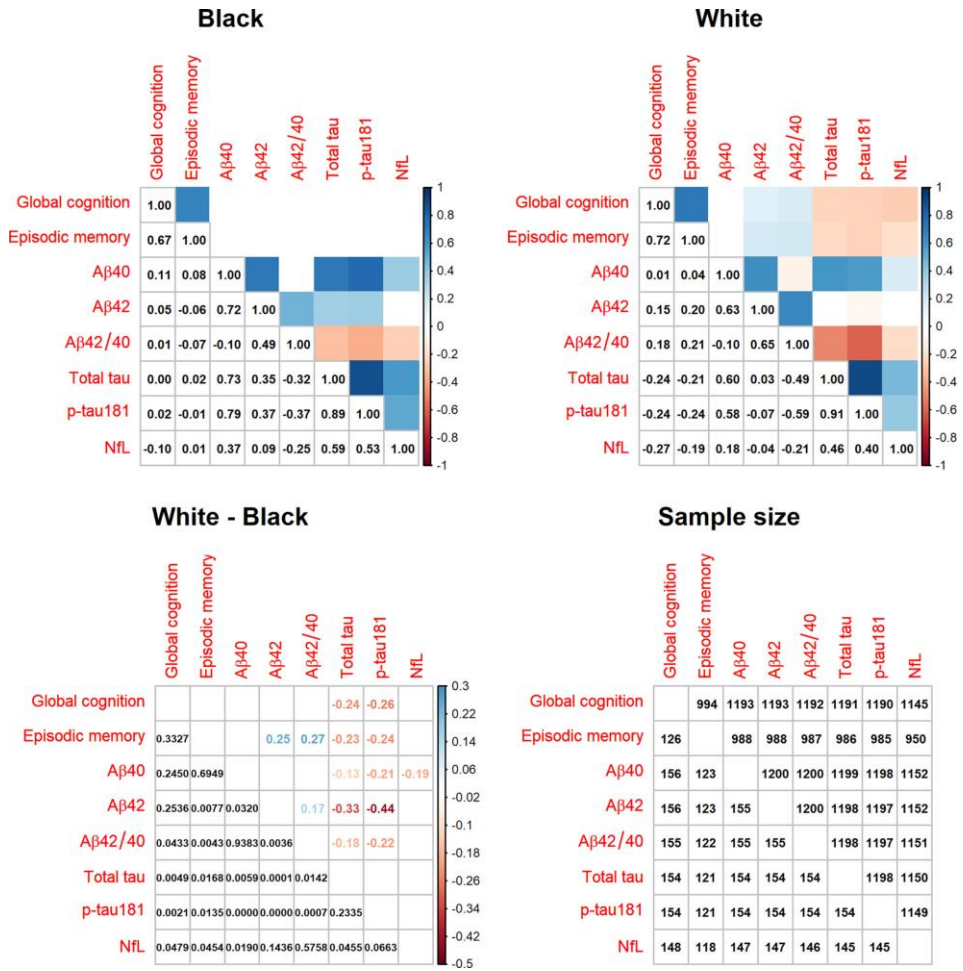
- Levels of some fluid biomarkers are associated with factors not directly related to the disease of interest (e.g., age, sex, race, ethnicity, comorbidities, medications)
- Especially when lower performing biomarker tests are used in a diverse clinical populations (e.g., Bouteloup *et al., Neurology* 2024), the signal:noise may be extremely low, such that the biomarker test result has little relationship to the disease and depends largely on non-disease related factors



Mielke, *et al. Nature Medicine* 2022

Evaluating the noise: differences in continuous relationships

- The correlations between CSF biomarkers and cognitive measures vary between Black and White individuals
- Rates of amyloid positivity may vary by race/ethnicity, even after accounting for many other variables
- Lower rates of amyloid positivity may exacerbate under-representation of Black and Hispanic individuals in clinical trials (Molina-Henry, *et al., Alzheimer's and Dementia* 2024)



Bonomi, Lu, *et al. Neurology* 2022

Evaluating the noise: classification accuracy

- Logistic regression models of amyloid status
  - Covariate-adjusted biomarker effects reflect the signal
  - Covariate effects may (or may not) reflect noise
  - The AUC reflects the signal:noise
- For biomarker tests with high signal:noise (AUC)
  - Biomarkers have stronger associations with amyloid status
  - Covariates have weaker associations with amyloid status
- For biomarker tests with low signal:noise (AUC)
  - Biomarkers have weaker associations with amyloid status
  - Covariates have stronger associations with amyloid status
- **High accuracy tests are needed for generalizability**

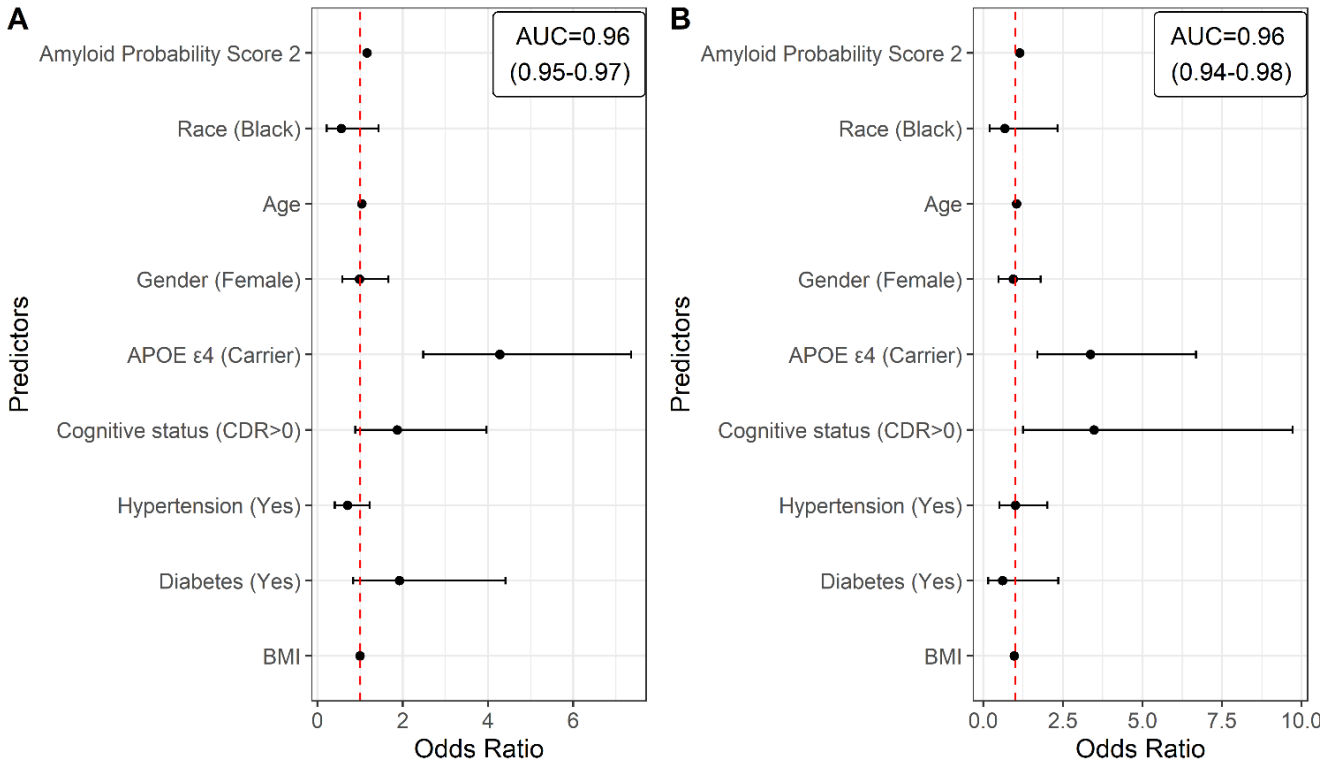
Classification of amyloid status

Plasma Aβ42/Aβ40, AUC 0.90 (0.85-0.96)			
Parameter	**Estimate**	**SE**	**p =**
Intercept	13.0	4.7	0.005
Plasma Aβ42/Aβ40 (pg/ml)	-220	46	<0.0001
Race (African American)	0.058	0.274	N.S.
Sex (female)	0.843	0.568	N.S.
Age (years)	0.109	0.04	0.007
APOE ε4 status (carrier)	0.865	0.269	0.001
Cognitive status (CDR>0)	1.11	0.41	0.007
Plasma p-tau181, AUC 0.85 (0.79-0.92)			
Parameter	**Estimate**	**SE**	**p =**
Intercept	-8.69	2.71	0.001
Ln (plasma p-tau181)	1.53	0.57	0.007
Race (African American)	-0.59	0.22	0.007
Sex (female)	-0.21	0.44	N.S.
Age (years)	0.072	0.035	0.04
APOE ε4 status (carrier)	0.87	0.23	0.0002
Cognitive status (CDR>0)	1.02	0.39	0.009
Plasma NfL, AUC 0.81 (0.74-0.89)			
Parameter	**Estimate**	**SE**	**p =**
Intercept	-6.20	2.41	0.01
Ln (plasma NfL)	-0.097	0.476	N.S.
Race (African American)	-0.65	0.22	0.003
Sex (female)	-0.50	0.42	N.S.
Age (years)	0.109	0.040	0.007
APOE ε4 status (carrier)	0.89	0.23	<0.0001
Cognitive status (CDR>0)	1.27	0.39	0.001

Schindler, Karikari, *et al. Neurology* 2022

A high accuracy blood test classifies amyloid status consistently

- Individuals in the Knight ADRC cohort (75 Black individuals, 687 White individuals)
- Race, sex, age, hypertension, diabetes, and BMI did not significantly affect classification of amyloid status by APS2 (plasma Aβ42/40 + %p-tau217)



CSF Aβ42/40 status

Amyloid PET status

Bui et al., in preparation

Needed: more representative cohorts

- Research cohorts traditionally have been relatively homogenous, with potentially lower biomarker variance due to non-disease related factors
- Ideally, cohorts used for biomarker validation would resemble the intended use population
  - Community-based for screening tests
  - Clinic-based for diagnostic tests
- However, biomarker validation requires high quality reference standards (CSF and PET), and it may be difficult to obtain CSF and PET in a truly representative cohorts
- Smaller, targeted, and carefully designed studies to evaluate the effects of certain conditions may be more efficient than very large studies of low frequency conditions

Conclusions

- We need fluid biomarkers that accurately and consistently reflect disease processes in all individuals
- High generalizability requires a high signal:noise to clearly “see” the underlying biological/clinical condition
- A variety of studies can be used to assess the signal:noise of different fluid biomarker tests
- We need more representative cohorts with reference standards to validate fluid biomarkers
- Smaller, carefully designed cohorts may also be helpful in answering specific questions

Acknowledgements

- **Our research participants and their supportive families**
- **Washington University:**
  - **Mentors:** Randall Bateman, Anne Fagan, David Holtzman, John Morris
  - **Collaborators:** Beau Ances, Andrew Aschenbrenner, Nico Barthelemy, Tammie Benzinger, Charles Chen, Brian Gordon, Mahendra Gupta, Sarah Hartz, Rachel Henson, Kanta Horie, Albert Lai, Yan Li, Jessica Mozersky, Madeline Paczynski, Chengjie Xiong
  - **Wash U MDC:** Joy Snider (Director), Randall Bateman, David Carr, Steven Dunham, Nupur Ghoshal, Jee-Young Han, David Holtzman, Justin Long, Eric McDade, John Morris, Erik Musiek, Madeline Paczynski, Jessica Spring, Cassandra Ward, Kyle Womack
- **BioFINDER:** Oskar Hansson, Shorena Janelidze, Gemma Salvado

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