

1 • BACKGROUND

- AD-related pathology and subtle cognitive change can begin years before clinical diagnosis.
- The **NIA-AA AT(N)** framework organizes biomarkers into amyloid, tau, and neurodegeneration; tau and neurodegeneration are closely linked to cognitive decline.

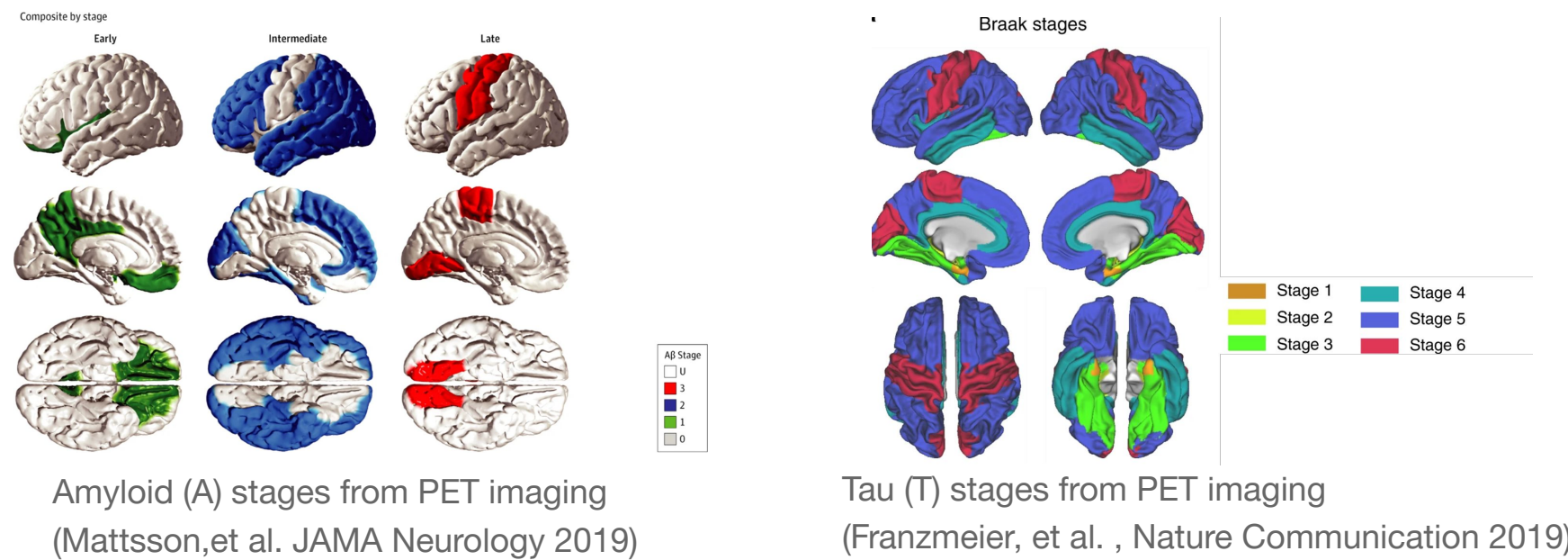


Figure 1. Amyloid (A) and Tau (T) Deposition Trajectories

- Resting-state functional connectivity is sensitive to early disruption in default-mode, salience, and medial temporal circuits.

- Subtype and Stage Inference (SuStaln)** probabilistically models both subtype and temporal stage from cross-sectional data; it has uncovered four tau-PET AD trajectories but has rarely been applied to cognitively normal (CN) cohorts.

Research Question

Can SuStaln modeling of resting-state functional connectivity identify reproducible preclinical trajectories and predict future conversion from cognitively normal status to MCI/AD?

To our knowledge, this is the first study combining PySuStaln with rs-FC in a purely CN aging cohort.

2 • METHOD – Functional Connectivity Feature Extraction

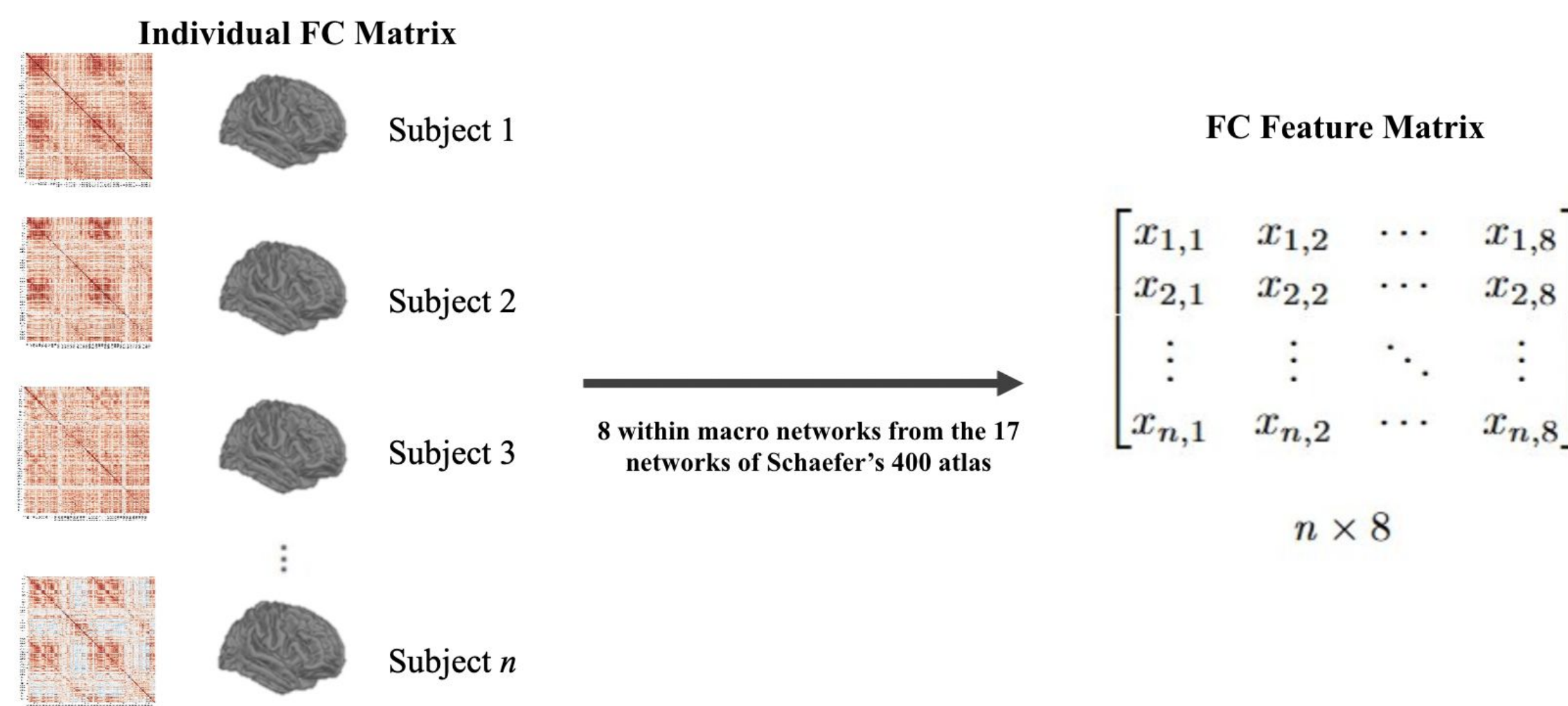


Figure 2. Schematic view of feature extraction from individual FC matrices.

- Cortex parcellated with the **Schaefer 2018** 400-region / 17-network atlas.
- Pearson correlation of regional **BOLD time-series** → **400x400 matrix** → **Fisher-Z** normalised.
- 17 networks collapsed into **8 macro-networks: Default Mode, Control, Limbic, Dorsal Attention, Somatomotor, Visual, Temporal, Salience/Ventral Attention.**
- Within-network mean** Fisher-Z → 8 features per subject → input to PySuStaln.

References

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- Young AL, et al. Uncovering the heterogeneity and temporal complexity of neurodegenerative diseases with Subtype and Stage Inference. Nature Communications. 2018;9(1):4273.
- Mattsson, N, et al. Staging β -Amyloid Pathology With Amyloid Positron Emission Tomography. JAMA Neurology 2019;76;(11):1319-1329.
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3 • METHOD II – Sustain Modeling

- SuStaln estimates both a participant's most likely subtype and disease stage based on the ordering of biomarker abnormality events. Here, SuStaln stage represents accumulated FC abnormality burden, not chronological age.
- ZscoreSustain** utilizing within-fold z-scaling to prevent data leakage.
- Candidate subtypes **K = 1 - 3 subtypes; random starts; 200,000 MCMC iterations.**
- 10-fold stratified cross-validation;** selection based on CVIC and FDR-corrected statistical tests on log-likelihood.

Table 1 Frequency of cognitive decline defined by MMSE, CDR-SUM, and predication change.

	Total (n = 472)
Conversion Types	
MMSE Decline (>2 pts)	39 (8.26%)
CDR-SUM Increase (>0.5)	39 (8.26%)
MMSE Decline (>2 pts) & CDR-SUM Increase (>0.5)	25 (5.3%)
At least one Predication Change (CN to MCI/AD)	134 (28.39%)
Final Predication Change (CN to MCI/AD)	56 (11.86%)

Linear mixed-effects model predicting MMSE (REML, random intercept by subject):

$$MMSE_{ij} = \beta_0 + \beta_1 time_{ij} + \beta_2 subtype_i + \beta_3 (time_{ij} \times subtype_i) + \beta_4 baselineMMSE_i + \beta_5 ageBaseline_i + \beta_6 sex_i + \beta_7 education_i + u_{0i} + \epsilon_{ij}.$$

4 • RESULTS – SuStaIn Identified Two Distinct FC Trajectories

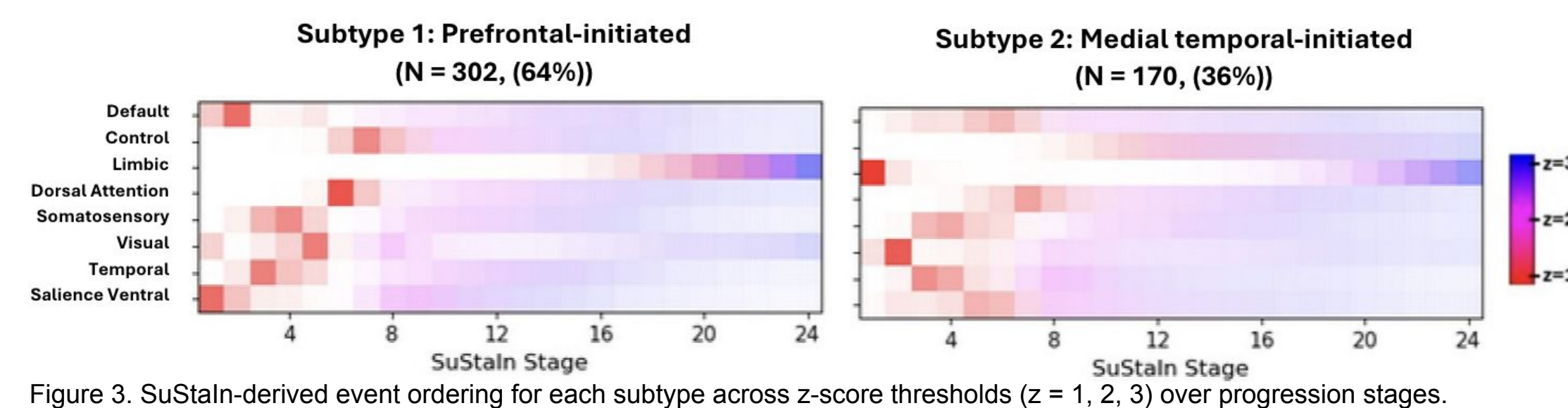


Figure 3. SuStaIn-derived event ordering for each subtype across z-score thresholds (z = 1, 2, 3) over progression stages.

Subtype 1 • Prefrontal-initiated N = 302 (64%) Early: Default Mode + Salience/Ventral Attention Middle: Somatomotor + Temporal Late: Dorsal Attention + Control Pattern: fronto-cingulo-parietal progression	Subtype 2 • Medial temporal-initiated N = 170 (36%) Early: Limbic + Temporal + Visual Middle: Somatomotor + Salience/Ventral Attention Late: Default Mode + Dorsal Attention + Control Pattern: medial temporal/occipital to fronto-parietal progression
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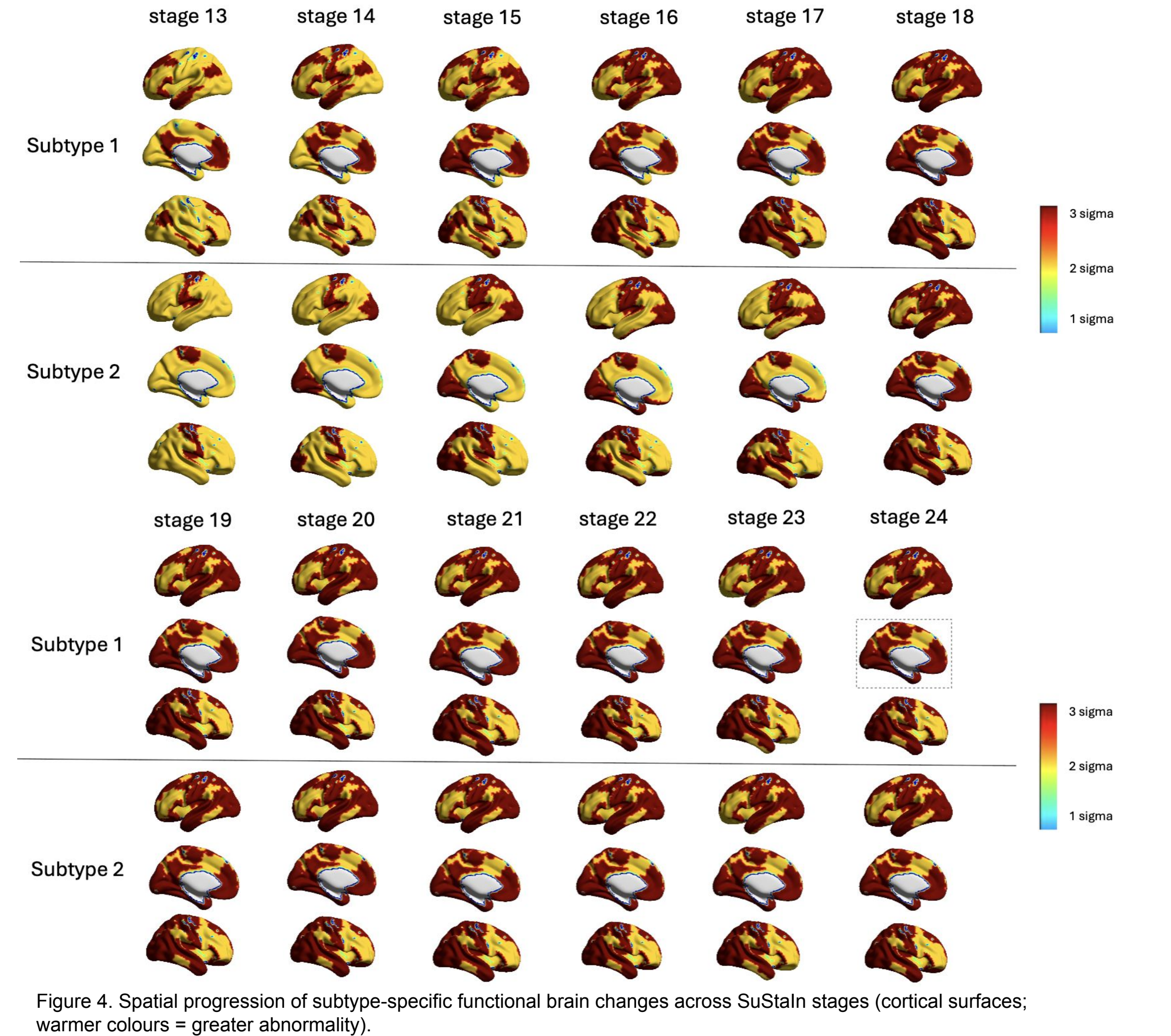
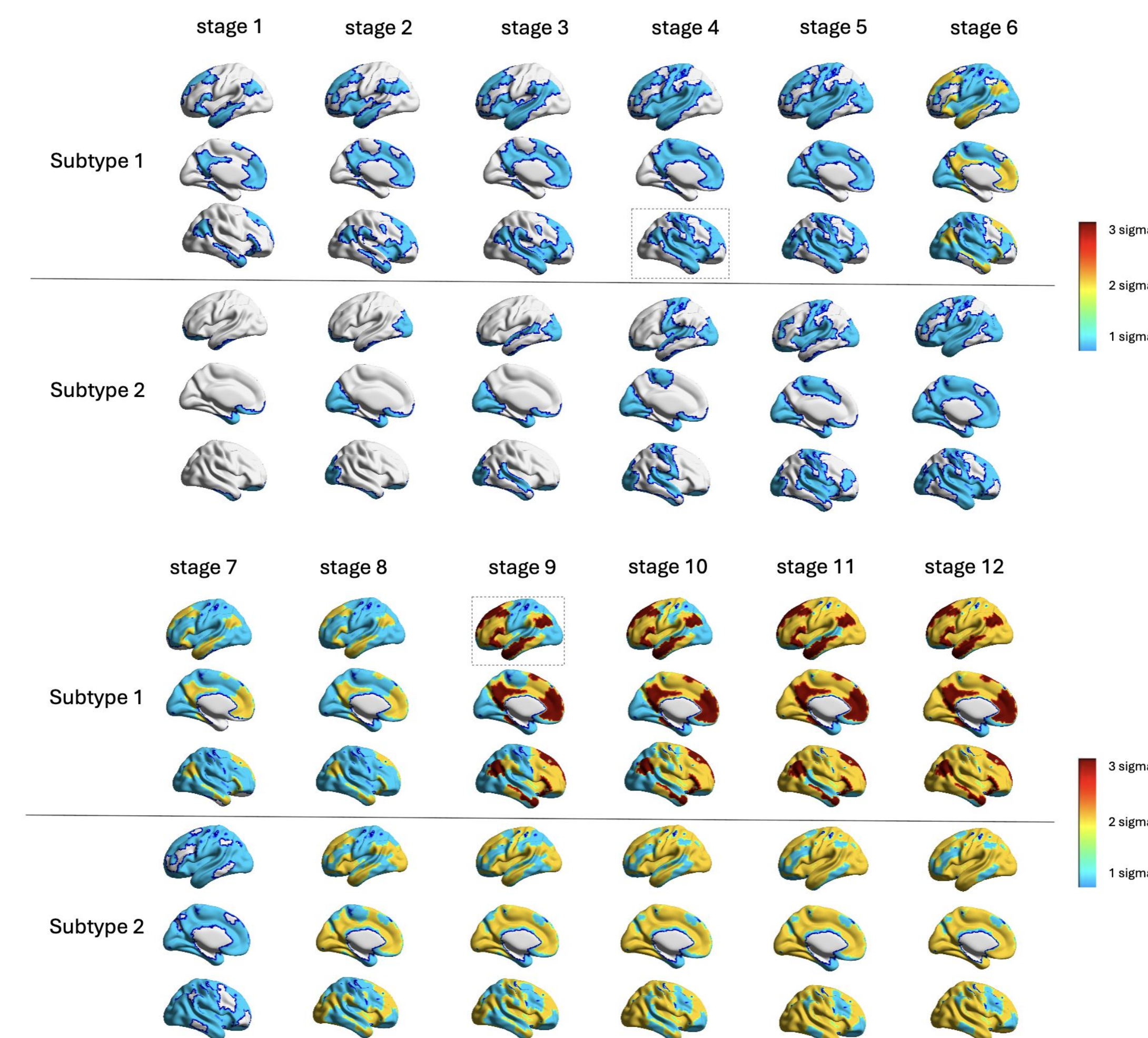


Figure 4. Spatial progression of subtype-specific functional brain changes across SuStaIn stages (cortical surfaces; warmer colours = greater abnormality).

5 • RESULTS – Baseline & Conversion

Table 2 Distribution of converters across SuStaIn subtypes.

Total (n = 472)	Subtype 1 (n = 368)	Subtype 2 (n = 104)	p value
Conversion Types			
MMSE Decline (>2 pts)	27 (7.34%)	12 (11.54%)	0.241
CDR-SUM Increase (>0.5)	31 (8.42%)	8 (7.69%)	0.97
MMSE Decline (>2 pts) & CDR-SUM Increase (>0.5)	18 (4.89%)	7 (6.73%)	0.623
At least one Predication Change (CN to MCI/AD)	104 (28.26%)	30 (28.85%)	1.000
Final Predication Change (CN to MCI/AD)	46 (12.5%)	10 (9.62%)	0.5277

Table 3 Mean disease stages across different conversion categories.

Conversion Types	Rest of Cohort	Converters	p value
MMSE Decline (>2 pts)	1.94 ± 2.43	3.10 ± 2.97	0.0049
CDR-SUM Increase (>0.5)	1.93 ± 2.44	3.15 ± 2.85	0.0018
MMSE Decline (>2 pts) & CDR-SUM Increase (>0.5)	1.96 ± 2.44	3.32 ± 3.06	0.0068
At least one Predication Change (CN to MCI/AD)	1.79 ± 2.38	2.65 ± 2.66	0.0001
Final Predication Change (CN to MCI/AD)	1.93 ± 2.46	2.79 ± 2.66	0.0032

6 • DISCUSSION & CONCLUSIONS

- Two reproducible FC trajectories** were identified in cognitively normal aging: a prefrontal/salience route and a medial temporal/visual route.
- Distinct early network involvement suggests multiple latent routes to degeneration**, detectable before measurable cognitive impairment.
- Observed FC patterns converge with known AD-sensitive circuits**, including default mode, salience, and medial temporal networks.
- Subtype 2 showed female predominance**, aligning with reported sex-related vulnerability in AD and warranting further replication.
- Baseline SuStaIn stage, not subtype, predicted future conversion**, supporting FC-based staging for risk enrichment in preclinical AD studies.

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