

Baseline Serum Proteomic Profiles Associated With The Rate Of One-year Cognitive Decline In Early-onset Alzheimer's Disease

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INTRODUCTION

Early-onset Alzheimer's disease (EOAD) is often considered rapidly progressive, but clinical trajectories vary substantially even among patients with similar baseline severity. We examined whether baseline serum proteomic profiles are associated with one-year cognitive decline in EOAD.

METHODS

- LEAF-AD is a multicenter nationwide cohort in Korea. Between 2021 and 2022, a total of 197 individuals with EOAD were enrolled. Among them, 155 completed a one-year follow-up assessment. To assess disease progression at an earlier stage, we narrowed the analysis to individuals with a baseline MMSE score of 18 or higher (n=101). The patients were divided into a rapid group (≥ 4 -point MMSE decline, n=13) and a slow group (no decline, n=13) after matching for age, sex, education, and baseline MMSE score (Figure 1).
- Blood samples were collected from the participants at the baseline visit. Serum samples were prepared, digested, and analyzed via LC-MS/MS using a Dionex UltiMate 3000 RSLCnano system coupled with an Orbitrap Exploris 480 mass spectrometer in data-independent acquisition (DIA) mode equipped with a FAIMS Pro interface. The resulting data were processed using DIA-NN (v1.8.1) in library-free mode against the UniProt human reference database, filtered at a 1% FDR, and normalized via quantile normalization before deposition into the PRIDE repository.
- Differentially expressed proteins (DEPs) were identified using an integrative statistical method that combined p-values from Student's t-test, Wilcoxon rank-sum test, and $\log_2$ median ratios via Stouffer's method against a permutation-based empirical null distribution, applying thresholds of a t-test p-value less than 0.1 and a strict cut-off for the absolute $\log_2$ median ratio.

RESULTS

Baseline demographic and clinical characteristics were comparable between groups, while follow-up MMSE differed significantly (Table 1). Overall, 904 proteins were identified; PCA showed partial separation of rapid and slow groups (Figure 2). Compared with the slow group, the rapid group exhibited 43 upregulated and 35 downregulated proteins. To identify highly robust candidates, we further filtered the DEPs by applying additional stringent criteria: being a single-copy gene, a p-value < 0.05, a $\log_2$ fold change > 1.5, and a missing value ratio $\leq 30\%$; **APOA2**, **PDE4DIP**, **NUCB1**, and **GPD1** were higher in the rapid group, whereas **VCAN** and **HP** were lower (Figure 3).

Table 1. Baseline demographic and clinical characteristics of the matched rapid and slow groups. Values are mean $\pm$ SD or n (%).

Characteristic	Rapidly	Slowly	p
N	13	13	
Age at baseline assessment, y	62.5 $\pm$ 5.6	62.1 $\pm$ 3.3	0.800
Sex, female n. (%)	8 (61.5)	7 (53.8)	1.000
Education, y	11.8 $\pm$ 4.3	13.4 $\pm$ 3.5	0.322
Onset age, y	58.0 $\pm$ 3.8	58.9 $\pm$ 3.7	0.571
APOE ϵ 4 carrier, n (%)	7 (53.8)	8 (61.5)	1.000
Hypertension, n (%)	5 (38.5)	3 (23.1)	0.673
Stroke, n (%)	0	0	NA
Cardiovascular disease, n (%)	1 (7.7)	1 (7.7)	1.000
Diabetes, n (%)	1 (7.7)	1 (7.7)	1.000
Dyslipidemia, n (%)	2 (15.4)	4 (30.8)	0.645
Baseline MMSE	22.8 $\pm$ 3.8	23.1 $\pm$ 3.1	0.822
Baseline CDR	0.73 $\pm$ 0.26	0.73 $\pm$ 0.26	1.000
Baseline CDR-SOB	3.8 $\pm$ 2.1	3.1 $\pm$ 2.0	0.347
1 year follow-up MMSE	17.0 $\pm$ 3.8	24.8 $\pm$ 3.5	<0.001

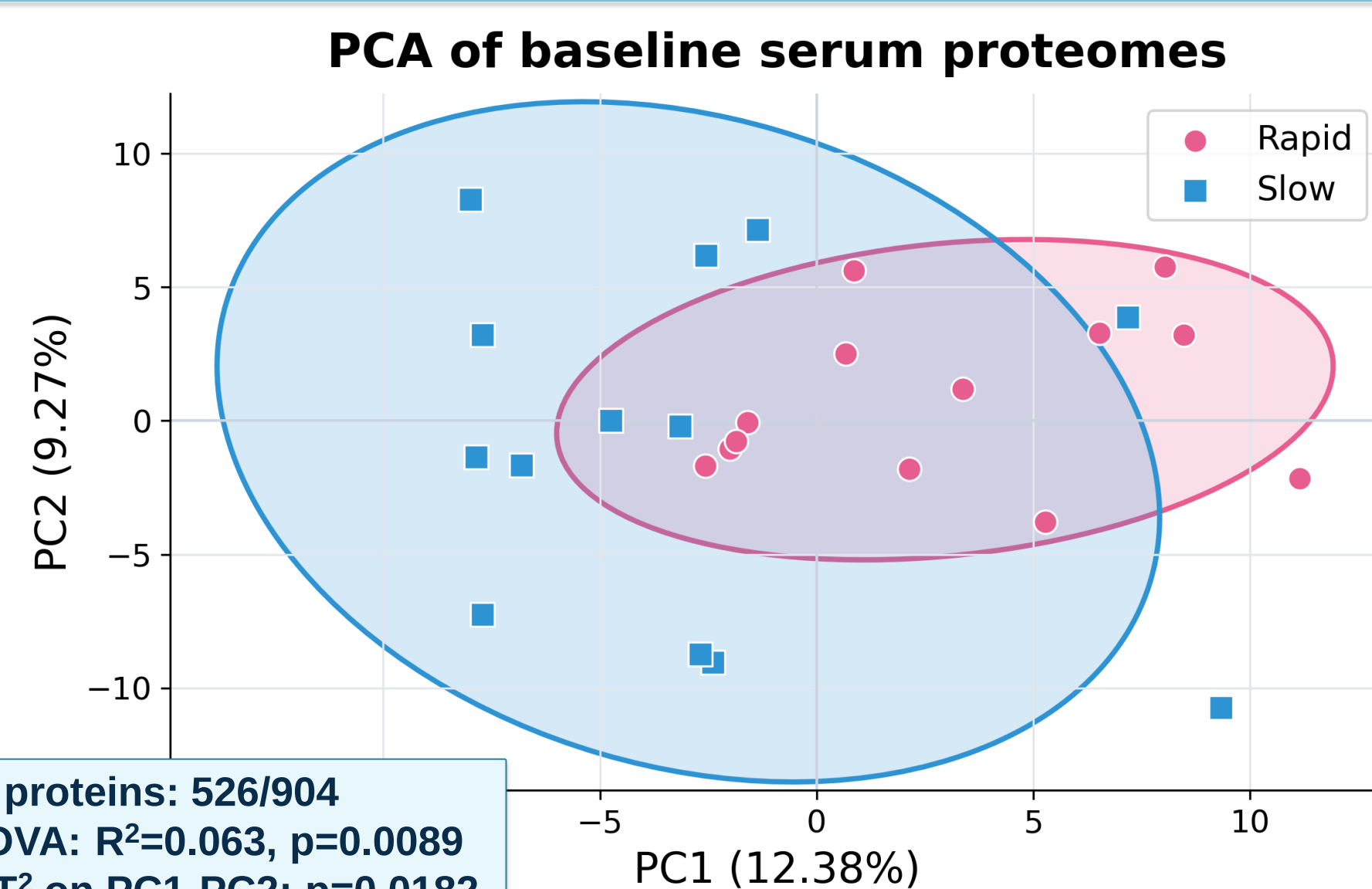


Figure 2. Principal component analysis of baseline serum proteomes.

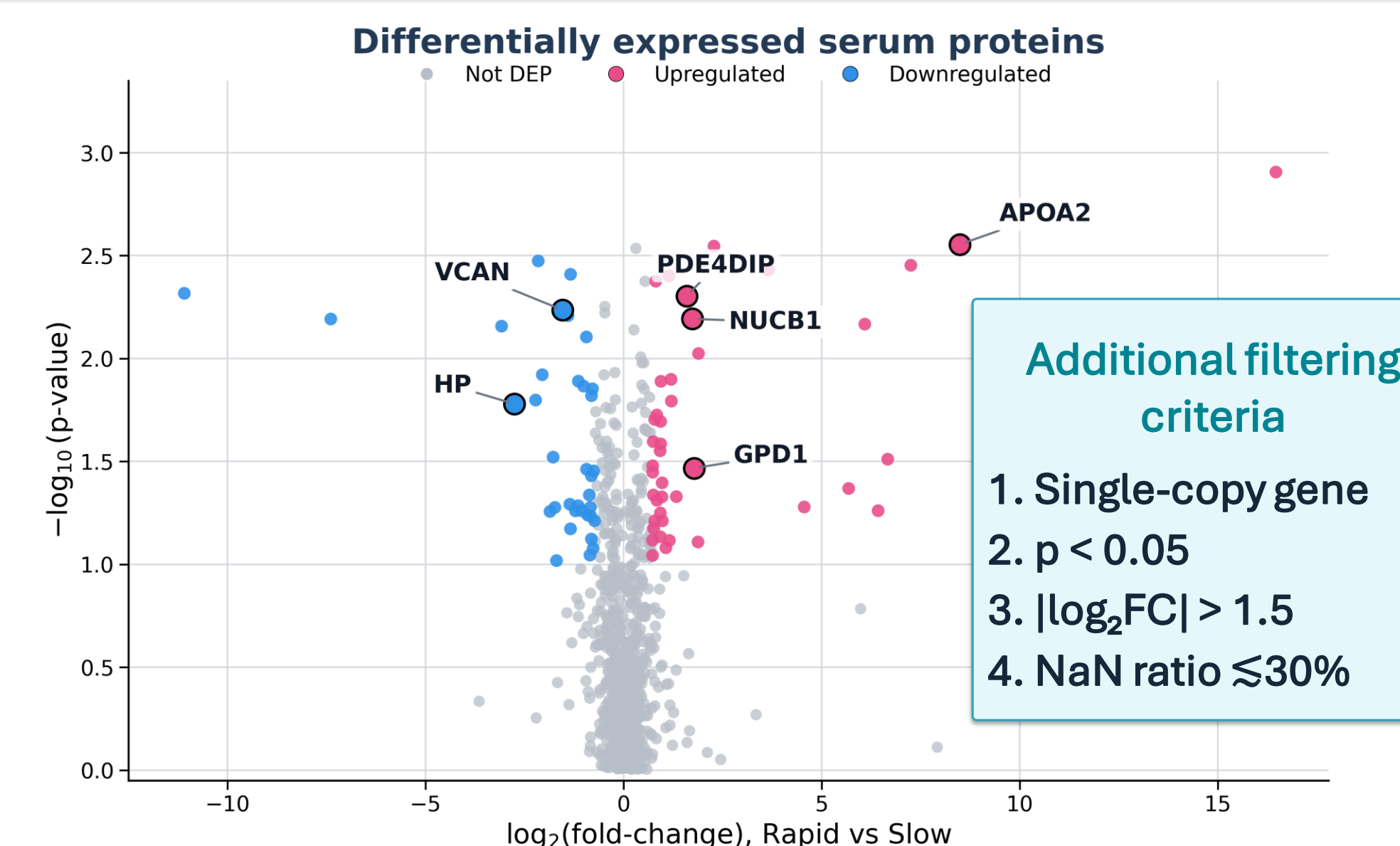


Figure 3. Volcano plot of differentially expressed proteins. Six labeled candidates are emphasized at their observed coordinates: APOA2, PDE4DIP, NUCB1, GPD1, VCAN, and HP.

HEATMAP & EXPLORATORY FUNCTIONAL ENRICHMENT

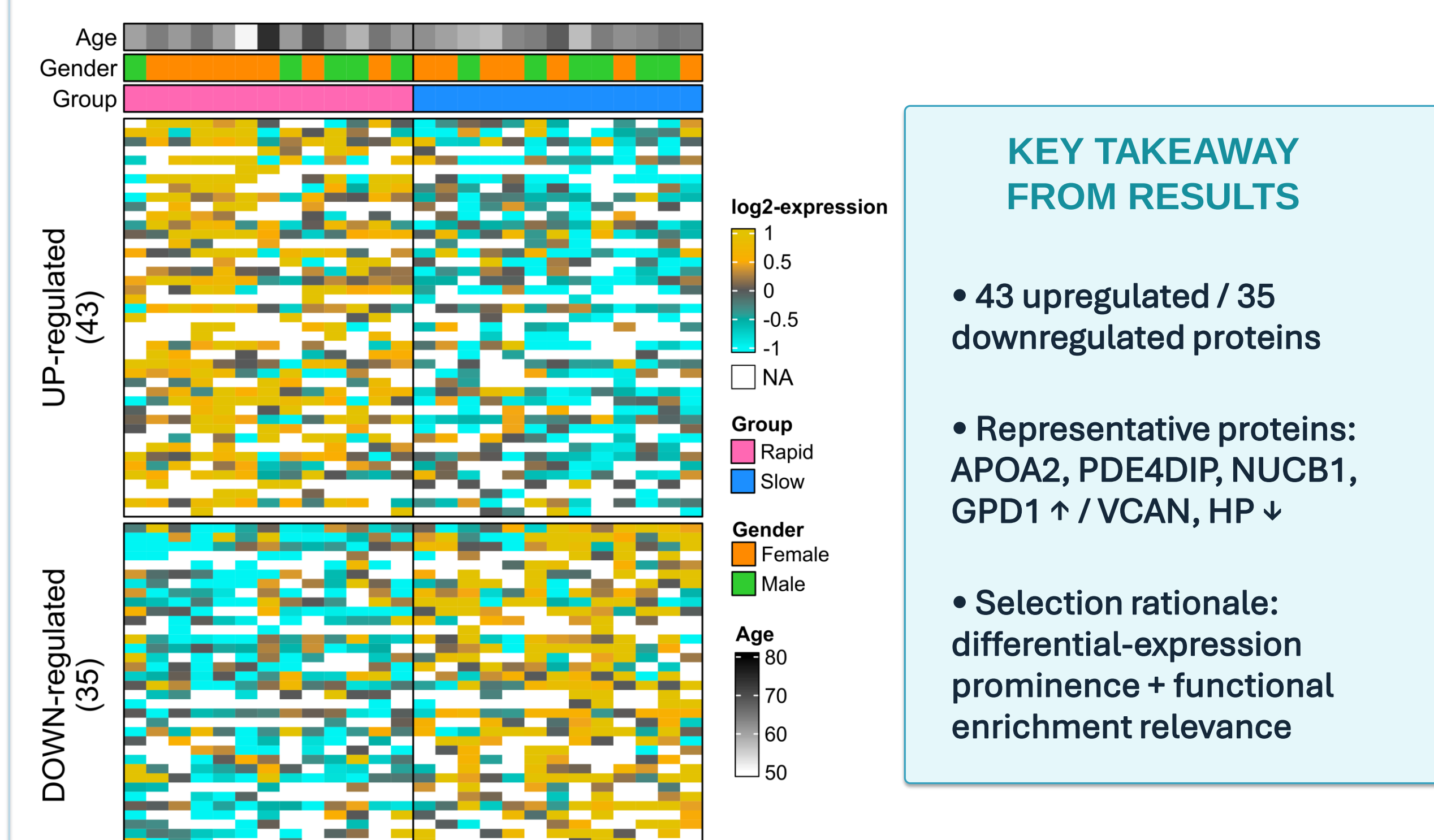


Figure 4. Heatmap of differentially expressed proteins distinguishing rapid and slow progression groups.

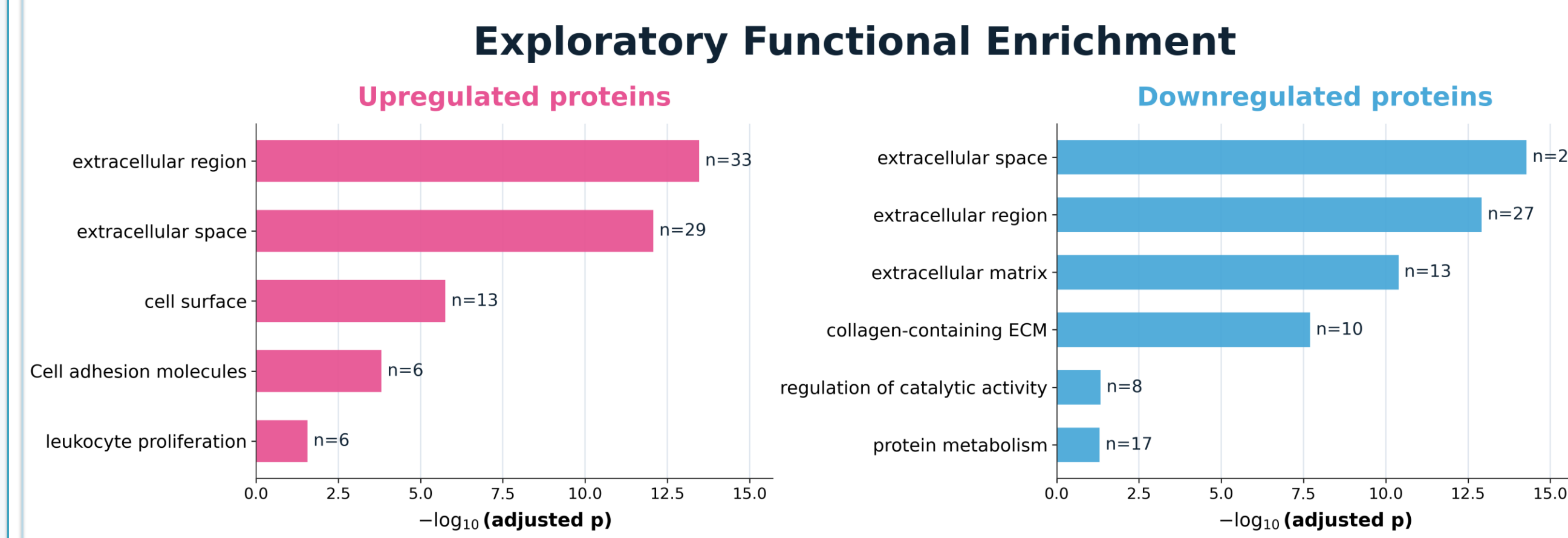


Figure 5. Exploratory functional enrichment summary of upregulated and downregulated proteins. Bars show $-\log_{10}(\text{adjusted } p)$; labels indicate the number of proteins mapped to each term.

Functional enrichment analysis

In the rapid group, both up- and down-regulated DEPs were commonly associated with the extracellular region and extracellular space. Specifically, up-regulated DEPs were primarily involved in leukocyte regulation, whereas down-regulated DEPs were related to the extracellular matrix (ECM) and protein metabolism

CONCLUSION

Among EOAD patients with similar baseline severity, baseline serum proteomic profiles differed according to cognitive decline rate. The dominant enrichment patterns pointed to immune-related and extracellular-matrix-related serum signatures. These peripheral protein signatures may help identify patients at risk of rapid progression and warrant validation in larger longitudinal cohorts.

LIMITATIONS

- Small sample size
- Exploratory nature of the enrichment analysis
- The need for validation in larger longitudinal cohorts

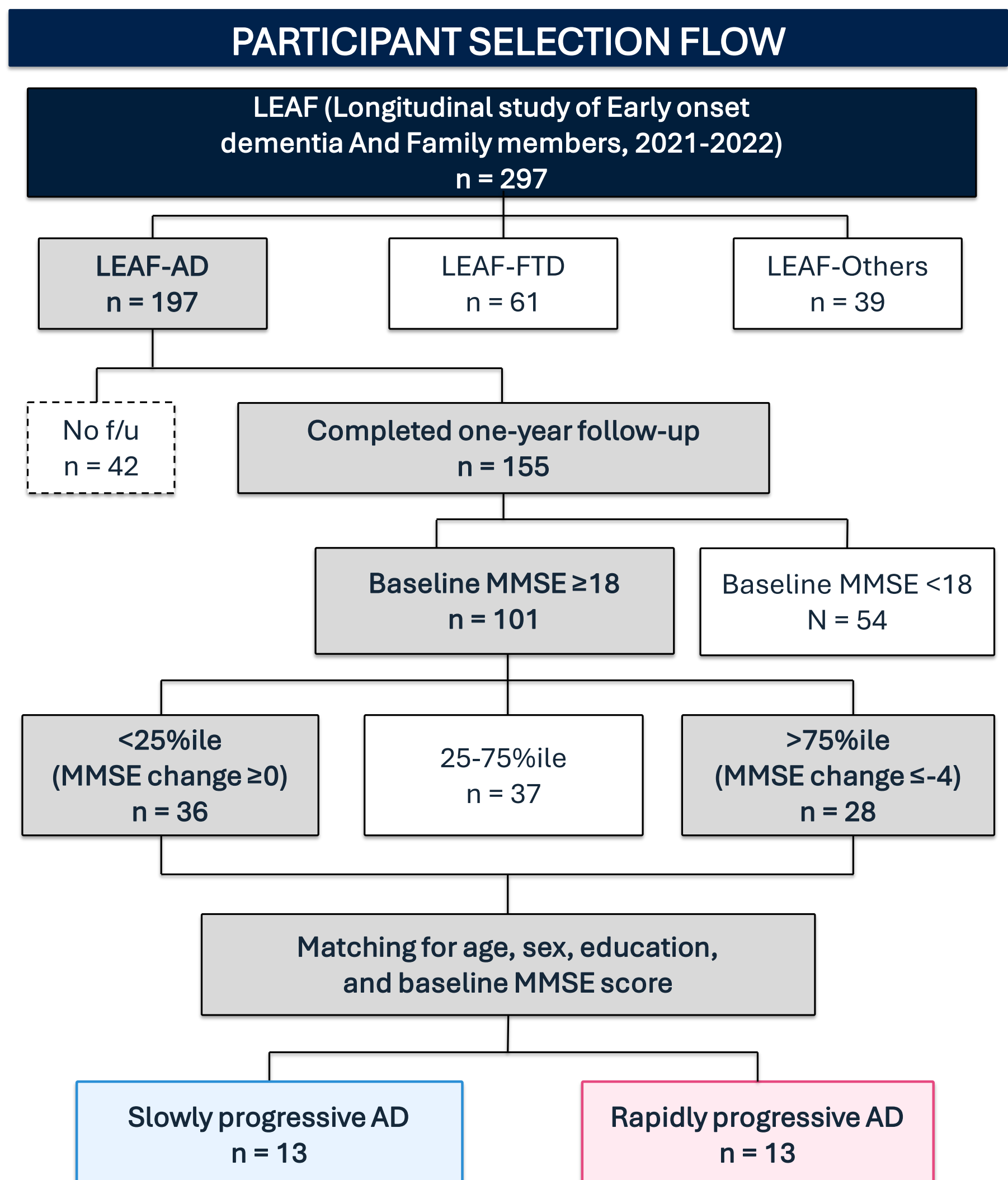


Figure 1. Participant selection and matching flow from the LEAF cohort.