

Epigenetic Factors Associated with the Course of Early-Onset Alzheimer's Disease



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Background

Early-onset Alzheimer's disease (EOAD) often exhibits a relatively rapid disease course; however, the rate and timing of progression vary considerably among individuals. As genetics alone cannot fully explain this variability, epigenetics, such as DNA methylation, which regulates gene expression in response to environmental factors, may be crucial. Furthermore, epigenetic age acceleration (EAA) may provide insights into the heterogeneity of disease progression. Therefore, we aimed to 1) identify DNA methylation patterns associated with the rapid progression, and 2) evaluate the relationship between EAA and disease course in EOAD.

METHODS

- Participants were recruited from the LEAF-AD, a Korean multicenter longitudinal EOAD cohort established in 2021.
- 197 EOAD participants enrolled in 2021-2022; 155 completed one-year follow-up.
- Early-stage subset: baseline MMSE ≥ 18 (n = 101)
- Rapid group : annual MMSE decline ≥ 4 (n = 13)
- Slow group : no decline in annual MMSE (n = 13)
- Each groups matched for age, sex, education, and baseline MMSE (Figure 1).
- DNA methylation profiling : Analyzed microarray data using beta values to represent methylation levels at each CpG site.
- DMC identification : Identified differentially methylated CpGs(DMC) via linear regression. ($|\Delta\text{mean}| \geq 0.2$ and $p < 0.05$)
- EAA calculation : Defined as the residual difference between chronological age and methylation-predicted biological age, which was estimated by uploading sample annotations and beta values(0-1) to the online DNA Methylation Age Calculator (<https://dnamage.clockfoundation.org>).

PARTICIPANT SELECTION AND MATCHING FLOW

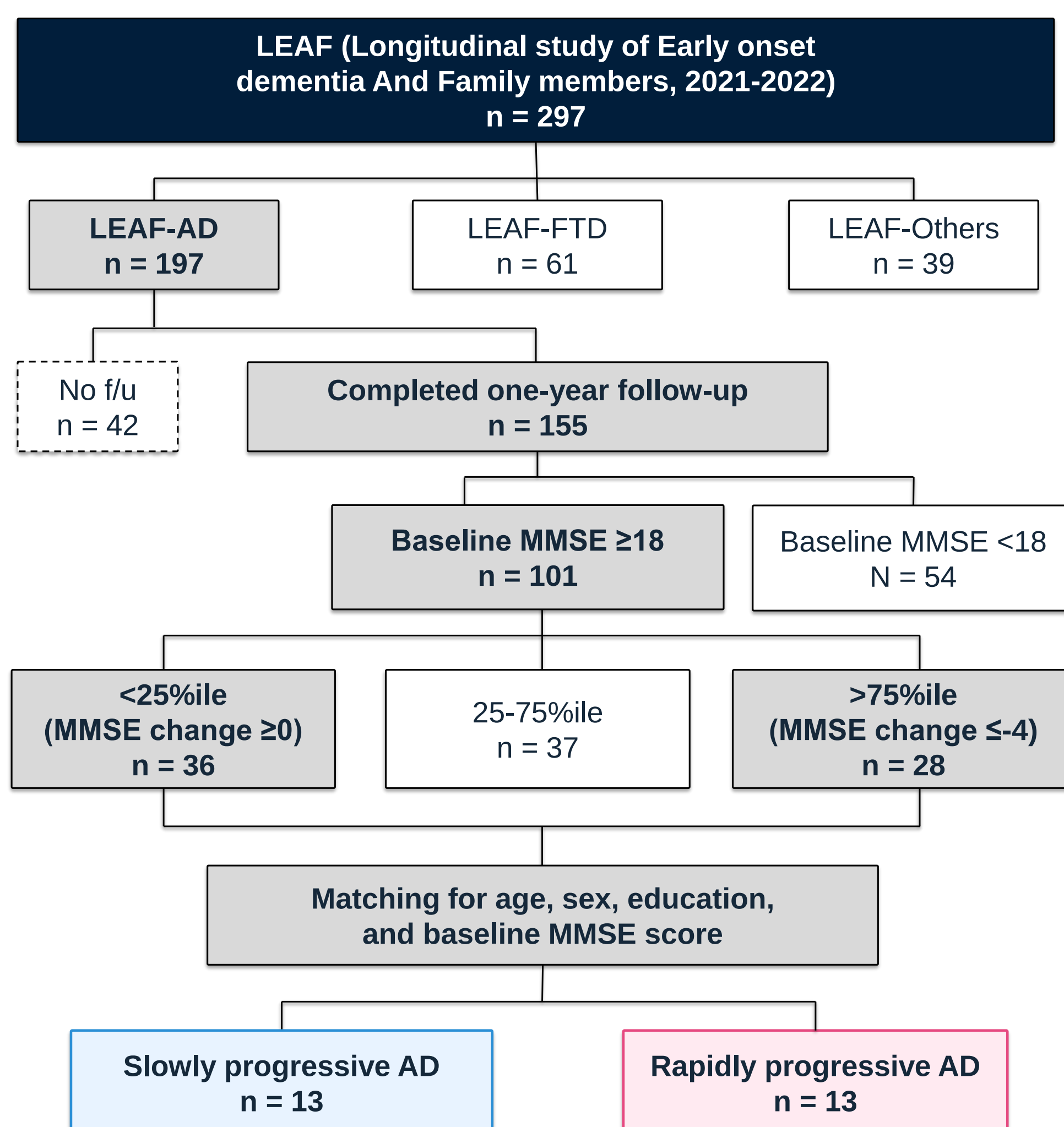


Figure 1. Participant selection and matching flow from the LEAF cohort.

RESULTS

Baseline demographic and clinical characteristics were comparable between groups, while follow-up MMSE differed significantly (Table 1). After quality control of 865,918 CpG sites, 18 significant DMCs were identified including 7 hypermethylated and 11 hypomethylated sites in the RP group compared to the SP group (Figure 2). Notably, a hypomethylated CpG site was located in the promoter region of the *DEF8* gene (Figure 3). No significant differences were observed in EAA between the two groups (Table 2).

Table 1. Demographic characteristics of participants

	Rapidly (N = 13)	Slowly (N = 13)	p
Age at baseline assessment, y	62.5 ± 5.6	62.1 ± 3.3	0.800
Sex, female n. (%)	8 (61.5)	7 (53.8)	1.000
Education, y	11.8 ± 4.3	13.4 ± 3.5	0.322
Onset age, y	58.0 ± 3.8	58.9 ± 3.7	0.571
APOE ε4 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 ± 3.8	23.1 ± 3.1	0.822
Baseline CDR	0.73 ± 0.26	0.73 ± 0.26	1.000
Baseline CDR-SOB	3.8 ± 2.1	3.1 ± 2.0	0.347
1 year follow-up MMSE	17.0 ± 3.8	24.8 ± 3.5	<0.001

Values are mean ± SD or n (%).

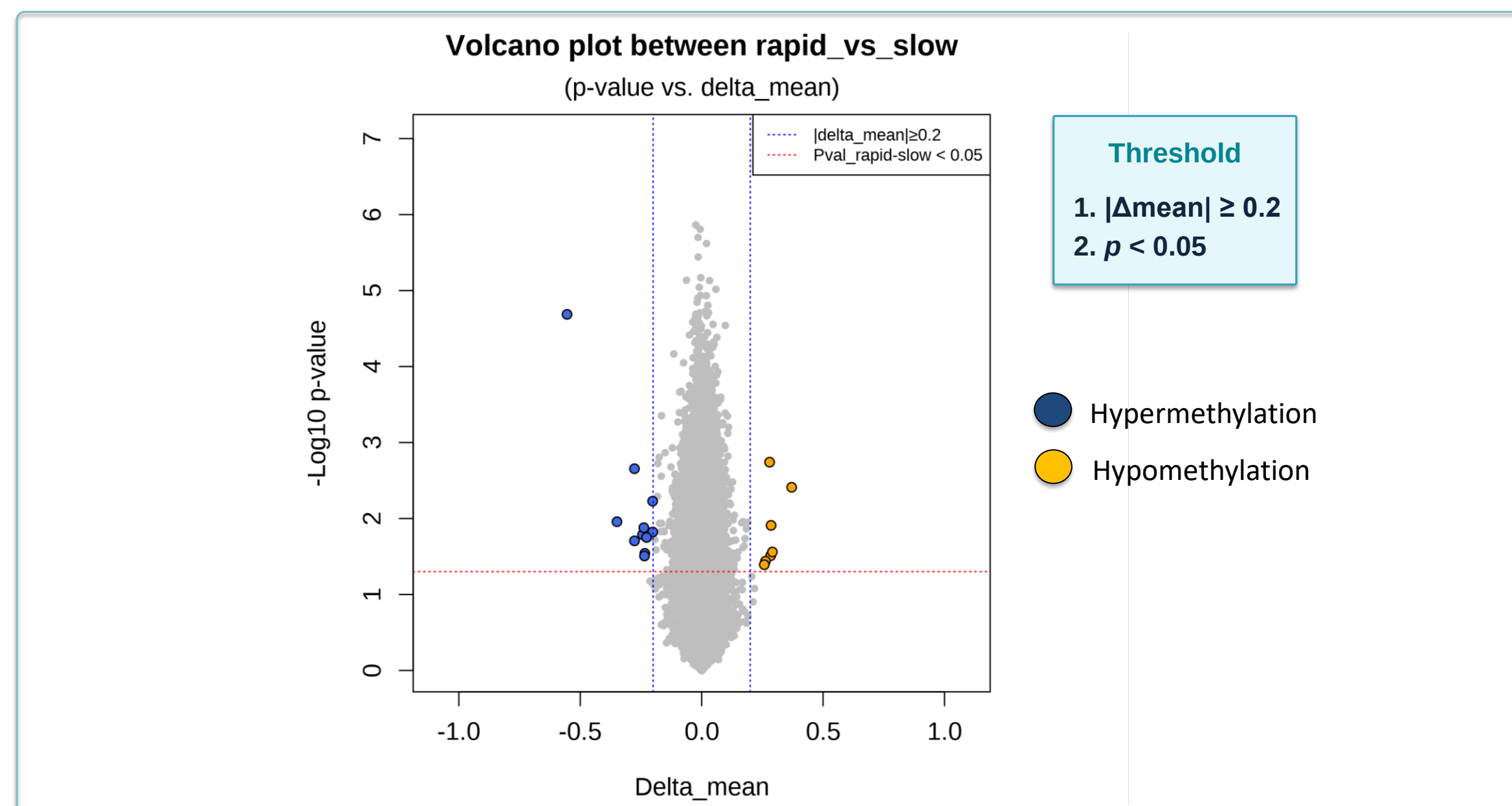


Figure 2. Volcano plot of differentially methylated CpGs.

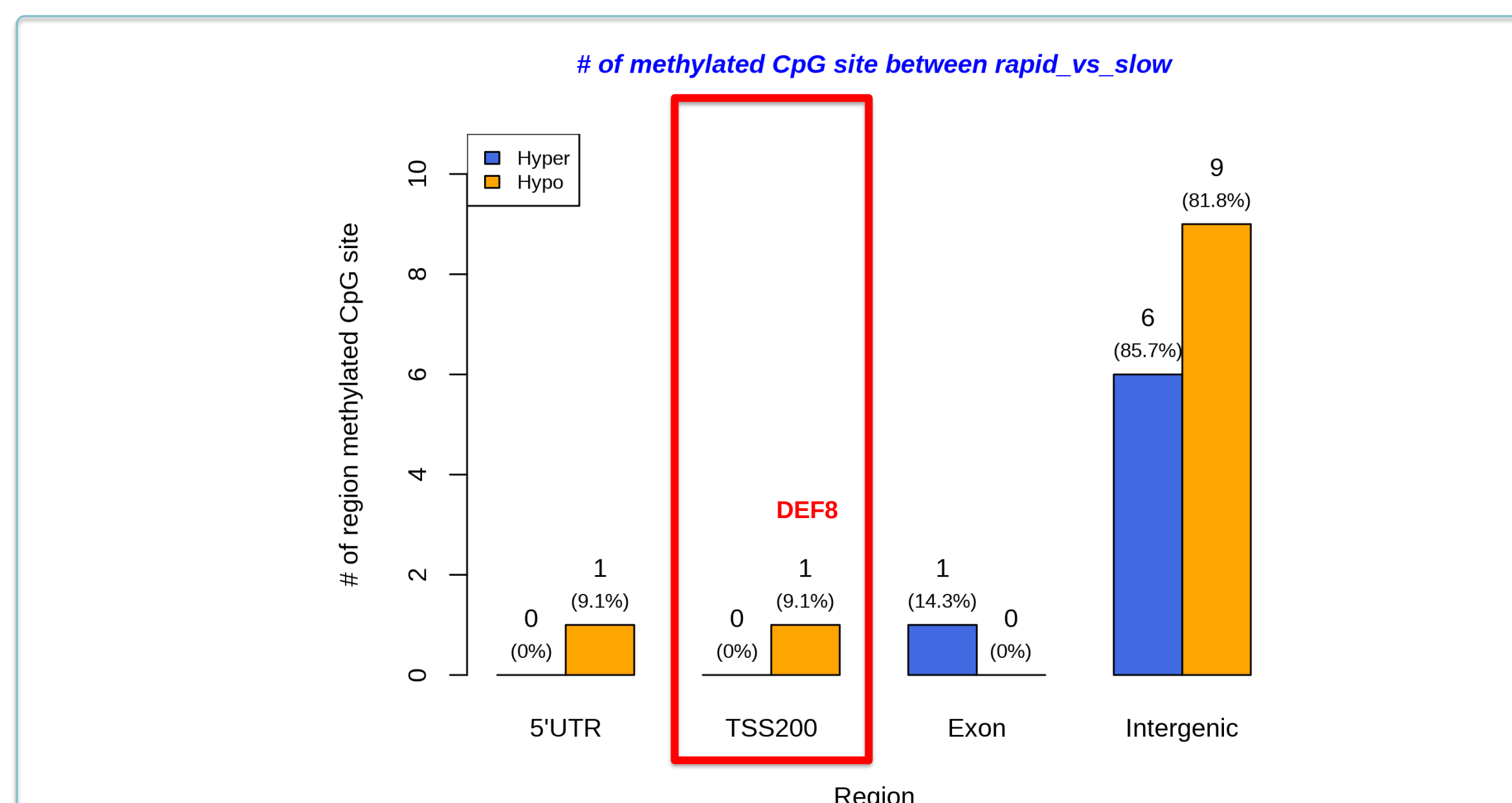


Figure 3. Genomic distribution of differentially methylated CpGs

Table 2. Comparison of epigenetic age acceleration(EAA) measures between the rapid and slow progression groups

Variables	Rapidly (N = 13)	Slowly (N = 13)	p
AgeAccelResidual	0.52 ± 4.35	-0.52 ± 5.26	0.589
IEAA	0.05 ± 3.32	-0.05 ± 4.42	0.951
EEAA	0.52 ± 4.35	-0.52 ± 5.26	0.589
AgeAccelResidualHannum	0.63 ± 2.59	-0.63 ± 3.90	0.340
AgeAccelPheno	2.25 ± 3.42	-2.25 ± 7.15	0.052
AgeAccelGrim	0.79 ± 4.95	-0.79 ± 3.53	0.358

HEATMAP

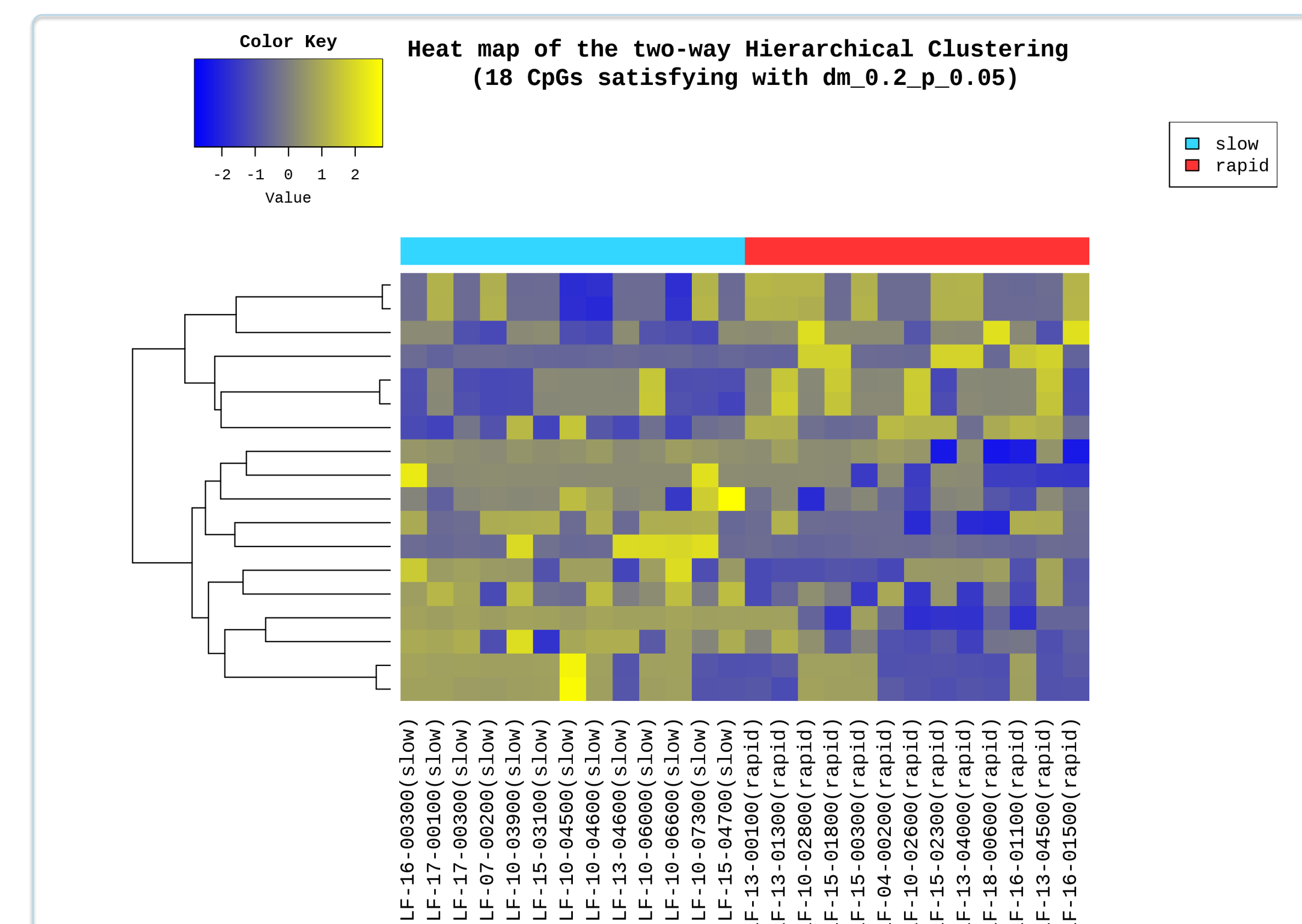


Figure 4. Heatmap of 18 significant CpGs separating rapid and slow progression groups. The heatmap demonstrates distinct DNA methylation profiles between the RP and SP groups based on the 18 significant DMCs.

CONCLUSION

In our study, hypomethylation of the *DEF8* promoter was associated with rapid progression in EOAD. *DEF8* is known to regulate lysosomal positioning and autophagy-mediated protein clearance. This hypomethylation may reflect a compensatory but insufficient response to severe proteotoxic stress in aggressive disease states. No significant differences were observed in EAA between the two groups.

LIMITATIONS

- The small matched sample size necessitates caution when interpreting these results.
- Peripheral DNA methylation patterns may not fully reflect epigenetic alterations in the central nervous system.
- Lack of concurrent transcriptome data to verify the actual impact of *DEF8* hypomethylation on gene expression.