

Molecular Subtypes And Age Cutoffs In Alzheimer’S Disease: A Multi-modal Proteomic Study From PUMCH Dementia Cohort

aaic2026.0e801a3

Yuhan Jiang^{1,2,a}, Xueying Yang^{3,2,a}, Yunfan You^{1,2}, Jialu Bao^{1,2}, Yuyue Qiu^{1,2}, Tianyi Wang^{1,2}, Yuanheng Li^{1,2}, Wenjun Wang^{1,2}, Xinyi Du^{1,2}, Dan Lei^{1,2}, Shanshan Chu^{1,2}, Wei Jin^{1,2}, Liling Dong^{1,2}, Meiqi Wu⁴, Li Huo⁴, Xinying Huang⁵, Feng Feng⁵, Ling Qiu⁶, Siyuan Wang^{7,b}, Chenhui Mao^{1,2,b}, Catherine C.L. Wong^{7,b}, Jing Gao^{1,2,b}

¹ Department of Neurology, PUMCH; ² State Key Laboratory for Complex, Severe and Rare Diseases, PUMCH; ³ Tsinghua-Peking Center for Life Sciences, Tsinghua University; ⁴ Nuclear Medicine, PUMCH; ⁵ Radiology, PUMCH; ⁶ Clinical Laboratory, PUMCH; ⁷ Clinical Research Institute, PUMCH. ^a Equal contribution; ^b Corresponding authors.

KEY TAKEAWAY

Pathogenic variant carriers exhibited an earlier age at onset, with 50 years identified as the genetic cutoff. Plasma proteomics defined S1/S2 and a 62.5-year biological cutoff, supporting a genetic- or molecularly defined framework.

INTRODUCTION

Biological problem

- AD is a biological continuum defined by amyloid and tau pathology, yet substantial phenotypic heterogeneity persists.¹
- EOAD and LOAD are usually separated at 60 or 65 years.²
- Compared with LOAD, EOAD shows a more aggressive course and greater neocortical atrophy with relative hippocampal sparing.^{3,4}
- These phenotypic differences suggest mechanisms not fully captured by amyloid-centric models.

Why revisit age?

- The chronological 60/65-year cutoff derives largely from sociological factors, such as retirement age, rather than biological relevance.⁵
- Pathogenic *APP*, *PSEN1* and *PSEN2* variants are core drivers of early-onset symptoms.⁶
- Systematic large-scale genetic evaluations defining a threshold remain limited.

Study aims

- Identify age-independent molecular subtypes using high-depth, untargeted plasma proteomics.
- Compare clinical background, core CSF biomarkers and neuroimaging atrophy across subtypes.
- Elucidate subtype-specific pathways and screen clinically relevant plasma biomarkers.
- Establish an optimal age cutoff based on pathogenic variants and molecular subtypes.

STUDY FRAMEWORK

We integrated genetics, clinical phenotyping, CSF biomarkers, structural MRI and plasma proteomics to test whether molecular classification better captures AD heterogeneity than age alone.

Study scope

797

biomarker-confirmed AD

162

AD proteomic cohort

56

healthy controls

Cross-sectional PUMCH dementia cohort and community volunteers, 2019–2025.

METHODS

Study design and participants

91

clinical EOAD < 60y

71

clinical LOAD ≥ 60y

56

healthy controls

Proteomic workflow

- Plasma from 91 EOAD, 71 LOAD and 56 HCs was profiled by dia-PASEF.
- After high-abundance protein depletion, 1,453 proteins were log2-transformed and quantile normalized.
- Clinical background, MMSE/MoCA/ADL, core CSF biomarkers and regional MRI atrophy were compared.

S1 and S2 were defined by plasma proteomic

S1 n=86

negative PC1; onset 54.7 y

S2 n=76

positive PC1; onset 64.3 y

- PCA was applied to the normalized 1,453-protein matrix.
- Unsupervised K-means clustering (centres = 2) defined S1 on the negative PC1 axis and S2 on the positive axis without clinical information
- GO enrichment was applied, and Spearman correlations identified mechanisms and subtype-linked biomarkers.
- ROC analysis with Youden's J statistic determined the biological cutoff.

RESULTS

Genetic age threshold

50 y

optimal genetic cutoff

OR 6.38

13.6% vs 2.4%; P < 0.001

Pathogenic/likely pathogenic variant carriers had earlier onset (P < 0.001) in 797 patients fulfilled AD biomarker criteria.

S1/S2 baseline profile

Measure	S1 (n=86)	S2 (n=76)	P
Onset age, y	54.7 ± 4.6	64.3 ± 6.6	<0.001
Duration, y	3.7 ± 1.8	3.5 ± 1.9	0.551
APOE ε4	36.4%	57.1%	0.105
CSF Aβ42/40	0.05	0.05	0.926
MMSE	11.0 ± 7.7	16.3 ± 7.9	<0.001
ADL	40.5 ± 11.2	35.5 ± 9.7	0.002

S1/S2 were similar in disease duration, education, medical histories, APOE ε4 and core CSF burden; cognition and daily function differed.

Clinical and MRI divergence

- S1 had significantly lower MMSE and MoCA and worse ADL than S2.
- S1 showed greater global grey matter, parietal and temporal atrophy; hippocampal, amygdala and entorhinal atrophy were comparable.

RESULTS

Divergent pathway biology

- 323 DEPs distinguished S1/S2.
- S1: adaptive immunity, immunoglobulin/keratin pathways and cellular oxidant detoxification.
- S2: innate immunity, complement activation, coagulation, hemostasis and wound healing.

Subtype-linked biomarkers

- Shared: HBA1, HBB, HBD and RANBP3L
- Opposite: CDC65
- S2-specific: IGHG2, IGHG4, USP19 and CHRD1L
- S1-specific proteins were screened by correlations with cognition, MRI and CSF.

Proteomic biological cutoff

Age of onset strongly predicted molecular subtype: 62.5 y | AUC 0.882 | sensitivity 97.7% | specificity 76.3%.

The traditional 65-year cutoff misclassified 42.1% of S2.

CONCLUSIONS

Molecular subtype synthesis

S1 | n=86 | onset 54.7 y

- More severe cognitive impairment and ADL deficits.
- More extensive parietal and temporal atrophy.
- Adaptive immunity, keratin pathways and oxidant detoxification.

S2 | n=76 | onset 64.3 y

- Less severe cognitive and functional impairment.
- Similar disease duration and core CSF amyloid/tau burden.
- Innate immunity, complement and coagulation dysfunction.

Genetic cutoff: 50 years | Proteomic biological cutoff: 62.5 years

Conclusions

- The heterogeneity of EOAD and LOAD is driven by distinct biological mechanisms rather than onset age alone.
- These findings support a shift from age-based classification to a genetic- or molecularly defined framework.
- This framework may facilitate precision medicine and targeted therapeutic strategies for AD.

Limitations

- HCs had higher education and a high reported family history of dementia, potentially attenuating patient-control proteomic differences.
- Cross-sectional subtypes and biomarker panels require independent, large-scale, multicentre longitudinal validation.

REFERENCES

1 Jack et al., *Alzheimers Dement.* 2024; 2 Mendez, *Neurol Clin.* 2017. 3 Palasi et al., *J Neurol.* 2015; 4 Mehta et al., *Cereb Cortex.* 2024. 5 Liu et al., *Chin Med J.* 2025; 6 Laniolelee et al., *PLoS Med.* 2017. 7 Seath et al., *Int Psychogeriatr.* 2024. 8 Casquero-Veiga et al., *Curr Opin Cell Biol.* 2025.

CONTACT

Siyuan Wang: wsy1015@163.com | Chenhui Mao: maochenhui@pumch.cn Catherine C.L. Wong: catclw321@126.com | Jing Gao: gj107@163.com

ALZHEIMER'S ASSOCIATION

AAIC26