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IsoAsp7-Aβ – A major Aβ variant in Alzheimer's disease, dementia with Lewy bodies and vascular dementia

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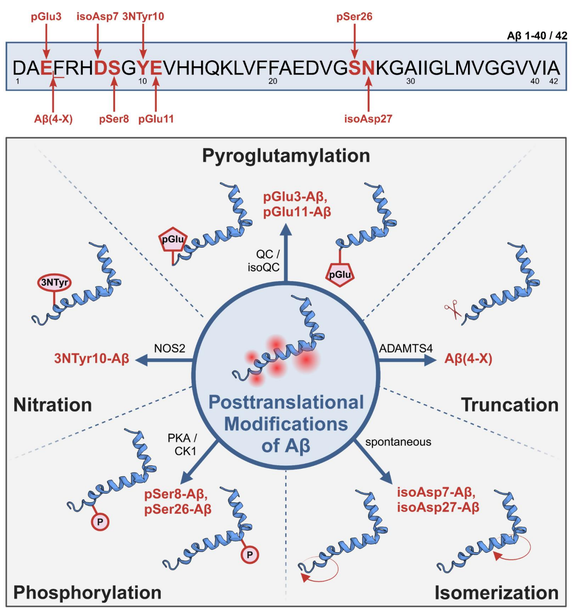
The formation of amyloid beta (Aβ) aggregates in brain is a neuropathological hallmark of Alzheimer's disease (AD). There is mounting evidence that specific post-translational modifications (PTMs) of Aβ contribute to its pathogenic profile and that Aβ plays a pathogenic role in other types of dementia. In this study, we examined the hypothesis that distinct types of dementia are characterized by specific patterns of Aβ PTMs.

Aβ modifications



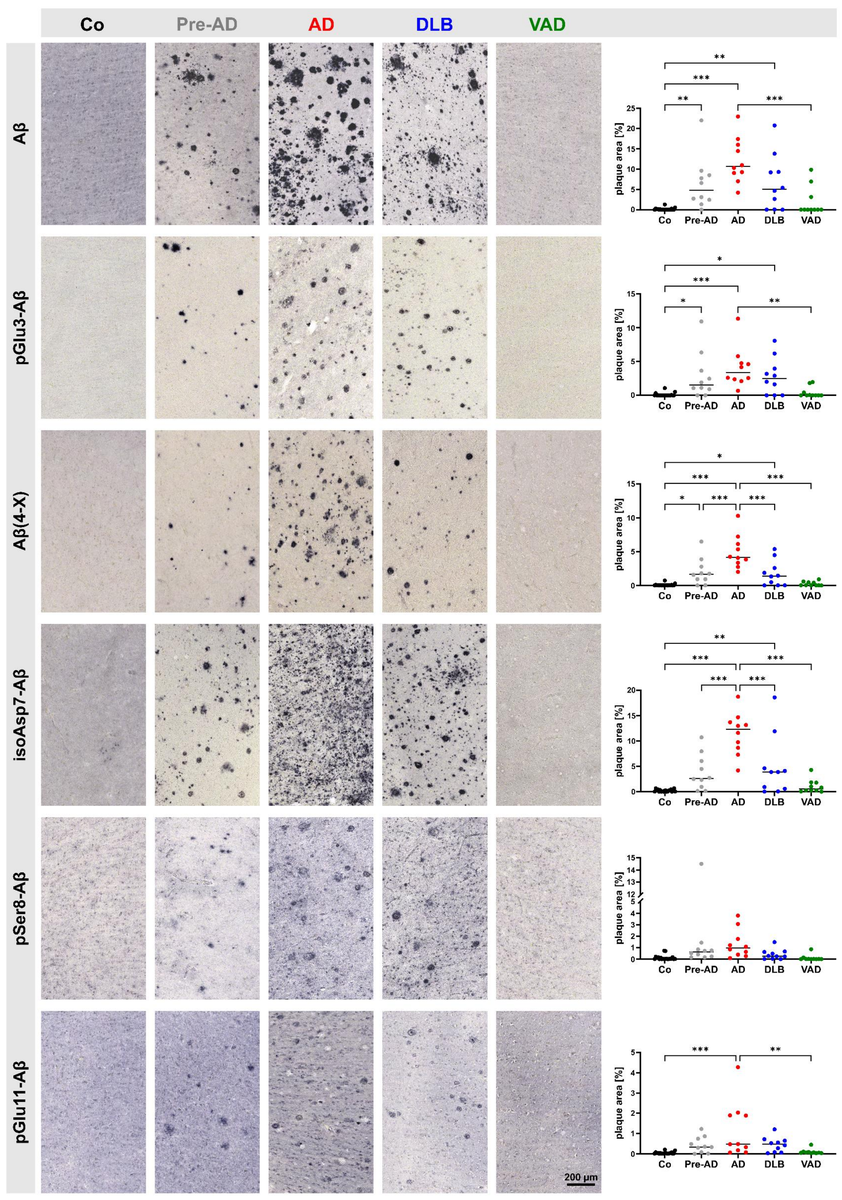
Aβ peptides comprise a heterogeneous group of peptides with distinct biophysical properties. Specific PTMs at various sites within the Aβ peptide sequence lead to an enhanced diversification in cell biological characteristics. We investigated the morphology and quantified the amount of total Aβ and post-translationally modified Aβ in the temporal cortex of different dementia conditions.

PTMs of Aβ catalyzed by the respective enzymes. PTMs presented here are depicted in red.[1-5] Overview and characterization of the clinical cases. Pre-AD - pre-symptomatic AD; DLB - dementia with Lewy bodies; VAD - vascular dementia

Types of dementia and control cases

	Control	Pre-AD	AD	DLB	VAD
cases	20	10	10	10	10
Braak	I - III	I - III	V, VI	I - IV	I - IV
Thal	0 - 1	2 - 5	5	0 - 5	0 - 3

Aβ PTMs in human temporal cortex

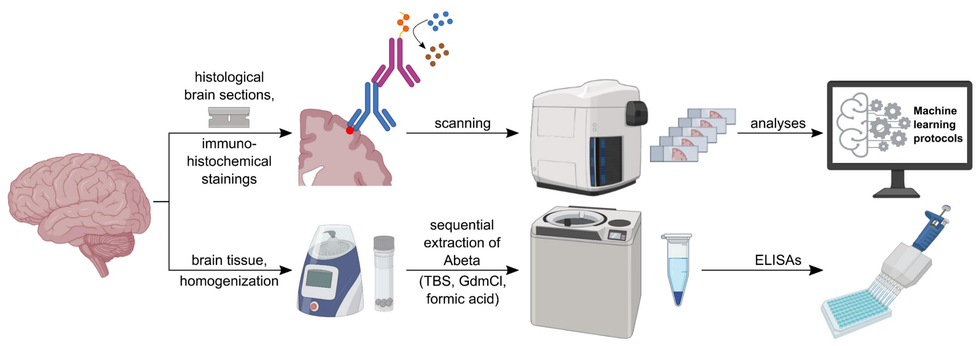


In human brain tissue, we identified the isoAsp7-Aβ variant as a highly abundant Aβ form in all clinical conditions, followed by Aβ(4-X), pGlu3-Aβ, pGlu11-Aβ and pSer8-Aβ. These Aβ variants were detected in distinct plaque types of compact, coarse-grained, cored and diffuse morphologies and, with varying frequencies, in cerebral blood vessels. Both total plaque load and abundance of Aβ variants were highest in AD cases, followed by DLB cases.
The 3NTyr10-, pSer26- and isoAsp27-Aβ variants were not found to be present in Aβ plaques but were detected intraneuronally (not shown). Quantifications with single labeling immunohistochemistry were consistent with ELISA quantifications (not shown).

Representative examples of immunohistochemical labeling of human cortical brain tissue from control cases and different clinical conditions using antibodies to detect specific post-translational Aβ modifications as indicated (left). Respective quantifications of Aβ plaque load as a percentage of brain area covered by plaques are presented (right).
Statistical significance: *p<0.05; **p<0.01; ***p<0.001 defined by One-Way ANOVAs followed by Tukey's multiple comparisons test; medians are indicated by horizontal lines.

Quantification and machine learning

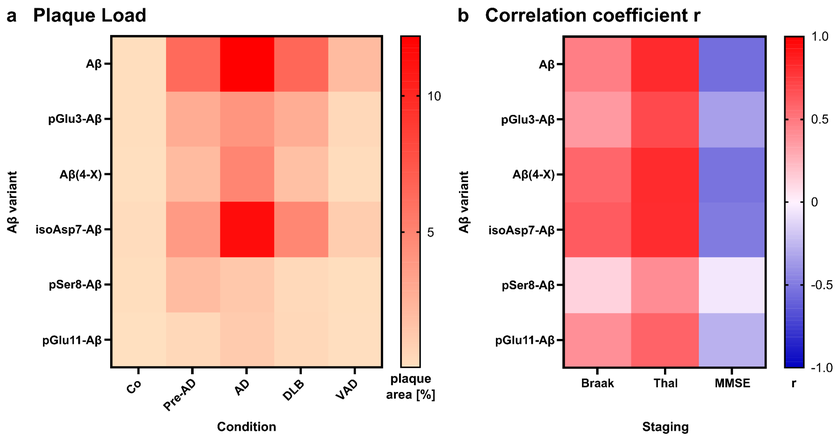
We employed monoclonal antibodies for comparative analyses of different Aβ PTMs in *post mortem* human brain tissue of Pre-AD, AD, DLB and VAD cases, and control subjects.



Workflow for the quantification of total Aβ and of Aβ variants by means of Zeiss arivis Cloud and Zeiss Zen Intellesis software.[5] TBS - Tris-buffered saline; GdmCl - guanidinium chloride

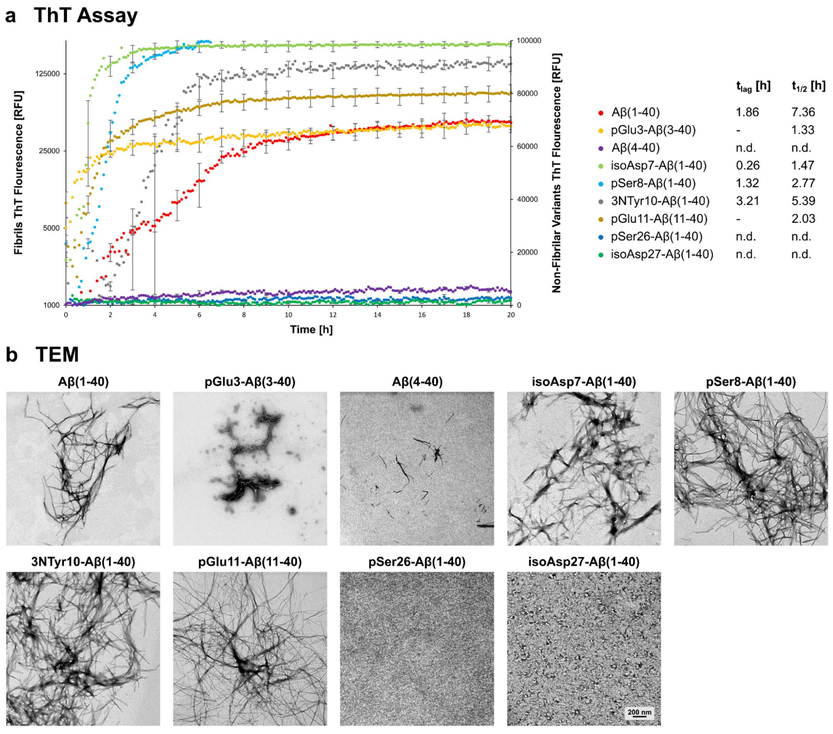
Correlation with clinical data

Histopathological findings were correlated with clinical data. We discovered a strong and moderate positive correlation between isoAsp7-Aβ and Thal phase and Braak stage, respectively, and a moderate negative correlation between isoAsp7-Aβ and MMSE score.



Heat maps for the plaque load of Aβ variants and the correlation coefficient r of the Pearson correlation analyses. **a** Immunohisto-chemically quantified plaque load of Aβ variants for the different conditions. **b** Correlation coefficient r for all correlations between the plaque load of Aβ variants and Braak and Thal staging and MMSE score.

Aggregation of Aβ variants



The Aβ variants detected in amyloid plaques showed ThT fluorescence with instant fibril formation for pGlu3-Aβ (1–40), isoAsp7-Aβ(1–40) and pGlu11-Aβ(1–40). To reveal specific characteristics of the fibrils derived from Aβ variants, transmission electron microscopy (TEM) was performed. TEM images confirmed the data of the fibrillation curves obtained in the ThT assay. Remarkably, pGlu3-Aβ(1–40) and isoAsp7-Aβ(1–40) fibrils appeared to be shorter and thicker than fibrils of the other Aβ variants.

ThT assay and TEM images of Aβ variants.
a Aggregation curves of time-dependent fibril formation of Aβ variants. t_{lag} - lag phase; $t_{1/2}$ - half maximum ThT fuorescence intensity time
b TEM images of fibrils derived from the respective Aβ variants.

A disease-specific pattern of post-translationally modified Aβ peptides was not identified. Instead, a consistent pattern of Aβ variants was observed across different clinical entities.

Aβ PTMs, and in particular the isoAsp7-Aβ variant, might be considered for diagnostic and therapeutic approaches in different types of dementia.

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1 Kummer, M. P. and Heneka, M. T. (2014). Truncated and modified amyloid-beta species. *Alzheimers Res Ther.* 6(3), 28.
2 Howell, S. *et al.* (1995). Neutral endopeptidase can hydrolyze β-amyloid(1-40) but shows no effect on β-amyloid precursor protein metabolism. *Peptides,* 16(4), 647-52.
3 Leissring, M. A. *et al.* (2003). Kinetics of amyloid beta-protein degradation determined by novel fluorescence- and fluorescence polarization-based assays. *J Biol Chem.* 278(39):37314-20.
4 Crescenzi, O. *et al.* (2002). Solution structure of the Alzheimer amyloid beta-peptide (1-42) in an apolar microenvironment. Similarity with a virus fusion domain. https://doi.org/10.2210/pdb1iyt/pdb
5 Created with BioRender.com