Neuroscience Letters 754 (2021) 135894

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Characterization of monomeric and soluble aggregated Aβ in Down's syndrome and Alzheimer's disease brains

Eleni Gkanatsiou [a,*], Charlotte Sahlin [b,c], Erik Portelius [a,d], Malin Johannesson [b], Linda Söderberg [b], Johanna Fälting [b], Hans Basun [b], Christer Möller [b], Tomas Odergren [b], Henrik Zetterberg [a,d,e,f], Kaj Blennow [a,d], Lars Lannfelt [b,c], Gunnar Brinkmalm [a,d]

[a] Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden
[b] BioArctic AB, Stockholm, Sweden
[c] Department of Public Health/Geriatrics, Uppsala University, Uppsala, Sweden
[d] Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden
[e] Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, United Kingdom
[f] UK Dementia Research Institute at UCL, London, United Kingdom

ARTICLE INFO

Keywords:
Beta amyloid
Alzheimer's disease
Down syndrome
Mass spectrometry

ABSTRACT

The major characteristics of Alzheimer's disease (AD) are amyloid plaques, consisting of aggregated beta amyloid (Aβ) peptides, together with tau pathology (tangles, neuropil treads and dystrophic neurites surrounding the plaques), in the brain. Down's syndrome (DS) individuals are at increased risk to develop AD-type pathology; most DS individuals have developed substantial pathology already at the age of 40. DS individuals have an extra copy of chromosome 21, harbouring the amyloid precursor protein gene (*APP*). Our aim was to investigate the Aβ peptide pattern in DS and AD brains to investigate differences in their amyloid deposition and aggregation, respectively. Cortical tissue from patients with DS (with amyloid pathology), sporadic AD and controls were homogenized and fractionated into TBS (water soluble) and formic acid (water insoluble) fractions. Immunoprecipitation (IP) was performed using a variety of antibodies targeting different Aβ species including oligomeric Aβ. Mass spectrometry was then used to evaluate the presence of Aβ species in the different patient groups. A large number of Aβ peptides were identified including Aβ1-X, 2-X, 3-X, 4-X, 5-X, 11-X, and Aβ peptides extended N terminally of the BACE1 cleavage site and ending at amino 15 in the Aβ sequence APP/Aβ(-X to 15), as well as peptides post-translationally modified by pyroglutamate formation. Most Aβ peptides had higher abundance in AD and DS compared to controls, except the APP/Aβ(-X to 15) peptides which were most abundant in DS followed by controls and AD. Furthermore, the abundancies of AβX-40 and AβX-34 were increased in DS compared with AD. Aβ1-40, Aβ1-42, and Aβ4-42 were identified as the main constitutes of protofibrils (IP'd using mAb158) and higher relative Aβ1-42 signals were obtained compared with samples IP'd with 6E10 + 4G8, indicating that the protofibrils/oligomers were enriched with peptides ending at amino acid 42. All Aβ peptides found in AD were also present in DS indicating similar pathways of Aβ peptide production, degradation and accumulation, except for APP/Aβ(-X to 15). Likewise, the Aβ peptides forming protofibrils/oligomers in both AD and DS were similar, implying the possibility that treatment with clinical benefit in sporadic AD might also be beneficial for subjects with DS.

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia, with an estimated 50 million people currently suffering from the disease. The major hallmarks of the disease are the presence of amyloid plaques in the brain, consisting of beta amyloid (Aβ) peptides [1] and neurofibrillary tangles, consisting of hyperphosphorylated tau. Aβ peptides are produced by enzymatic cleavage of amyloid precursor protein (APP). The APP gene is located at chromosome 21. Down's syndrome (DS) is a genetic disorder with an extra copy of chromosome 21 leading to a

* Corresponding author at: Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, The Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden.
E-mail address: eleni.gkanatsiou@gu.se (E. Gkanatsiou).

https://doi.org/10.1016/j.neulet.2021.135894
Received 2 September 2020; Received in revised form 6 April 2021; Accepted 7 April 2021
Available online 10 April 2021

Table 1
Demographics.

No	Diagnosis	Region	Sex	Age (Years)	Braak NFT	Amyloid score	Braak a-syn	Pmd (hh:mm)	Weight (g)	APOE
1	DS	inferior frontal gyrus	f	58	6	*	0	09:30	664	33
2	DS	inferior frontal gyrus	f	67	6	*	0	11:00	769	33
3	DS	inferior frontal gyrus	m	64	5	*	0	07:09	790	33
4	DS	middle frontal gyrus	f	70	6	C	0	08:55	699	33
5	AD	medial temporal gyrus	m	67	5	C	0	08:20	1263	33
6	AD	medial temporal gyrus	f	87	4	C	0	06:15	910	33
7	AD	medial temporal gyrus	m	89	4	*	0	04:56	1172	33
8	AD	medial temporal gyrus	m	80	4	C	0	04:00	1288	33
9	control	inferior frontal gyrus	f	53	0	0	0	07:25	1180	33
10	control	inferior frontal gyrus	f	72	1	A	0	06:50	1165	33
11	control	medial temporal gyrus	f	60	1	0	0	06:50	1201	32
12	control	medial temporal gyrus	f	91	1	B	0	04:15	1054	32

In the table is shown the diagnosis of each subsect, the region used for analysis, the sex, the age at death, the Braak tau phases, amyloid score, Braak phase for Lewy bodies, the post mortem delay (Pmd), the weight of tissues and finally the APOE status. The asterisks represent amyloid pathology in the subjects, despite no score was given by a pathologist.

life-long overproduction of Aβ [2]. Today around 6 million people have DS worldwide and it is estimated that the first amyloid deposits in the brain are present already in childhood [3] and that two thirds have developed dementia in their 60 s [4].

APP processing can be categorized into the non-amylogenic pathway [5], where APP is cleaved by α- and γ-secretase, and the amylogenic pathway where APP is cleaved by β- and γ-secretase [6]. α-Secretase (members of the "A disintegrin and metalloproteinase domain", ADAM, family) cleaves between amino acids (aa) 15/16 and 16/17 (α site) in the Aβ sequence, producing AβX-15, AβX-16, and Aβ17-X (numbering according to the Aβ sequence). The major cleavage site of β-secretase (encoded by "beta-site amyloid precursor protein-cleaving enzyme 1", BACE1) is N-terminally of aa 1, producing Aβ1-X, but can also cleave between aa 10/11 (β' site) producing AβX-10 and Aβ11-X. If α- and β-secretase act on the same APP molecule, short Aβ peptides, including Aβ 1-15 and Aβ1-16, are produced [7]. γ-Secretase is a membrane-bound protease complex consisting of at least four essential components: the homologous presenilin-1 and -2 (PS1 and 2), nicastrin, Aph-1 and Pen-2 [8] and is responsible for the production of AβX-37, 38, 39, 40, 42, and 43 [9]. In the amylogenic pathway, longer and more hydrophobic peptides are produced (Aβ1-37,…, 43) of which Aβ1-42 and Aβ1-43 are the most aggregation-prone. In addition, the gene of another APP-processing enzyme, *BACE2,* is also located at chromosome 21. BACE2 is a homologue of BACE1 (β-secretase) and is involved in the production of AβX-19, AβX-20, and AβX-34 [10–12].

Since the initial identification of Aβ as the major component of amyloid plaques [1,13], Aβ has been at the centre of AD research, with the amyloid cascade hypothesis as the model for the sequence of the occurring events [14]. The hypothesis postulates that there is an imbalance between the production and clearance of Aβ peptides which leads to a build-up of Aβ peptides in the brain and the subsequent formation of oligomers/protofibrils and finally insoluble amyloid plaques. It is not yet clear whether dimeric Aβ, or oligomeric/protofibrils assemblies of Aβ are the most toxic species in the brain; all have been reported to confer toxicity [15–17].

DS and AD have many features in common, such as genetics, pathogenesis, and some clinical manifestations. The major genetic risk factor for sporadic AD is the *APOE* ε4 allele, a genetic risk also observed in DS patients, although to a lesser extent [18]. DS also shares similarities with familial AD forms, as APP plays an important role in amyloid depositions in both DS and AD [2]. Further, positron emission tomography (PET) studies have shown that the distribution of Pittsburgh compound B (a radio-ligand for PET imaging of Aβ pathology) binding in DS is generally similar to Aβ cortical accumulation in sporadic AD (although in DS it appears first in the stratium, which is is more similar to autosomal dominant AD) [19–21]. Apart from the amyloid pathology, the presence of neurofibrillary tangles (consisting of the protein tau) also seems to follow the same pattern in AD and DS, but with higher density in DS [22].

Our aim was to compare the Aβ peptide patterns in DS, AD, and control brains using mass spectrometry (MS). Our hypothesis was that the Aβ peptide pattern and the composition of protofibrils are similar in AD and DS, suggesting a similar amyloidogenic process in the two conditions.

2. Materials and methods

2.1. Protein extraction of human post mortem tissue

Post-mortem human brain tissue was obtained from The Netherlands Brain Bank (NBB), Netherlands Institute for Neuroscience, Amsterdam. All material has been collected from donors whose written informed consent for brain autopsy and the use of the material and clinical information for research purposes has been obtained by the NBB. The informed consent form of the NBB meets all current legal and ethical requirements for brain autopsy, tissue storage and use of tissue and clinical data for scientific research worldwide. The study followed the Helsinki Declaration and was approved by the regional ethics committees at the University of Gothenburg.

In the study two groups of patients with AD pathology were selected based on their Braak stage (≥5); AD (n = 4) and DS (trisomy 21 with amyloid positivity) (n = 4). A control group (n = 4) was selected with Braak stage ≤1, no cerebral abnormalities observed in autopsy and no cognitive complain during lifetime. All DS subjects were clinically diagnosed with dementia and showed amyloid positivity in neuropathological examination. Amyloid positivity was examined by silver stain and Congo red staining, although for some subjects the amyloid scoring was not available during the time of autopsy. These subjects, even they do not have any amyloid score given by a pathologist, they do have many senile plaques (sp) (often with a core) and congophilic plaques (cp). All samples were selected based on the absence of *APOE-ε*4 allele. None of the subjects showed Lewy bodies during autopsy. The AD subjects were on average ten years older compared with DS and controls. However, the subjects were selected based on pathology data and the amyloid time is likely to be similar for both AD and DS. Detailed demographic information is shown in Table 1.

Fresh frozen cerebral tissue (150 mg pieces, consisting of both grey and white matter) from the frontal lobe (either inferior frontal gyrus or middle frontal gyrus) was homogenized in 750 μL tris(hydroxymethyl) aminomethane (Tris) buffered saline (TBS), pH 7.6, containing complete protease inhibitor by 2 × 10 strokes using a Potter-Elvehjelm homogenisator. The homogenate (~800 μL) was transferred to a new tube and centrifuged at 16,000×*g* for 1 h at +4 °C. The supernatant (TBS fraction) was transferred to a new tube and stored at −80 °C until further use. The TBS pellet was resuspended in 1 mL of 70 % (v/v) formic acid (FA), followed by further homogenization in a TissueLyser (Qiagen) for 2 min

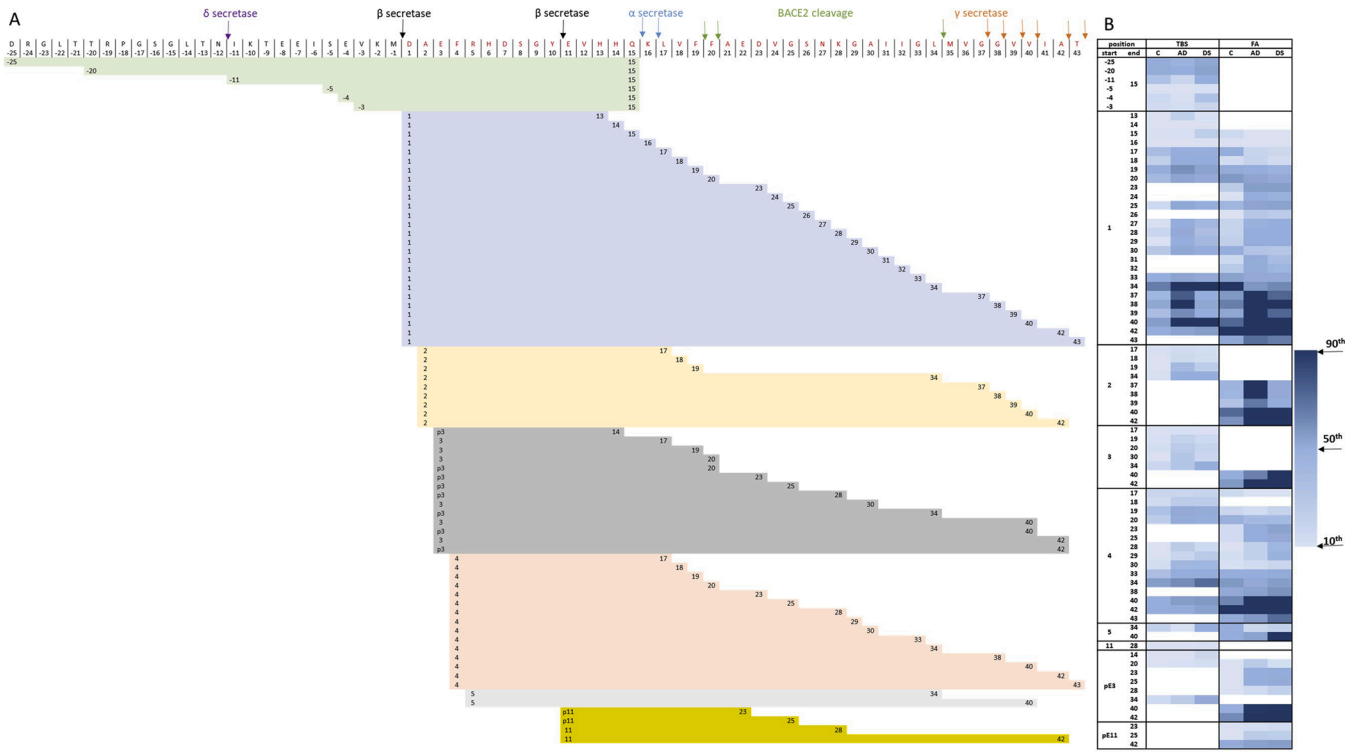

Fig. 1. Overview of the Aβ peptides identified by nanoflow LC-ESI-MS (A) and their respective signal intensity in each fraction (B), with the lightest colour to represent the signal intensity of the 10th percentile and the darkest colour to represent the signal of the 90th percentile.

at 30 Hz and subsequent sonication for 30 s. The homogenate was centrifuged again at 31,000×*g* for 1 h at +4 °C and the supernatant (FA fraction) was dried in a vacuum centrifuge.

2.2. Immunoprecipitation

Prior to IP, TBS fractions were diluted to 40 mL with phosphate buffered saline (PBS) and each sample was divided into two aliquots (20 mL each). The dried FA fractions were reconstituted in 400 µL 70 % FA, shaken for 30 min at room temperature and centrifuged again at 31,000×*g* for 1 h at +4 °C. The supernatant was removed and neutralized with 8 mL 0.5 M Tris and divided into two aliquots (4.2 mL each).

IP was performed with a KingFisher magnetic particle processor (Thermo Fisher Scientific) as previously described with some modifications [23]. Briefly, two different antibody combinations were prepared, a) 4 µg of the anti-Aβ antibodies 6E10 and 4G8 were separately added to 25 µL of Dynabeads M-280 sheep anti-mouse suspension, and b) 8 µg of the anti-oligomeric Aβ antibody mAb158 [24] was added to a 50 µL of Dynabeads M-280 sheep anti-mouse suspension. After 1 h incubation of beads and antibodies, the beads were washed, and the beads-antibody complex (6E10 and 4G8) were combined (50 µL total volume), while the mAb158 was resuspended in its initial volume (50 µL in total). 50 µL of 6E10 + 4G8/beads or 50 µL of mAb158/beads were added to each sample from both diluted TBS-soluble and neutralized FA-soluble fraction. Finally, 20 % (v/v) Triton X-100 was added to each sample [final concentration 0.2 % (v/v)] and incubated over night at +4 °C. The beads/FA fraction was transferred to the KingFisher for automatic washing (in 0.2 % Triton X-100, PBS, pH 7.6, and 50 mM ammoniumbiocarbonate) and eluted in 0.5 % FA. The eluate was then dried in a vacuum centrifuge and the dried samples were stored at −80 °C pending MS analysis.

2.3. Mass spectrometry

Prior to MS analysis, samples were reconstituted in 5 µL 0.1 % FA/20 % acetonitrile in water (v/v/v). Analysis was performed using a matrix-assisted-laser-desorption/ionization-time-of-flight/time-of-flight (MALDI-TOF/TOF) instrument (UltraFleXtreme, Bruker Daltonics) operated in reflector mode with *m/z* range 1500−5000. MALDI samples were prepared using the seed layer method (using α-cyano-4-hydroxycinnamic acid) as previously described [25], consuming 2 µL sample. An average of 10,000 shots were acquired for each spectrum (2000 at a time using a random walk mode). Individual peak areas were normalized to the sum of all Aβ peak areas before further analysis. At different stages in the sample extraction and preparation, quality control experiments were performed, by IP of both CSF and brain samples, to ensure that the protein extraction and the IP performed normally.

The 3 µL left from MALDI preparation were dried and reconstituted in 7 µL 8 % FA/8 % acetonitrile in water (v/v/v), for analysis by nanoflow liquid chromatography (LC) coupled to electrospray ionization (ESI) hybrid quadrupole–orbitrap tandem MS (Dionex Ultimate 3000 system and Q Exactive, both Thermo Fisher Scientific) in a similar way as described previously [26]. Samples were loaded onto a reversed phase Acclaim PepMap 100 C18 trap column (length: 20 mm; inner diameter: 75 µm; particle size: 3 µm; pore size: 100 Å) which was used for online desalting, and subsequently onto a reversed-phase Acclaim PepMap RSLC column (length: 150 mm, inner diameter: 75 µm; particle size: 2 µm; pore size: 100 Å), which was used for separation (both Thermo Fisher Scientific). Mobile phases were 0.1 % FA in water (v/v) (A) and 0.1 % FA/84 % acetonitrile in water (v/v/v) (B). The separation was performed at a flow rate of 300 nL/min by applying a linear gradient of 3–40 % B for 50 min at 60 °C. The mass spectrometer was operated in positive ion mode and set to acquire spectra between 350 and 1800 mass-to-charge (*m/z*) units. Both MS and MS/MS acquisitions were obtained at a resolution setting of 70,000 using 1 microscan, target values of 10^6, and maximum injection time of 250 ms. MS/MS acquisitions were obtained using higher-energy collision-induced dissociation (HCD) using a normalized collision energy (NCE) setting of 25, exclusion of singly charged ions and ions with unassigned charge. Database search (including isotope and charge deconvolution) was performed with

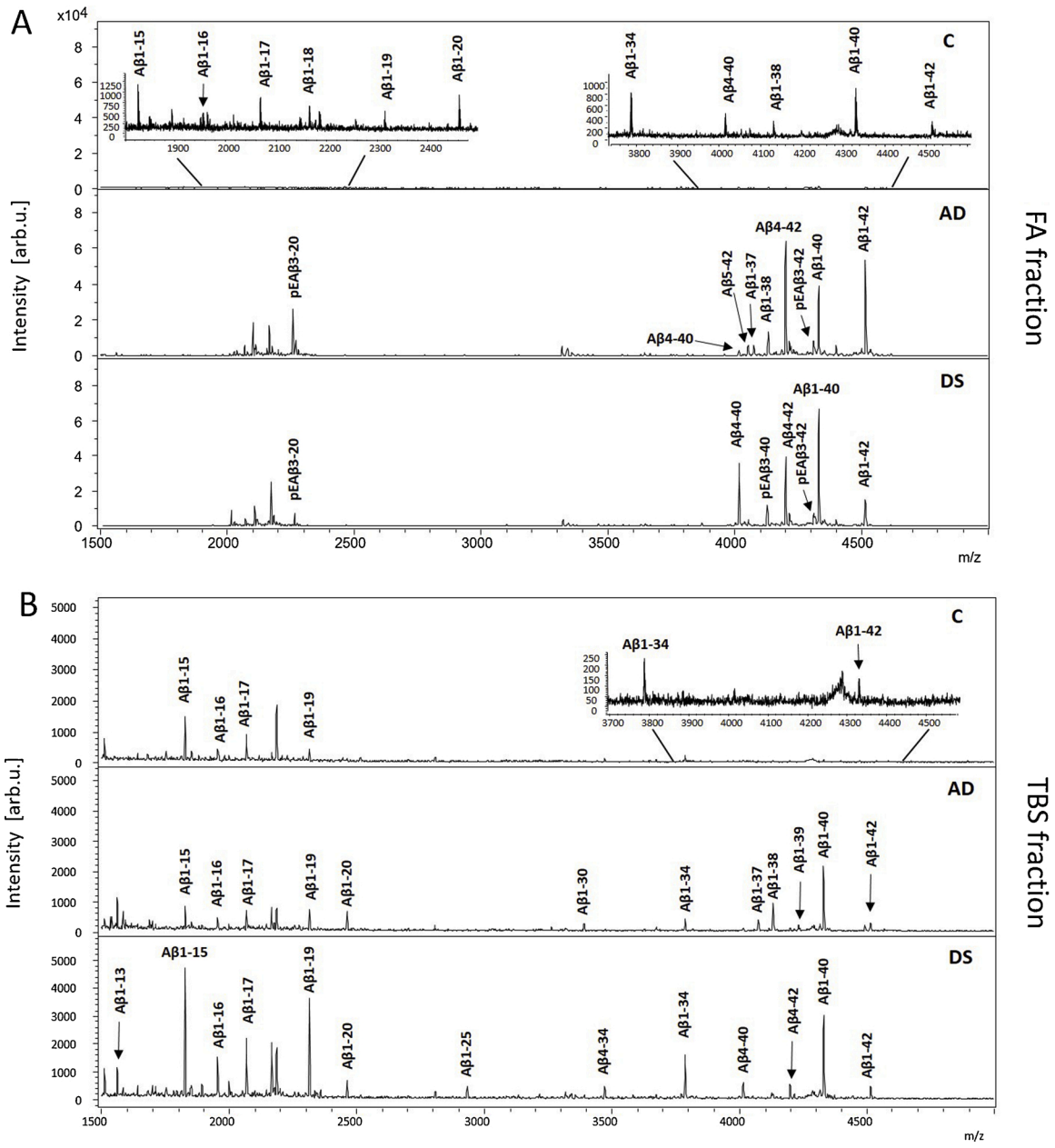

Fig. 2. MALDI spectra of Aβ peptides in controls (C), AD and DS, in FA fraction (A) and TBS fraction (B).In both FA and TBS fractions AD and DS exhibit a similar Aβ pattern, while controls have much lower signal intensity, and higher relative abundance of shorter peptides compared to AD and DS.

PEAKS Studio v8.5 (Bioinformatics Solutions Inc.) against a custom-made APP database. All Aβ peptides identified were quantified (label free) by manual peak picking in Skyline v19.1.0.193.

It should be noted that although it is possible to study non-covalently bound complexes with ESI-MS, this requires special conditions, both in the sample preparation and in the LC–MS setup to ensure that the complexes are not dissociated in the process. Moreover, a specific problem with Aβ is that oligomers are present at low concentrations, which makes them hard to detect in reasonable amounts of tissue [27]. Therefore, we chose to monitor Aβ monomers also in this case.

2.4. Statistical analysis

Statistical analysis was performed using GraphPad Prism v8.1.2. Since the groups were not normally distributed the Mann-Whitney *U* test was used to test the statistical significance. Due to the small sample size, the best (and only) statistically significant difference that can been achieved is $p = 0.0286$, which requires complete separation between the groups.

3. Results

3.1. The Aβ peptide composition differs between FA and TBS fractions

Using IP with 6E10 + 4G8, directed at the N-terminal region and mid-region of Aβ, respectively (both antibodies bind to monomeric as well as fibrillar Aβ) [28], we identified (by LC-ESI-MS/MS) a total of 76 Aβ peptides, starting both N-and C-terminally of the BACE1 cleaving site, ranging from –25 to 11 and ending at positions ranging from aa 13 to 43 relative to the Aβ sequence (Fig. 1). Twenty-nine of these peptides were found in both the FA and the TBS fractions, as well as in all groups and the mass spectrometric signals (both for MALDI-MS and nanoflow LC-ESI-MS) were generally higher in the FA fraction compared to the TBS fraction (Fig. 2). Pyroglutamate formation as post-translational

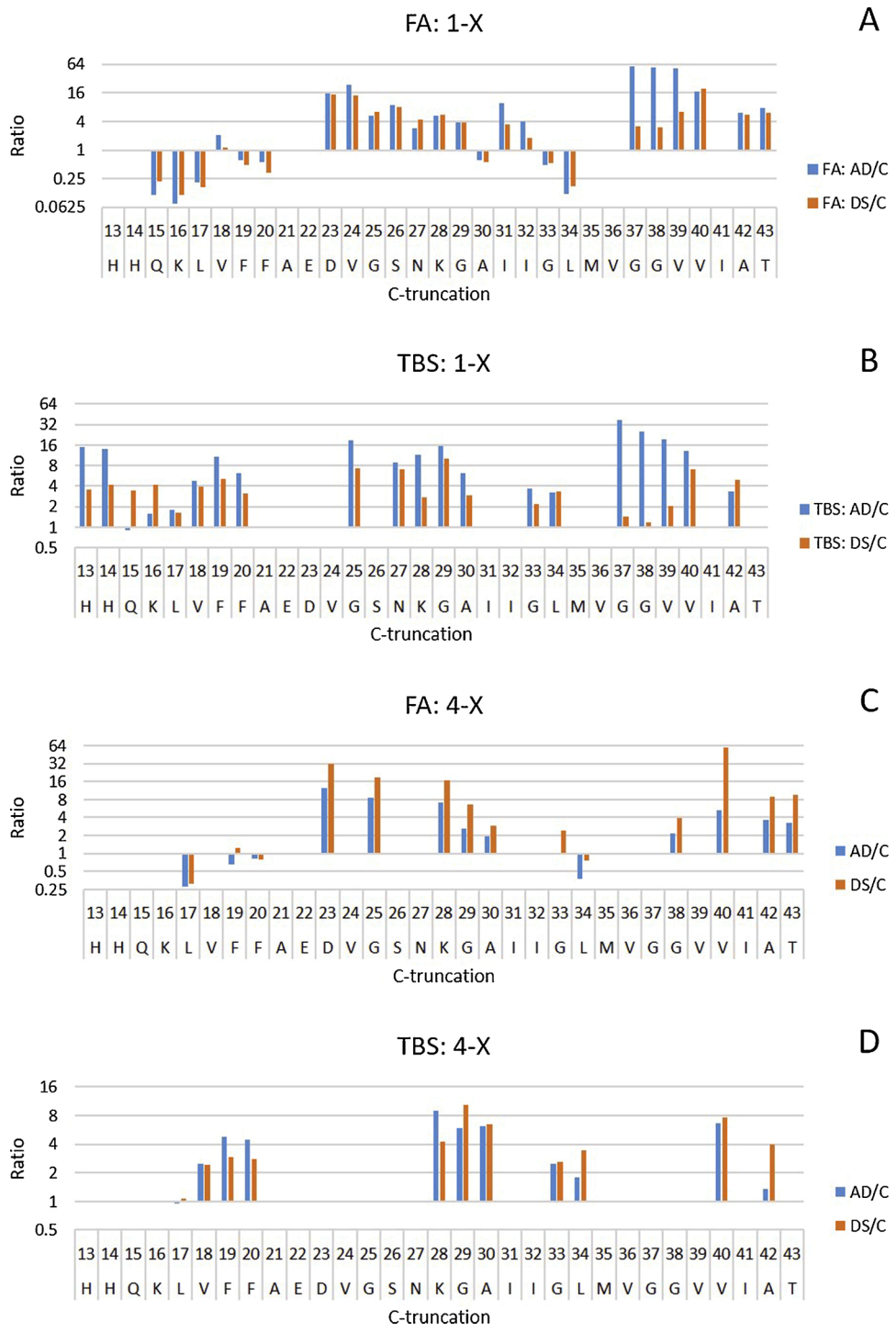

Fig. 3. Peptides detected using nanoflow LC-ESI-MS for the two major N-terminal starting positions (Aβ1-X and Aβ4-X). The ratios of AD-to-controls (AD/C) (A, B) and DS/C (C, D) exhibit similar regulations for the disease groups compared to controls, for the TBS (B, D) and FA (A, C) fractions.

modification of amino acids E3 and E11 was also observed. The most intense signals were observed for Aβ1-40, Aβ1-42, and Aβ4-42. As expected, the FA fraction contained a higher relative amount of the more hydrophobic peptides; *e.g.*, Aβ1-43 and Aβ4-43 were only detected in this fraction, while the TBS fraction contained a larger relative proportion of the shorter and more hydrophilic peptides, such as Aβ1-13 and Aβ1-14 (Fig. 2).

3.2. The Aβ peptide pattern is generally similar in AD and DS

To explore the relative abundance of the Aβ peptides between the different patient groups, we investigated the DS-to-Control (DS-to-C) ratio and the AD-to-C ratio for each observed peptide using nanoflow LC-ESI-MS. This was done by (for each peptide) calculating the average peak area of each group (DS, AD, and C) and then calculating the ratios between these averages (DS/C and AD/C). A fold-change pattern was then acquired showing the increase or decrease in the relative abundance of all detected Aβ peptides. The most abundant Aβ peptides were

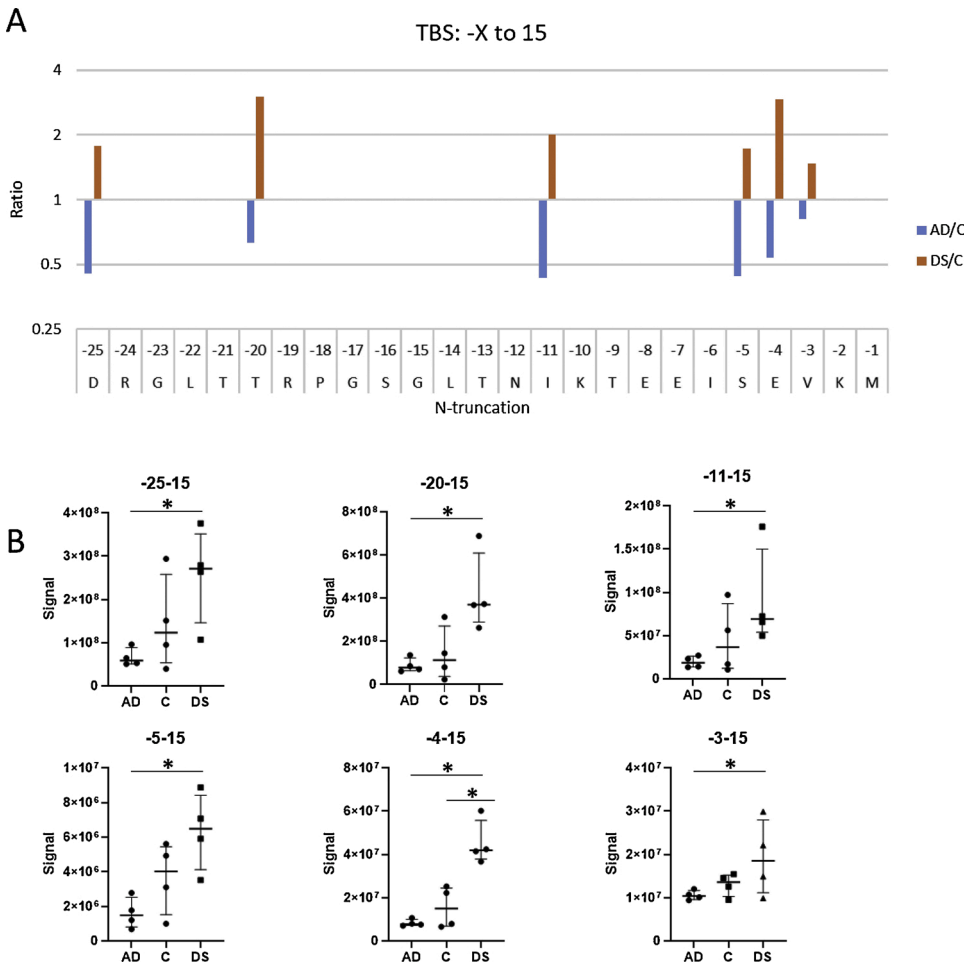

Fig. 4. APP/Aβ(-X to 15) peptides show a different regulation for AD and DS compared with controls (C), with highest abundance in DS, followed by controls and AD. The ratios AD/C and DS/C are shown in (A). In (B) scatterplots for each peptide are shown. Data was acquired by nanoflow LC-ESI-MS. * p = 0.0286.

those starting at positions 1 and 4 in both the FA (Fig. 3A and C) and TBS (Fig. 3B and D) fractions and the change in AD and DS compared with controls were in the same direction. Also, most Aβ peptides starting at other positions changed in the same direction for AD and DS *vs.* controls for both the FA and TBS fractions, (Suppl. Fig. 1). There were some exceptions to this behaviour; *e.g.*, Aβ 1-15 in the TBS fraction and Aβ 4-19 in the FA fraction. In the FA fraction, the relative abundance of shorter Aβ peptides, Aβ 1-15, 16, 17, 19, 20, 34, Aβ4-17, 20, 34, and Aβ5-34, was higher in controls compared with the patient groups, which was not the case for the TBS fraction. Although DS patients have an extra copy of *APP*, a higher relative abundance of Aβ peptides compared with AD patients was generally not observed.

3.3. BACE1 site-spanning APP/Aβ peptides have different regulation pattern in AD and DS compared with controls

Peptides spanning the BACE1 cleavage site were only observed in the TBS fraction and using nanoflow LC-ESI-MS. These APP/Aβ peptides have different N-termini but they all ended at position 15 in the Aβ sequence (Fig. 4). All these N-terminally extended peptides, and also Aβ 1-15, exhibited a regulation pattern that was opposite between AD and DS (Fig. 3); DS had higher abundance compared to controls, while AD had lower abundance than controls.

3.4. N-truncated AβX-34, AβX-40, and AβX-42 peptides are more abundant in DS compared with AD

The AβX-34 peptides were more abundant in DS compared with AD in the TBS fraction. Moreover, the abundance in both AD and DS as compared with controls was higher in the FA fraction but lower in the TBS fraction (Fig. 5A).

Similarly, AβX-40 and AβX-42 peptides were generally more abundant in both AD and DS compared with controls (Figs. 3 and 5B, C). However, the abundance was higher in DS relative to AD in the FA fractions (Fig. 5B, C). Especially, peptides starting at aa 4 (Aβ4-X) were increased in DS compared with AD (Fig. 5D).

3.5. Protofibrils consist of Aβ1-40, Aβ1-42 and Aβ4-42, with higher relative abundance in AD and DS compared with controls

Using the antibody mAb158 and both MALDI-MS (data not shown) and nanoflow LC-ESI-MS, we demonstrated that oligomers/protofibrils contain Aβ1-40, Aβ1-42, and Aβ4-42. The antibody binds to the oligomers/protofibrils during the IP, but during elution in acidic conditions the protofibrils are dissociated into monomeric Aβ. Apart from the identity of the detected Aβ peptides (Fig. 6A), the relative abundance of aggregated Aβ1-40, Aβ1-42, and Aβ4-42 in the oligomeric/protofibril form was higher in both AD and DS compared with controls. The relative abundance between AD and DS did not differ for Aβ1-40 and Aβ1-42, but Aβ4-42 was higher in DS compared with AD. A similar tendency was also observed for Aβ4-42 IP'd with 6E10 + 4G8 in TBS, see Fig. 3.

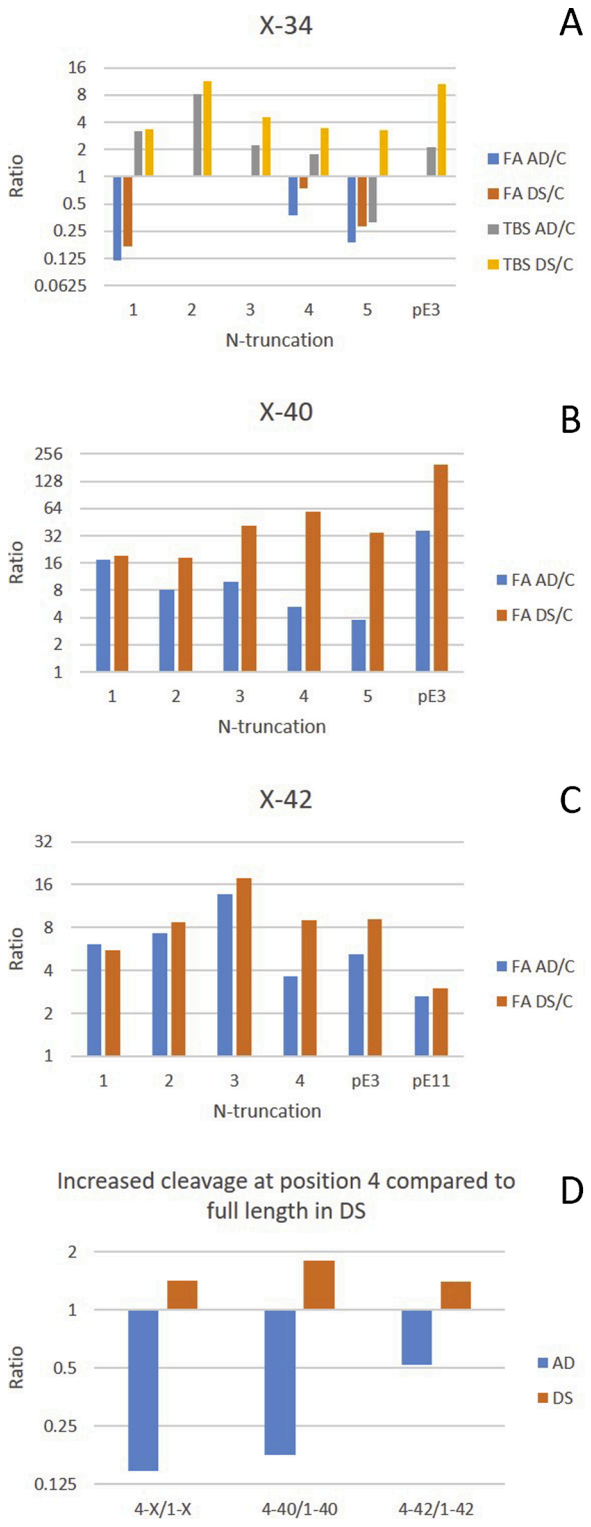

Fig. 5. The ratios of AD-to-controls (AD/C) and DS/C of the N-truncated peptides ending at (A) aa 34, (B) aa 40, and (C) aa 42, are increased in DS compared to AD. (D) The ratios of the sum of the intensities of all peptides starting at aa 4 to the sum of the intensities of all peptides starting at aa 1, together with the ratios of 4–40/1–40 and 4–42/1–42 are increased in DS compared to AD. Data was acquired by nanoflow LC-ESI-MS.

3.6. The relative level of soluble Aβ1-42 is higher in oligomers compared with monomers in both AD and DS

The TBS fractions contained Aβ1-40, Aβ1-42, and Aβ4-42 as shown by IP using either mAb158 or 6E10 + 4G8. Aβ1-42 extracted from protofibrils (mAb158) had a higher relative abundance for both AD and DS compared with controls (Fig. 6B). Contrary to this, Aβ1-40 IP'd using 6E10 + 4G8 had a higher relative abundance in controls compared with oligomeric Aβ1-40 (mAb158), while no difference was observed between the two different forms in AD and DS. There was no difference observed in the abundance between 6E10 + 4G8-IP'd Aβ and oligomeric Aβ4-42 in any of the groups.

4. Discussion

Understanding of the APP and Aβ metabolism and the molecular pathways leading to Aβ aggregation has been central in the efforts to understand the pathology of both AD and DS. DS individuals have an extra copy of chromosome 21, which apart from *APP* also contains *BACE2* and many other genes [29] Aβ peptides are produced from proteolytic cleavages of APP, and the extra copy of the *APP* gene may result in a 50 % overexpression of APP and thus 50 % more Aβ resulting in an imbalance in DS individuals between the production and the clearance of Aβ peptides, eventually leading to increased plaque formation.

The goal of this study was to investigate the Aβ peptide pattern in DS patients and make a comparison with both AD and controls. Although different cortical regions were used, possible differences from an Aβ perspective should be minor. To make this comparison, Aβ was extracted from brain tissue into two fractions; a TBS fraction and subsequently an FA fraction. This enabled separate analyses of the water soluble Aβ peptides, which would be obscured by the much larger amount of aggregated Aβ, if the fractions were combined.

In general, more hydrophilic peptides, (aa $\leq$34) were observed at higher abundance (or only) in the TBS fraction (Fig. 1B). This is likely due to the fact that the shorter, more hydrophilic, peptides are less prone to aggregation. It is intriguing that many of these peptides are possibly produced by α-secretase or BACE2. Moreover, Aβ1-23 to Aβ1-32, which are produced differently, were more abundant in the FA fraction (Fig. 1B). The longer, more hydrophobic Aβ peptides, ending at 37–43, were much more abundant in the FA fraction, most likely reflecting their aggregation propensity.

For the more hydrophilic peptides we noticed a group dependent difference in the Aβ composition between the TBS and FA fractions (Fig. 3 and Suppl. Fig. 1). In the TBS fraction all of these peptides had higher abundance in AD and DS compared with controls, suggesting an increased α-secretase activity or increased activity of other proteases such as endothelin-converting enzyme, plasmin, matrix metalloproteases, or BACE2 [30]. For the FA fraction these peptides had lower abundance in AD and DS compared with controls, suggesting a decreased activity for α-secretase and the other proteases. In addition, a recent study [31] showed that the abundance of the BACE2-cleaved peptides Aβ1-19, 20, 34 was increased in cerebral organoids of DS patients compared with controls. This pattern was also found in the TBS fraction of the present study. Moreover, Aβ1-34 was generally more abundant in DS compared with AD in the TBS fractions (Fig. 5A).

The longer, more hydrophobic Aβ peptides, AβX-37 to AβX-43, were all more abundant in AD and DS compared with controls in both FA and TBS fractions due to aggregation tendency and/or inefficient clearance. A difference between AD and DS was observed only for the AβX-40 peptides and Aβ4-42 of which Aβ4-42 was increased in DS patients compared to AD. (Figs. 3, 5, and Suppl. Fig. 2). Aβ1-37, 38, 39 were markedly elevated in AD compared with DS. This might be due to that two of the AD patients also suffered from CAA [23]; the potential association is, however, presently speculative. Although there are differences observed between AD and DS, in general both groups had similar alterations compared with controls.

The group of peptides spanning the BACE1 site, APP/Aβ(-X to 15), have not previously been detected in brain. These peptides, and possibly Aβ 1-15, were found at increased levels in DS compared with controls,

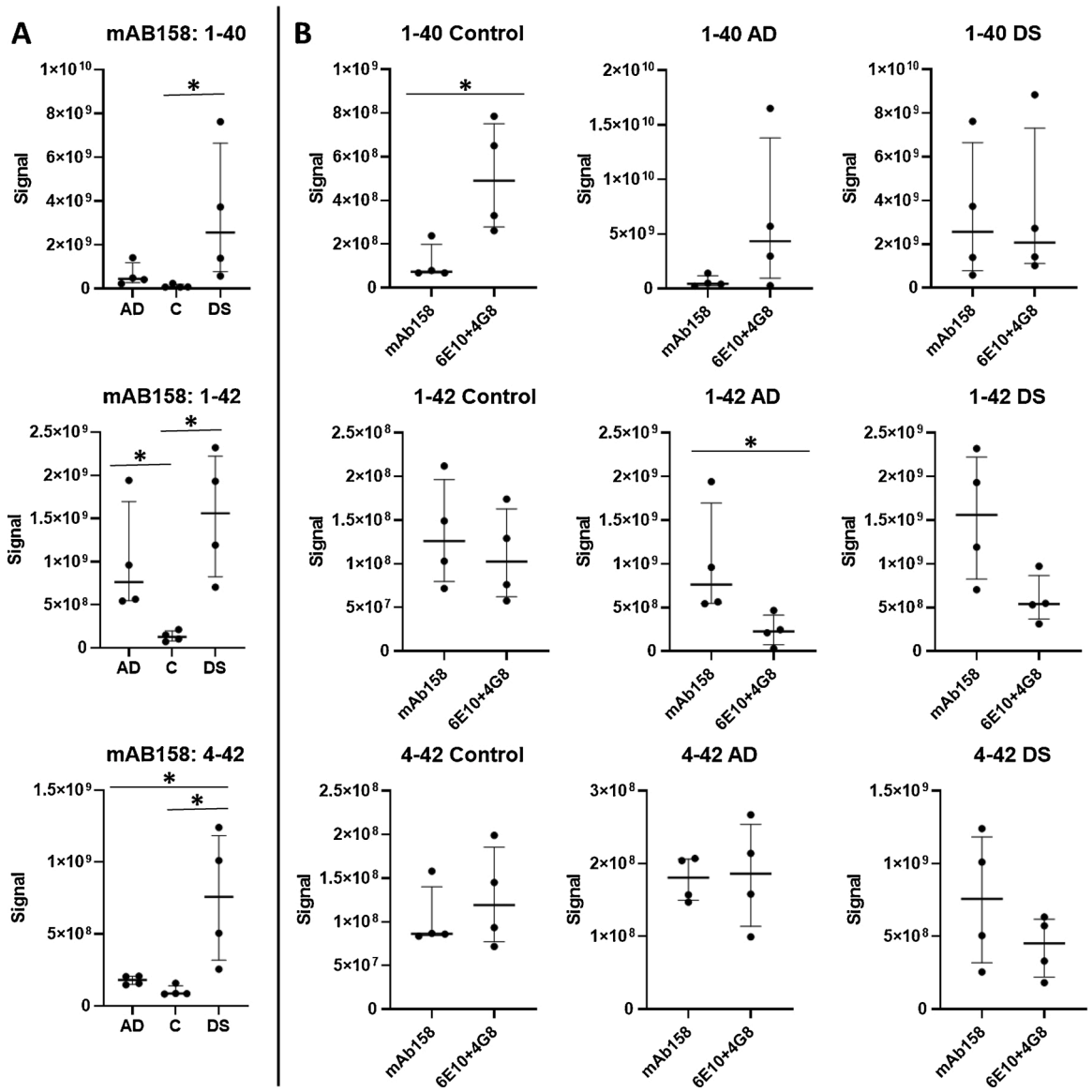

Fig. 6. (A) The relative abundance of the Aβ1-40, Aβ1-42 and Aβ4-42 peptides identified using mAb158 for AD, DS, and controls (C) have increased signal intensity for Aβ1-42 and Aβ4-42 in DS and AD compared to controls. (B) The relative abundance of the Aβ1-40, Aβ1-42 and Aβ4-42 peptides captured by either mAb158 or 6E10 + 4G8 are shown individually for all three patient groups. The signal intensity is increased for oligomeric/protofibril Aβ1-42 in both AD and DS and for Aβ4-42 in DS compared to controls. Data was acquired by nanoflow LC-ESI-MS. * $p = 0.0286$.

while for AD the amounts were decreased. Aβ 1-15 belongs both to the Aβ1-X and APP/Aβ(-X to 15) groups and the observed amount may be the result of two superimposed pathways The reason for the different levels of APP/Aβ(-X to 15) in DS compared with AD is not clear. Possibilities include different BACE1 activity and/or different α-secretase activity. This finding requires validation in a different cohort.

To assess oligomeric/protofibrillic Aβ content in brain the mAb158 antibody was utilised, which has previously been shown to bind preferably to oligomers [24]. Here we showed that their main components were Aβ1-40, Aβ1-42, and Aβ4-42, and similarly to Aβ IP'd using 6E10 + 4G8, all three peptides were more abundant in AD and DS compared with controls. This result supports the conclusion that the qualitative Aβ composition differences between AD and DS are minor.

From Fig. 6B, it is clear that for AD and DS Aβ peptides ending at aa 42 were relatively more abundant in the oligomers/protofibrils compared with species in monomeric form. This finding is consistent with what is typically observed in plaques and is a strong indication that mAb158 indeed selectively binds to oligomers/protofibrils rather than monomeric Aβ peptides.

The main limitation of the study is the small cohort; unfortunately, access to well characterised DS brain material was very limited, which also limits the value of the statistical evaluation. The second limitation is that the quantification was performed in a so-called label-free manner, *i.e.*, without added stable-isotope-labelled standards. One reason for conducting the study this way is that in order to take advantage of standards, they should be incorporated in the sample before homogenization. Since full incorporation into the plaques is not possible, their use will be very limited.

5. Conclusions

The Aβ peptide pattern in brain tissue was found to be similar in AD and DS compared with controls, indicating a similar pathway of Aβ peptide production, degradation and accumulation. The exception was the N-terminally extended APP/Aβ(-X to 15) peptides, which were more abundant DS compared to controls, while in AD their abundance was

lower than for controls, suggesting different BACE1 and/or α-secretase activities in AD and DS. Likewise, no qualitative difference was found for Aβ peptides forming protofibrils/oligomers in AD and DS. The similarities between sporadic AD and DS regarding Aβ/APP hint that monitoring DS patients from early age might contribute to our understanding of plaque formation and finally neurodegeneration in sporadic AD. This also implies the possibility that treatment with clinical benefit in sporadic AD will also be beneficial for subjects with DS. However, with such a small cohort, all results must be interpreted with caution and it is imperative to validate the results in additional studies with larger number of samples.

Authors' contributions

EG, CS, EP, MJ, LS, JF, HB, CM, TO, HZ, KB, LL, and GB drafted the study design. MJ and CS performed the protein extraction of human post mortem tissue and TBS fractionation. EG carried out sample processing, MS data collection, analysis and interpretation. EP and GB assisted with MS data analysis and interpretation. All authors participated in writing the manuscript.

CRediT authorship contribution statement

Eleni Gkanatsiou: Methodology, Validation, Formal analysis, Investigation, Writing - original draft. **Charlotte Sahlin:** Methodology, Validation, Investigation, Writing - review & editing. **Erik Portelius:** Methodology, Validation, Formal analysis, Writing - review & editing. **Malin Johannesson:** Methodology, Validation, Investigation, Writing - review & editing. **Linda Söderberg:** Methodology, Validation, Writing - review & editing. **Johanna Fälting:** Methodology, Validation, Writing - review & editing. **Hans Basun:** Methodology, Validation, Writing - review & editing. **Christer Möller:** Methodology, Validation, Writing - review & editing. **Tomas Odergren:** Methodology, Validation, Writing - review & editing. **Henrik Zetterberg:** Methodology, Validation, Writing - review & editing. **Kaj Blennow:** Methodology, Validation, Writing - review & editing. **Lars Lannfelt:** Methodology, Validation, Writing - review & editing. **Gunnar Brinkmalm:** Methodology, Software, Validation, Formal analysis, Writing - review & editing.

Declaration of Competing Interest

The authors declare the following competing interests:

HZ has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program.

Acknowledgements

We thank the Netherlands Brain Bank for providing the brain tissue. The study was supported by grants from the Swedish Research Council (#2017-00915), the European Research Council, the Swedish Alzheimer Foundation (#AF-742881), Hjärnfonden, Sweden (#FO2017-0243), Stiftelsen för Gamla Tjänarinnor, the Knut and Alice Wallenberg Foundation, the Torsten Söderberg Foundation, the Swedish state under the agreement between the Swedish government and the County Councils, the ALF-agreement (#ALFGBG-715986), and Demensfonden.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.neulet.2021.135894.

References

[1] C.L. Masters, G. Simms, N.A. Weinman, G. Multhaup, B.L. McDonald, K. Beyreuther, Amyloid plaque core protein in Alzheimer disease and Down syndrome, Proc. Natl. Acad. Sci. U. S. A. 82 (12) (1985) 4245–4249, https://doi.org/10.1073/pnas.82.12.4245.

[2] F.K. Wiseman, T. Al-Janabi, J. Hardy, A. Karmiloff-Smith, D. Nizetic, V. L. Tybulewicz, et al., A genetic cause of Alzheimer disease: mechanistic insights from Down syndrome, Nat. Rev. Neurosci. 16 (9) (2015) 564–574, https://doi.org/10.1038/nrn3983.

[3] D.M. Mann, Alzheimer's disease and Down's syndrome, Histopathology 13 (2) (1988) 125–137, https://doi.org/10.1111/j.1365-2559.1988.tb02018.x.

[4] M. McCarron, P. McCallion, E. Reilly, N. Mulryan, A prospective 14-year longitudinal follow-up of dementia in persons with Down syndrome, J. Intellect. Disabil. Res. 58 (1) (2014) 61–70, https://doi.org/10.1111/jir.12074.

[5] F.S. Esch, P.S. Keim, E.C. Beattie, R.W. Blacher, A.R. Culwell, T. Oltersdorf, et al., Cleavage of amyloid beta peptide during constitutive processing of its precursor, Science 248 (4959) (1990) 1122–1124, https://doi.org/10.1126/science.2111583.

[6] D.J. Selkoe, Alzheimer's disease: genes, proteins, and therapy, Physiol. Rev. 81 (2) (2001) 741–766, https://doi.org/10.1152/physrev.2001.81.2.741.

[7] E. Portelius, E. Price, G. Brinkmalm, M. Stiteler, M. Olsson, R. Persson, et al., A novel pathway for amyloid precursor protein processing, Neurobiol. Aging 32 (6) (2011) 1090–1098, https://doi.org/10.1016/j.neurobiolaging.2009.06.002.

[8] B. De Strooper, Aph-1, Pen-2, and Nicastrin with Presenilin generate an active gamma-Secretase complex, Neuron 38 (1) (2003) 9–12, https://doi.org/10.1016/s0896-6273(03)00205-8.

[9] C. Haass, C. Kaether, G. Thinakaran, S. Sisodia, Trafficking and proteolytic processing of APP, Cold Spring Harb. Perspect. Med. 2 (5) (2012), https://doi.org/10.1101/cshperspect.a006270 a006270.

[10] R. Fluhrer, A. Capell, G. Westmeyer, M. Willem, B. Hartung, M.M. Condron, et al., A non-amyloidogenic function of BACE-2 in the secretory pathway, J. Neurochem. 81 (5) (2002) 1011–1020, https://doi.org/10.1046/j.1471-4159.2002.00908.x.

[11] X.P. Shi, K. Tugusheva, J.E. Bruce, A. Lucka, G.X. Wu, E. Chen-Dodson, et al., Beta-secretase cleavage at amino acid residue 34 in the amyloid beta peptide is dependent upon gamma-secretase activity, J. Biol. Chem. 278 (23) (2003) 21286–21294, https://doi.org/10.1074/jbc.M209859200.

[12] X. Sun, G. He, W. Song, BACE2, as a novel APP theta-secretase, is not responsible for the pathogenesis of Alzheimer's disease in Down syndrome, FASEB J. 20 (9) (2006) 1369–1376, https://doi.org/10.1096/fj.05-5632com.

[13] G.G. Glenner, C.W. Wong, Alzheimer's disease and Down's syndrome: sharing of a unique cerebrovascular amyloid fibril protein, Biochem. Biophys. Res. Commun. 122 (3) (1984) 1131–1135, https://doi.org/10.1016/0006-291x(84)91209-9.

[14] J. Hardy, Alzheimer's disease: the amyloid cascade hypothesis: an update and reappraisal, J. Alzheimers Dis. 9 (Suppl. 3) (2006) 151–153, https://doi.org/10.3233/jad-2006-9s317.

[15] B. O'Nuallain, D.B. Freir, A.J. Nicoll, E. Risse, N. Ferguson, C.E. Herron, et al., Amyloid beta-protein dimers rapidly form stable synaptotoxic protofibrils, J. Neurosci. 30 (43) (2010) 14411–14419, https://doi.org/10.1523/JNEUROSCI.3537-10.2010.

[16] A.B. Reiss, H.A. Arain, M.M. Stecker, N.M. Siegart, L.J. Kasselman, Amyloid toxicity in Alzheimer's disease, Rev. Neurosci. 29 (6) (2018) 613–627, https://doi.org/10.1515/revneuro-2017-0063.

[17] O. Sofola-Adesakin, M. Khericha, I. Snoeren, L. Tsuda, L. Partridge, pGluAbeta increases accumulation of Abeta in vivo and exacerbates its toxicity, Acta Neuropathol. Commun. 4 (1) (2016) 109, https://doi.org/10.1186/s40478-016-0380-x.

[18] I.T. Lott, E. Head, Dementia in Down syndrome: unique insights for Alzheimer disease research, Nat. Rev. Neurol. 15 (3) (2019) 135–147, https://doi.org/10.1038/s41582-018-0132-6.

[19] T. Annus, L.R. Wilson, Y.T. Hong, J. Acosta-Cabronero, T.D. Fryer, A. Cardenas-Blanco, et al., The pattern of amyloid accumulation in the brains of adults with Down syndrome, Alzheimers Dement. 12 (5) (2016) 538–545, https://doi.org/10.1016/j.jalz.2015.07.490.

[20] B.L. Handen, A.D. Cohen, U. Channamalappa, P. Bulova, S.A. Cannon, W.I. Cohen, et al., Imaging brain amyloid in nondemented young adults with Down syndrome using Pittsburgh compound B, Alzheimers Dement. 8 (6) (2012) 496–501, https://doi.org/10.1016/j.jalz.2011.09.229.

[21] P.J. Lao, T.J. Betthauser, A.T. Hillmer, J.C. Price, W.E. Klunk, I. Mihaila, et al., The effects of normal aging on amyloid-beta deposition in nondemented adults with Down syndrome as imaged by carbon 11-labeled Pittsburgh compound B, Alzheimers Dement. 12 (4) (2016) 380–390, https://doi.org/10.1016/j.jalz.2015.05.013.

[22] P.R. Hof, C. Bouras, D.P. Perl, D.L. Sparks, N. Mehta, J.H. Morrison, Age-related distribution of neuropathologic changes in the cerebral cortex of patients with Down's syndrome. Quantitative regional analysis and comparison with Alzheimer's disease, Arch. Neurol. 52 (4) (1995) 379–391, https://doi.org/10.1001/archneur.1995.00540280065020.

[23] E. Gkanatsiou, E. Portelius, C.E. Toomey, K. Blennow, H. Zetterberg, T. Lashley, G. Brinkmalm, A distinct brain beta amyloid signature in cerebral amyloid

angiopathy compared to Alzheimer's disease, Neurosci. Lett. 701 (2019) 125–131, https://doi.org/10.1016/j.neulet.2019.02.033.
[24] H. Englund, D. Sehlin, A.S. Johansson, L.N. Nilsson, P. Gellerfors, S. Paulie, et al., Sensitive ELISA detection of amyloid-beta protofibrils in biological samples, J. Neurochem. 103 (1) (2007) 334–345, https://doi.org/10.1111/j.1471-4159.2007.04759.x.
[25] E. Portelius, A.J. Tran, U. Andreasson, R. Persson, G. Brinkmalm, H. Zetterberg, et al., Characterization of amyloid beta peptides in cerebrospinal fluid by an automated immunoprecipitation procedure followed by mass spectrometry, J. Proteome Res. 6 (11) (2007) 4433–4439, https://doi.org/10.1021/pr0703627.
[26] G. Brinkmalm, E. Portelius, A. Ohrfelt, N. Mattsson, R. Persson, M.K. Gustavsson, et al., An online nano-LC-ESI-FTICR-MS method for comprehensive characterization of endogenous fragments from amyloid beta and amyloid precursor protein in human and cat cerebrospinal fluid, J. Mass Spectrom. 47 (5) (2012) 591–603, https://doi.org/10.1002/jms.2987.
[27] G. Brinkmalm, W. Hong, Z. Wang, W. Liu, T.T. O'Malley, X. Sun, et al., Identification of neurotoxic cross-linked amyloid-beta dimers in the Alzheimer's brain, Brain 142 (5) (2019) 1441–1457, https://doi.org/10.1093/brain/awz066.
[28] A.C. Klaver, L.M. Patrias, J.M. Finke, D.A. Loeffler, Specificity and sensitivity of the Abeta oligomer ELISA, J. Neurosci. Methods 195 (2) (2011) 249–254, https://doi.org/10.1016/j.jneumeth.2010.12.001.
[29] K. Gardiner, A.C. Costa, The proteins of human chromosome 21, Am. J. Med. Genet. C Semin. Med. Genet. 142C (3) (2006) 196–205, https://doi.org/10.1002/ajmg.c.30098.
[30] E. Portelius, H. Zetterberg, J. Gobom, U. Andreasson, K. Blennow, Targeted proteomics in Alzheimer's disease: focus on amyloid-beta, Expert Rev. Proteomics 5 (2) (2008) 225–237, https://doi.org/10.1586/14789450.5.2.225.
[31] I. Alic, P.A. Goh, A. Murray, E. Portelius, E. Gkanatsiou, G. Gough, et al., Patient-specific Alzheimer-like pathology in trisomy 21 cerebral organoids reveals BACE2 as a gene dose-sensitive AD suppressor in human brain, Mol. Psychiatry (2020), https://doi.org/10.1038/s41380-020-0806-5.