Apolipoprotein D: a potential biomarker for cerebral amyloid angiopathy

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Aims: We investigated the potential of apolipoprotein D (apoD) as cerebrospinal fluid (CSF) biomarker for cerebral amyloid angiopathy (CAA) after confirmation of its association with CAA pathology in human brain tissue. Methods: The association of apoD with CAA pathology was analysed in human occipital lobe tissue of CAA (n = 9), Alzheimer’s disease (AD) (n = 11) and healthy control cases (n = 11). ApoD levels were quantified in an age- and sex-matched CSF cohort of CAA patients (n = 31), AD patients (n = 27) and non-neurological controls (n = 67). The effects of confounding factors (age, sex, serum levels) on apoD levels were studied using CSF of non-neurological controls (age range 16–85 years), and paired CSF and serum samples. Results: ApoD was strongly associated with amyloid deposits in vessels, but not with parenchymal plaques in human brain tissue. CSF apoD levels correlated with age and were higher in men than women in subjects >50 years. The apoD CSF/serum ratio correlated with the albumin ratio. When controlling for confounding factors, CSF apoD levels were significantly lower in CAA patients compared with controls and compared with AD patients (P = 0.0008). Conclusions: Our data show that apoD is specifically associated with CAA pathology and may be a CSF biomarker for CAA, but clinical application is complicated due to dependency on age, sex and blood–CSF barrier integrity. Well-controlled follow-up studies are required to determine whether apoD can be used as reliable biomarker for CAA.

Keywords: Alzheimer disease, amyloid, apolipoprotein D, biomarkers, blood–CSF barrier, cerebral amyloid angiopathy, cerebrospinal fluid
Introduction

Cerebral amyloid angiopathy (CAA) is characterized by the accumulation of amyloid-β (Aβ) in the cerebral vasculature and is a major cause of intracerebral haemorrhages (ICH) and cognitive decline in the elderly [1]. CAA pathology of any severity is present in 55–59% of demented and 28–38% of nondemented elderly. The prevalence of CAA pathology is particularly high in patients with Alzheimer’s disease (AD), accounting for 47–100% of AD patients [2]. Two types of CAA have been recognized. In CAA type-1, CAA develops both in capillaries and larger vessels, whereas in CAA type-2, CAA pathology is restricted to medium-to-large sized blood vessels [3]. In vivo diagnosis of CAA is based on the modified Boston criteria, which are based on radiological markers for CAA, i.e. the presence of lobar haemorrhages or cortical superficial siderosis with (definite CAA) or without (probable or possible CAA) neuropathological demonstration of CAA [4]. MRI analysis, however, does not visualize the principal neuropathological cornerstone of CAA, i.e. deposition of Aβ in cortical and leptomeningeal vessels. In addition, only late-stage manifestations of CAA (i.e. haemorrhages) are visualized, and therefore MRI analysis is not optimal for early detection of CAA. Therefore, biomarkers that are more directly related to the cerebrovascular Aβ pathology or that detect earlier stages of CAA are dearly needed. Cerebrospinal fluid (CSF) biomarkers may serve as such markers. The potential of CSF biomarkers is supported by findings of decreased Aβ40 and Aβ42 levels in CSF of sporadic CAA patients compared with age-matched control subjects. Of these two biomarkers, CSF Aβ40 is the most specific since Aβ42 levels are also decreased in CSF from AD patients [5,6]. Moreover, previous studies have shown that CSF Aβ40 and Aβ42 are also decreased in patients with familial CAA, even in the presymptomatic phase of the disease [7].

We previously identified apolipoprotein D (apoD) by mass spectrometry as a potential CAA-associated protein in human brain tissue (unpublished data), which suggested that apoD may be a biomarker for CAA. ApoD is a lipid transport protein of the lipocalin family, and known as a multifunctional protein with a major role as antioxidant and in neuroprotection [8]. ApoD is expressed at high levels in the human brain, where it is predominantly produced by astrocytes and oligodendrocytes [9]. In this study, we aimed to confirm the association of apoD with CAA pathology in human brain tissue and to investigate the potential biomarker value of CSF apoD levels for the diagnosis of CAA. In addition, because studies on human brain indicate that apoD levels are dependent on age and sex, we assessed whether CSF apoD levels are related to age and sex and other potential confounding factors.

Materials and methods

Patients and biological fluids

To study the diagnostic potential of apoD, we obtained CSF samples from CAA (n = 31) and AD patients (n = 27) and age- and sex-matched controls (n = 67) from the Radboud University Medical Center (RUMC; Nijmegen, The Netherlands), and from Massachusetts General Hospital (MGH; Boston, MA, USA; n = 15 patients with CAA). Patient characteristics are summarized in Table 1. Clinical diagnosis of CAA was based on the modified Boston criteria [4], and was scored as definite (autopsy-confirmed; n = 2), probable (n = 7 with, and n = 20 without supporting pathology), or possible CAA (n = 2). Clinical diagnosis of AD patients was based on the NINCDS-ADRDA criteria [10], since most samples were collected before introduction of more recent diagnostic criteria [11]. The prevalence of CAA pathology in these AD patients is not known. Control subjects had undergone lumbar puncture as part of the diagnostic process yet were confirmed as not having a neurodegenerative disease.

To study the relation of apoD with age and sex, we used CSF samples from control subjects in the age range 16–85 years (n = 102; 49% men). To study the relation of apoD with blood-CSF barrier integrity, we used paired serum and CSF samples (n = 41). The paired CSF and serum samples were selected based on the availability of albumin levels. The ratio of albumin levels in CSF vs. serum (Qalb) was used as measure for blood–CSF barrier integrity.

CSF was obtained by lumbar puncture via standard procedures and collected in polypropylene tubes, centrifuged and stored at −80°C. Use of CSF and serum was approved by the local Medical Ethical Committees, and informed consent was obtained from all subjects or their caregivers.

Human brain tissue

We used human post mortem tissue to study the association of apoD with CAA pathology. Human occipital lobe tissue
was obtained from the Netherlands Brain Bank (NBB), Netherlands Institute for Neuroscience (NIN), Amsterdam. All brain tissues were collected from donors with written informed consent for brain autopsy and the use of the material and clinical information for research purposes has been obtained by the NBB. Tissue from three groups of cases was used, composed of (1) cognitively healthy control cases without any Aβ or AD-related pathology (n = 11), (2) AD cases with severe plaque pathology but without Aβ accumulation in vessels (n = 11) and (3) severe CAA type-1 cases with severe capillary CAA (n = 9). Details, including staging of AD pathology conform the ABC criteria [12], are described in Table S1.

### Immunohistochemistry

Immunohistochemistry (IHC) was performed as described previously [13]. In short, 5 μm sections of formalin fixed, paraffin embedded tissue were used. Endogenous peroxidase activity was quenched using methanol containing 0.3% H₂O₂ and antigen retrieval was performed by heating in an autoclave in citrate buffer. Anti-Aβ (clone IC16, kind gift from Prof. C. Korth, Heinrich Heine University, Düsseldorf, Germany) and anti-apoD (Proteintech group, Rosemont, IL, USA; cat no. 10520-1-AP) were used as primary antibodies, and anti-apoD (Proteintech group, Rosemont, IL, USA; cat no. 10520-1-AP) were used as primary antibodies, and anti-apoD (Proteintech group) overnight at 4°C. After washing, membranes were incubated for 3 h with an HRP-labelled secondary antibody (EnVision; Agilent, Santa Clara, CA, USA). After washing in PBS, slides were incubated with DAB and nuclei were counter-stained using haematoxylin.

### SDS-PAGE and Western blotting

Sections of snap frozen tissue were lysed in reducing, SDS-containing sample buffer using a 1:20 tissue weight to lysis buffer ratio. Protein samples were separated on stain-free precast SDS-PAGE gradient gels (Bio-Rad, Hercules, CA, USA) and transferred to PVDF membranes (Merck Millipore, Billerica, MA, USA) by western blotting. PVDF membranes were blocked using Odyssey blocking buffer (LI-COR, Bad Homburg, Germany) and subsequently incubated with anti-apoD antibody (Proteintech group) overnight at 4°C. After washing, membranes were incubated for 3 h with an IRDye 800 CW-labelled secondary antibody (LI-COR). ApoD staining was visualized using an Odyssey imaging system and total protein load using a chemidoc EZ imager (Bio-Rad), the densitometric values of which were used to normalize for total protein input.

### Immunofluorescence

The pretreatment and antibody incubation of tissue slices for immunofluorescence staining was similar as described for IHC. In addition, amyloid was visualized using Thioflavin-S (1% in dH2O) and anti-mouse Alexa 555 and anti-rabbit Alexa 647 (Thermo Fisher Scientific, Waltham, MA, USA) were used as secondary antibodies. Auto fluorescence was quenched using 0.1% Sudan black in 70% ethanol. After mounting, representative pictures were taken with a Leica DMi8 inverted fluorescence microscope equipped with a Leica DFC300 G camera.
Quantification was performed using Image J software (NIH, Bethesda, MD, USA).

**ApoD ELISA**

We quantified apoD levels in CSF and serum using the Human apoD ELISA<sup>PRO</sup> kit (Mabtech, Nacka Strand, Sweden) according to the manufacturers’ instructions. CSF samples were diluted 600 times and serum samples 6000 times. The mean coefficient of variation (CV) for duplicate measures was <15% for CSF samples and <21% for serum samples. Total protein levels in CSF were determined using Pierce<sup>TM</sup> BCA protein assay kit (Thermo Fisher Scientific). Five quality controls, consisting of pooled CSF samples, were used in each assay to check and correct for any inconsistencies between plates.

**Statistical analyses**

We analysed data using GraphPad Prism software version 5.03 (GraphPad Software, Inc., La Jolla, CA, USA) and SPSS Statistics software version 22.0 (IBM, Amsterdam, The Netherlands). We used Kolmogorov–Smirnov and the Shapiro–Wilk test to analyse normality of data. We assessed homogeneity of sex distribution in patient cohorts using the Chi-square test and for age using one-way analysis of variance (ANOVA) with Bonferroni’s post hoc test. Statistical outliers (significance level set at \( P < 0.01 \)) were identified using Grubbs’ outlier test. For all analyte comparisons and correlation analyses nonparametric tests were used (Kruskal–Wallis with Dunn’s post hoc test for multiple comparisons. Mann–Whitney’s t-test for comparison of two groups and Spearman \( r (r_S) \) for correlations). Analysis of covariance (ANCOVA) was applied to control for possible confounding factors.

**Results**

**Association of apoD with CAA pathology**

We analysed the expression pattern of apoD and association with Aβ pathogenesis in human occipital lobe tissue of CAA type-1 \((n = 9)\), AD without CAA pathology \((n = 11)\), and control cases \((n = 11)\). Staining for Aβ confirmed the presence of Aβ plaque pathology in the AD cases, and CAA type-1 pathology in the CAA cases, whereas control cases were devoid of Aβ pathology (Figure 1A, a–d). ApoD immunoreactivity was overall present as a granular staining, which was similar in AD cases and controls (Figure 1A, e–f). In CAA cases, granular apoD staining was also observed surrounding capillaries with CAA pathology that extended into the parenchyma (dysoxic CAA), and additionally, apoD staining could be observed in glial end feet (Figure 1A, h). In AD cases, we observed little or no apoD immunoreactivity in association with plaques. In contrast, in CAA cases we observed strong apoD staining in approximately one third of the arterioles that were positive for Aβ pathology (Figure 1A, g). The apoD staining in these arterioles could be observed both in the inner, endothelial cell layer and the outer layer of the vessel wall (Figure 1A, g). The association of apoD with CAA-positive vessels, but not with plaques, was further confirmed using a triple fluorescence staining.
in which, in addition to apoD staining, thioflavin and anti-Aβ were used to visualize Aβ pathology (Figure 1B). Finally, quantitative analysis of apoD in brain tissue of these cases was performed using immunoblotting (Figure 1C), but no significant differences in total apoD levels were found between controls, AD and CAA cases.
Correlation of CSF apoD levels with age and sex

We observed a positive correlation between age and CSF apoD levels in control subjects in the age range of 16 to 85 years ($r_{SP} = 0.48$, $P < 0.0001$, $n = 102$). This correlation was stronger in men ($r_{SP} = 0.60$, $P < 0.0001$) than in women ($r_{SP} = 0.42$, $P = 0.0020$) (Figure 2). In subjects above 50 years of age, no significant correlation of CSF apoD levels with age was observed ($r_{SP} = 0.18$, $P = 0.12$, $n = 74$). Higher apoD levels were observed in men older than 50 years (mean $9.0 \pm 3.0 \mu g/ml$) as compared with women of comparable age (mean $6.8 \pm 2.3 \mu g/ml$; $P = 0.0007$) or younger men (mean $5.2 \pm 1.6 \mu g/ml$; $P < 0.0001$).

Correlation between apoD levels and blood–CSF barrier integrity

CSF apoD levels correlated with total protein levels in controls in the age range of 16 to 85 years ($r_{SP} = 0.59$, $P < 0.0001$, $n = 102$) and in patients with CAA and AD and age- and sex-matched controls ($r_{SP} = 0.60$, $P < 0.0001$, $n = 125$; Figure 3A). To investigate whether apoD protein in the CSF may be (partly) derived from blood, we quantified apoD levels in paired CSF and serum samples. Mean CSF apoD levels were $7.8 \pm 4.9 \mu g/ml$, and mean serum apoD levels were $128 \pm 31 \mu g/ml$ in these samples. CSF and serum apoD levels did not correlate ($r_{SP} = 0.19$, $P = 0.23$), but a strong correlation between the apoD CSF/serum ratio (QapoD) and Qalb was observed ($r_{SP} = 0.61$, $P < 0.0001$, $n = 41$; Figure 3B).
The potential of CSF apoD as biomarker for CAA

CSF apoD levels were similar in controls (mean 7.9 ± 2.9 µg/ml), CAA patients (mean 7.4 ± 3.0 µg/ml) and AD patients (mean 9.4 ± 5.4 µg/ml; P = 0.14), also after exclusion of one AD patient with extremely high CSF apoD levels (33.5 µg/ml; identified as statistically significant outlier (P < 0.01)). CSF apoD levels corrected for total protein (apoD/TP) tended to be different between controls, CAA and AD patients, but this did not reach statistical significance (P = 0.10; Figure 4). However, when only CSF samples from RUMC patients were included, apoD/TP levels were significantly decreased in CSF of CAA patients (mean 7.1 ± 2.6 µg/mg, n = 16; P = 0.0068) as compared with both controls (mean 9.4 ± 2.9 µg/mg, n = 67) and AD patients (mean 9.3 ± 2.2 µg/mg, n = 27), indicating that the centre of origin is a possible confounding factor. For most AD patients, we had APOE genotype data available (Table 1), and we additionally tested whether carrying one or two ε4 alleles was related to CSF apoD levels. No differences were found in CSF apoD or apoD/TP levels between AD patients with different APOE genotypes, or between AD non-ε4 and ε4 carriers (data not shown). APOE status was therefore not considered to be a confounding factor. Finally, we analysed CSF apoD levels in all controls, AD and CAA patients, taking along multiple possible confounding factors (age, sex, TP and centre of origin) by ANCOVA. To obtain normally distributed data for this analysis, the significant outlier in the AD group had to be excluded and data were log transformed. ANCOVA analysis confirmed significant different apoD levels between groups (P = 0.0008).

Discussion

The modified Boston criteria, that are currently applied for diagnosis of CAA, are not optimal. Importantly, these criteria are not directly related to deposition of Aβ in the cerebrovasculature and are not suitable for early diagnosis of CAA. Findings of decreased CSF Aβ40 levels in sporadic and familial CAA patients, even in the presymptomatic phase of the disease [5–7], support the potential of CSF biomarkers for early diagnosis of CAA and suggest that proteins that are specifically associated with CAA pathology may be good biomarker candidates for this purpose. On the basis of this, we hypothesized that apoD may be such a candidate biomarker that may aid in the diagnosis of CAA.

Using IHC and immunofluorescence techniques, we found that apoD is specifically associated with CAA pathology in human post mortem brain tissue. This association was particularly observed in arterioles affected by CAA. The presence of apoD in CAA-affected vessels has been suggested previously, but in contrast to our results, in these studies strong immunoreactivity of apoD was also found in parenchymal amyloid deposits in AD brain [14,15]. This inconsistency with previous studies may be explained by differences in the methodology used to stain apoD in brain tissue. To strengthen our findings, we also tested an apoD antibody (clone 8CD6) similar to the antibody used in a previous study on our 5 µm sections, and included a formic acid pretreatment protocol as described in this previous study [15]. Again, we observed that apoD was absent in plaques, whereas it was observed in association with CAA pathology, although the staining was less consistent using this antibody (data not shown). These findings indicate that apoD is expressed in vessels affected by
CAA, and that its expression in plaques is lower, but cannot be fully excluded. Our IHC data suggested that apoD staining is present both in the endothelial cell layer and in the glia limitans. These findings may point to a problem in perivascular drainage of proteins similar to Aβ. Little is, however, known about the mechanism of apoD clearance. It has been reported that Basigin (BSG/CD147) is a potential apoD receptor, involved in apoD internalization [16], but this has not been studied in relation to Aβ drainage. It is currently also not known why apoD would preferably accumulate in vascular rather than parenchymal Aβ deposits. Several reports indicate that the composition of vascular and parenchymal deposits differ, both concerning Aβ peptide variants as well as associated non-Aβ proteins [13,17–19], which may influence the association of apoD with these deposits. Despite the association of apoD with CAA pathology, we observed no difference in total apoD levels in brain tissue of CAA cases as compared with AD and control cases. This is in line with findings in a previous study [13], and may be explained by similar, abundant granular apoD expression throughout the tissue of all cases, and the fact that we did not exclusively study cerebral vascular isolations for this goal, but whole-brain tissue instead.

We used our immunohistochemical observations as a basis to study apoD as potential CSF biomarker for CAA. In line with the specific association of apoD with CAA pathology, we could show that apoD levels in CSF are specifically decreased in patients with CAA as compared with both controls and AD patients. Previously, it has been shown that CSF apoD levels are associated with cortical brain atrophy in cognitively healthy elderly, via a mechanism independent of Aβ. It has been reported that Basigin (BSG/CD147) is a potential apoD receptor, involved in apoD internalization [16], but this has not been studied in relation to Aβ drainage. It is currently also not known why apoD would preferably accumulate in vascular rather than parenchymal Aβ deposits. Several reports indicate that the composition of vascular and parenchymal deposits differ, both concerning Aβ peptide variants as well as associated non-Aβ proteins [13,17–19], which may influence the association of apoD with these deposits. Despite the association of apoD with CAA pathology, we observed no difference in total apoD levels in brain tissue of CAA cases as compared with AD and control cases. This is in line with findings in a previous study [13], and may be explained by similar, abundant granular apoD expression throughout the tissue of all cases, and the fact that we did not exclusively study cerebral vascular isolations for this goal, but whole-brain tissue instead.

We used our immunohistochemical observations as a basis to study apoD as potential CSF biomarker for CAA. In line with the specific association of apoD with CAA pathology, we could show that apoD levels in CSF are specifically decreased in patients with CAA as compared with both controls and AD patients. Previously, it has been shown that CSF apoD levels are associated with cortical brain atrophy in cognitively healthy elderly, via a mechanism independent of Aβ [20], which would suggest a more general role of apoD as marker of neurodegeneration. This is also supported by increased CSF apoD levels in AD [21,22]. However, in contrast, in other studies decreased CSF apoD levels were found in AD and other neurodegenerative diseases [23–25]. Furthermore, in a recent study it was shown that apoD levels may not simply be related to neurodegeneration in general, since unlike AD brains, apoD levels were not increased in brains of frontotemporal dementia patients [26]. Furthermore, in contrast to previous studies, we observed unchanged CSF apoD levels in AD patients as compared with controls.

These conflicting results on CSF apoD levels in various studies may be related to the dependency of CSF apoD on several (confounding) factors, as investigated in our study. First, we observed a correlation of CSF apoD levels with age. A relation of apoD with age has been a consistent finding in studies on brain tissue (reviewed in [8]), but in contrast, in most CSF studies such a correlation could not be established [21,22,27]. In our study we included subjects with a wide age range, which may explain the different findings as compared with these other CSF studies. Indeed, in our control cohort with patients older than 50 years, we did not observe a significant correlation of CSF apoD levels with age, suggesting that age may not be a main factor affecting apoD levels in patient groups with diseases that present at older age, like sporadic CAA and AD. Second, we observed higher CSF apoD levels in men than in women. These findings are in agreement with some [22], but not with other studies [21,27,28]. Differences in apoD levels based on sex are not unexpected, since the promoter of the APOD gene does contain three oestrogen-responsive elements [29], which may indicate sex-dependent regulation of apoD levels. Third, we found a strong correlation between CSF apoD and total protein levels, the latter are a proxy for the integrity of the blood–CSF barrier. This was supported by a strong correlation between the QapoD and Qalb. ApoD levels in blood are, based on our measures, on average 10 times higher than in CSF, indicating that a substantial amount of apoD in the CSF may be derived from the blood. However, since CSF apoD levels did not correlate with serum apoD levels, it is likely that CSF apoD is also partly derived from an intrathecal source. Calculation of the apoD index (i.e. the ratio of QapoD/Qalb) could not be performed at this time given the lack of sufficient data and materials, but this would be useful in future studies to correct for transport of apoD across the blood–CSF barrier as a result of decreased barrier integrity and may further define the biomarker potential of apoD for CAA. There is evidence supporting that the blood-CSF and blood–brain barriers may be disrupted in CAA patients [30]. Finally, we observed that mean apoD levels were higher in CSF from CAA patients collected in Boston vs. those collected in Nijmegen, which suggests that some, currently unknown, preanalytical factors may affect CSF apoD levels. Therefore, standardization of preanalytical protocols is likely to be important before apoD can be introduced as CAA biomarker.

In conclusion, normalized apoD levels in the CSF are decreased in CAA patients and the quantification of
apoD in CSF may thus have potential as a biomarker for CAA, but only when carefully controlling for confounding factors. In addition, development of standardized preanalytical protocols may be required. The strength of our study is that we studied apoD association with CAA pathology in parallel with its biomarker potential for CAA in CSF and extensively studied the effect of several potential confounding factors on CSF apoD levels. A limitation of our study is that the prevalence of CAA pathology in our AD patient cohort is not known. The co-existence of CAA in AD patients may affect CSF apoD levels.

Acknowledgements

HBK and MMV designed the study. DCH, IK and AAMV performed experiments. HBK, DCH, AJMR and MMV analysed and/or interpreted data. AJMR, SMG, FHBMS and CJMK contributed to the acquisition of patient data and samples. HBK drafted the manuscript. All authors critically revised the manuscript.

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Conflict of Interest

The authors have no conflicts of interest to disclose.

References

1. Viswanathan A, Greenberg SM. Cerebral amyloid angiopathy in the elderly. *Ann Neurol* 2011; 70: 871–80
2. Keuge HA, Carare RO, Friedland RP, Ince PG, Love S, Nicoll JA, et al. Population studies of sporadic cerebral amyloid angiopathy and dementia: a systematic review. *BMC Neurol* 2009; 9: 3
3. Attens J, Jellinger K, Thal DR, Van Nostrand W. Review: sporadic cerebral amyloid angiopathy. *Neuropathol Appl Neurobiol* 2011; 37: 75–93
4. Linn J, Halpin A, Damaerel P, Ruhl J, Giese AD, Dihgans M, et al. Prevalence of superficial siderosis in patients with cerebral amyloid angiopathy. *Neurology* 2010; 74: 1346–50
5. Verbeek MM, Kremer BP, Rikkert MO, Van Domburg PH, Skehan ME, Greenberg SM. Cerebrospinal fluid amyloid beta(40) is decreased in cerebral amyloid angiopathy. *Ann Neurol* 2009; 66: 245–9
6. Renard D, Castelnovo G, Wacongne A, Le Floch A, Thouvenot E, Mas J, et al. Interest of CSF biomarker analysis in possible cerebral amyloid angiopathy cases defined by the modified Boston criteria. *J Neurol* 2012; 259: 2429–33
7. van Etten ES, Verbeek MM, van der Grond J, Zielman R, van Roojen S, van Zet E, et al. beta-Amyloid in CSF: biomarker for preclinical cerebral amyloid angiopathy. *Neurology* 2017; 88: 169–76
8. Dassati S, Waldner A, Schweigreiter R, Apolipoprotein D takes center stage in the stress response of the aging and degenerative brain. *Neurobiol Aging* 2014; 35: 1632–42
9. Elliott DA, Weickert CS, Garner B. Apolipoproteins in the brain: implications for neurological and psychiatric disorders. *Clin Lipidol* 2010; 51: 555–73
10. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer’s disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer’s Disease. *Neurology* 1984; 34: 939–44
11. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR Jr, Kawas CH, et al. The diagnosis of dementia due to Alzheimer’s disease: recommendations from the National Institute on Aging-Alzheimer’s Association workgroups on diagnostic guidelines for Alzheimer’s disease. *Alzheimers Dement* 2011; 7: 263–9
12. Montine TJ, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Dickson DW, et al. National Institute on Aging-Alzheimer’s Association guidelines for the neuropathologic
assessment of Alzheimer’s disease: a practical approach. Acta Neuropathol 2012; 123: 1–11
13 Hondius DC, Bingenhuis KN, Morrema THJ, van der Schors RC, van Nierop P, Bugiani M, et al. Proteomics analysis identifies new markers associated with capillary cerebral amyloid angiopathy in Alzheimer’s disease. Acta Neuropathol Commun 2018; 6: 46
14 Navarro A, Del Valle E, Astudillo A, Gonzalez del Rey C, Tolvia J. Immunohistochemical study of distribution of apolipoproteins E and D in human cerebral beta amyloid deposits. Exp Neurol 2003; 184: 697–704
15 Desai PP, Ikonomovic MD, Abrahamson EE, Hamilton RL, Isanski BA, Hope CE, et al. Apolipoprotein D is a component of compact but not diffuse amyloid-beta plaques in Alzheimer’s disease temporal cortex. Neurobiol Dis 2005; 20: 574–82
16 Najyb O, Brissette L, Rassart E. Apolipoprotein D internalization Is a Basigin-dependent mechanism. J Biol Chem 2015; 290: 16077–87
17 Antonios G, Saiepour N, Bouter Y, Richard BC, Paetau A, Verkkoniemi-Ahola A, et al. N-truncated Abeta starting with position four: early intraneuronal accumulation and rescue of toxicity using NT4X-167, a novel monoclonal antibody. Acta Neuropathol Commun 2013; 1: 56
18 Reinert J, Martens H, Huettenrauch M, Kolbow T, Lannfelt L, Ingelsson M, et al. Abeta38 in the brains of patients with sporadic and familial Alzheimer’s disease and transgenic mouse models. J Alzheimers Dis 2014; 39: 871–81
19 Manousopoulou A, Gatherer M, Smith C, Nicoll JAR, Woelk CH, Johnson M, et al. Systems proteomic analysis reveals that clusterin and tissue inhibitor of metalloproteinases 3 increase in leptomeningeal arteries affected by cerebral amyloid angiopathy. Neurophathol Appl Neurobiol 2017; 43: 492–504
20 Mattsson N, Insel P, Nosheny R, Trojanowski JQ, Shaw LM, Jack CR Jr, et al. Effects of cerebrospinal fluid proteins on brain atrophy rates in cognitively healthy older adults. Neurobiol Aging 2014; 35: 614–22
21 Terrisse L, Poitier J, Bertrand P, Merched A, Visvikis S, Siest G, et al. Increased levels of apolipoprotein D in cerebrospinal fluid and hippocampus of Alzheimer’s patients. J Neurochem 1998; 71: 1643–50
22 Craig-Schapiro R, Kuhn M, Xiong C, Pickering EH, Liu J, et al. Multiplexed immunoassay panel identifies novel CSF biomarkers for Alzheimer’s disease diagnosis and prognosis. PLoS One 2011; 6: e18850
23 Ohal Z, Kalman J, Toth ME, Zvara A, Santha M, Ivitz E, et al. Proteomic analysis of cerebrospinal fluid in Alzheimer’s disease: wanted dead or alive. J Alzheimers Dis 2015; 44: 1303–12
24 Kroksveen AC, Guldbrandsen A, Vedeler C, Myhr KM, Opsahl JA, Berven FS. Cerebrospinal fluid proteome comparison between multiple sclerosis patients and controls. Acta Neurol Scand Suppl 2012; 126: 90–6
25 Kroksveen AC, Aasebo E, Vethe H, Van Pesch V, Franciotta D, Teunissen CE, et al. Discovery and initial verification of differentially abundant proteins between multiple sclerosis patients and controls using iTRAQ and SID-SRM. J Proteomics 2013; 78: 312–25
26 Bhatia S, Kim WS, Shepherd CE, Halliday GM. Apolipoprotein D Upregulation in Alzheimer’s Disease but Not Frontotemporal Dementia. J Mol Neurosci 2019; 67: 125–32
27 Reindl M, Knipping G, Wicher I, Dillitz E, Egg R, Deisenhammer F, et al. Increased intrathecal production of apolipoprotein D in multiple sclerosis. J Neuroimmunol 2001; 119: 327–32
28 Ogata Y, Charlesworth MC, Higgins L, Keegan BM, Vernino S, Muddiman DC. Differential protein expression in male and female human lumbar cerebrospinal fluid using iTRAQ reagents after abundant protein depletion. Proteomics 2007; 7: 3726–34
29 Lambert J, Provost PR, Marcel YL, Rassart E. Structure of the human apolipoprotein D gene promoter region. Biochim Biophys Acta 1993; 1172: 190–2
30 Freeze WM, Jacobs HIL, Schreuder F, van Oostendrugge R, Backes WH, Verhey FR, et al. Blood-brain barrier dysfunction in small vessel disease related intracerebral hemorrhage. Front Neurol 2018; 9: 926

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

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