BRAIN
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CSF proteomics in autosomal dominant Alzheimer's disease highlights parallels with sporadic disease

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Autosomal dominant Alzheimer's disease (ADAD) offers a unique opportunity to study pathophysiological changes in a relatively young population with few comorbidities. A comprehensive investigation of proteome changes occurring in ADAD could provide valuable insights into AD-related biological mechanisms and uncover novel biomarkers and therapeutic targets. Furthermore, ADAD might serve as a model for sporadic AD, but in-depth proteome comparisons are lacking. We aimed to identify dysregulated CSF proteins in ADAD and determine the degree of overlap with sporadic AD. We measured 1472 proteins in CSF of *PSEN1* or *APP* mutation carriers ($n = 22$) and age- and sex-matched controls ($n = 20$) from the Amsterdam Dementia Cohort using proximity extension-based immunoassays (PEA). We compared protein abundance between groups with two-sided *t*-tests and identified enriched biological pathways. Using the same protein panels in paired plasma samples, we investigated correlations between CSF proteins and their plasma counterparts. Finally, we compared our results with recently published PEA data from an international cohort of sporadic AD ($n = 230$) and non-AD dementias ($n = 301$). All statistical analyses were false discovery rate-corrected. We detected 66 differentially abundant CSF proteins (65 increased, 1 decreased) in ADAD compared to controls ($q < 0.05$). The most strongly upregulated proteins (fold change >1.8) were related to immunity (CHIT1, ITGB2, SMOC2), cytoskeletal structure (MAPT, NEFL) and tissue remodelling (TMSB10, MMP-10). Significant CSF-plasma correlations were found for the upregulated proteins SMOC2 and LILR1B. Of the 66 differentially expressed proteins, 36 had been measured previously in the sporadic dementias cohort, 34 of which (94%) were also significantly upregulated in sporadic AD, with a strong correlation between the fold changes of these proteins in both cohorts ($r_s = 0.730$, $P < 0.001$). Twenty-nine of the 36 proteins (81%) were also upregulated among non-AD patients with suspected AD co-pathology. This CSF proteomics study demonstrates substantial biochemical similarities between ADAD and sporadic AD, suggesting involvement of the same biological processes. Besides known AD-related proteins, we identified several relatively novel proteins, such as TMSB10, MMP-10 and SMOC2, which have potential as novel biomarkers. With shared pathophysiological CSF changes, ADAD study findings might be translatable to sporadic AD, which could greatly expedite therapy development.

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Introduction

Autosomal dominant Alzheimer's disease (ADAD) is a rare (<1%) form of Alzheimer's disease (AD) caused by genetic mutations in amyloid precursor protein (*APP*), presenilin-1 (*PSEN1*) or presenilin-2 (*PSEN2*).[1] ADAD has played a crucial role in identifying key pathological mechanisms underlying AD and the time course of AD-related brain changes.[1,2] A thorough investigation of the biochemical changes occurring in ADAD could further improve our understanding of its complex pathophysiology, as well as help to identify novel biomarkers and therapeutic targets.

ADAD and sporadic AD are largely comparable in terms of phenotype, clinical course and neuropathology,[1,3,4] and biochemical and structural measures have similar longitudinal profiles.[3,5] Owing to the relatively young age at symptom onset in ADAD,[6] age-related comorbidities which frequently confound sporadic AD studies are less common.[7] Furthermore, the near complete penetrance of ADAD genetic mutations enables identification of individuals destined to develop AD on a predictable timescale, providing a valuable framework for prevention trials.[1,8] Given their similarities, findings from ADAD studies might translate well to sporadic AD, but in-depth proteome comparisons are lacking.

In recent years, the field of proteomics has seen substantial technological improvements. Novel multiplexing immunoassays, such as proximity extension assays (PEA), enable highly sensitive measurements of thousands of proteins of interest simultaneously in minute sample volumes.[9,10] These platforms have proven to be powerful tools to identify proteome changes and novel biomarkers in various diseases, including sporadic AD.[11-15] In contrast, proteomics studies of ADAD are scarce and mostly small mass spectrometry-based studies focusing on presymptomatic mutation carriers,[16-19] highlighting the need for an updated study of protein changes in ADAD.

While blood is gaining popularity in biomarker research for its convenience in clinical settings, CSF is probably a more suitable medium to investigate the biological mechanisms underlying AD.[20] CSF is in direct contact with the brain, and proteins released from brain cells traffic freely to the CSF. Accordingly, 67% of CSF proteins have been detected in brain tissue.[21] Furthermore, the CSF proteome is relatively unaffected by protein expression in other organ systems, facilitating a more brain-specific protein profile.[20]

In the present study, we investigated the abundance of over 1400 proteins in CSF of ADAD mutation carriers and controls using PEA. We aimed to delineate the biological processes involved in ADAD pathophysiology and uncover novel biomarkers and therapeutic targets. Using paired plasma samples, we determined which dysregulated CSF proteins correlate with their counterparts in blood and warrant further investigation as potential blood biomarkers. Finally, to determine the degree of similarity in ADAD and sporadic AD on the proteome level, we compared our results with recently published PEA data from a large cohort of sporadic AD and non-AD dementias.[22]

Materials and methods

Subjects

Autosomal dominant Alzheimer's disease cohort

CSF samples were available for 22 carriers of a pathogenic mutation in *APP* or *PSEN1*, as well as 20 age- and sex-matched controls from the Amsterdam Dementia Cohort (ADC) biobank.[23] A paired plasma sample, collected on the same day as CSF, was available for 21 mutation carriers. All participants underwent standardized neurological, cognitive and laboratory assessments, regardless of clinical diagnosis as previously described.[23] The presence or absence of an ADAD-related pathogenic mutation was determined using PCR-based amplification of the appropriate exon followed by Sanger sequencing. Mutation carriers were considered presymptomatic or symptomatic [either mild cognitive impairment (MCI) or AD dementia] based on international consensus criteria.[24,25] The control group consisted of individuals with subjective cognitive decline (SCD) in whom cognitive and laboratory investigations were normal. The Mini-Mental State Examination (MMSE) was used as a global measure of cognitive functioning.

Sporadic dementias cohort

The sporadic dementias cohort and corresponding PEA measurements, which were used for comparisons with our ADAD dataset, have been described in detail elsewhere.[22] Here, we briefly

highlight the most important methodological components relevant to the present study.

The sporadic dementias cohort included CSF samples from patients with sporadic AD ($n = 230$), non-AD dementia [either dementia with Lewy bodies (DLB, $n = 110$) or frontotemporal dementia (FTD, $n = 191$)] and controls ($n = 195$). Clinical diagnoses were assigned based on international consensus criteria.[24-28] Controls had SCD with normal cognitive and laboratory investigations. Owing to the frequent occurrence of AD co-pathology among patients with non-AD dementias,[29] this group was further separated into those with a CSF AD biomarker profile (see later), indicative of AD pathology, and those with normal CSF AD biomarkers. A subset of diagnoses was confirmed either by autopsy or, in the case of FTD, by the presence of an FTD-related genetic mutation.[30] The majority of CSF samples were from the ADC; additional samples were collected through the Center for Neurodegenerative Disease Research at the University of Pennsylvania ('UPenn'), Erasmus University Medical Center ('Erasmus') and the Goizueta Alzheimer's disease Research Center at Emory University ('Emory').

Ethical approval

All studies described were approved by local medical ethical committees and all participants or their caregivers provided written informed consent according to the Declaration of Helsinki. Laboratory staff were blinded to the clinical and genetic status of samples.

Sample collection and protein profiling

CSF and plasma samples were collected by lumbar puncture and venipuncture, respectively, and were processed and stored in accordance with international consensus guidelines.[31,32] For each sample, a fresh aliquot was used, i.e. without previous freeze-thaw cycles.

In the ADAD cohort, levels of CSF amyloid-β_{42} (Aβ_{42}), total tau (t-tau) and phosphorylated tau-181 (p-tau) were analysed locally using commercially available kits [enzyme-linked immunosorbent assay (ELISA) (Innotest); Aβ_{42}, hTau-Ag and phospho-tau(181P) (Fujirebio or Elecsys); Aβ_{42}, t-tau and phospho-tau(181P) biomarker assays (Roche Diagnostics GmbH)]. Elecsys assay results were transformed to equivalent Innotest values using conversion formulas, which were previously developed in our centre using Passing-Bablok regression analyses.[33] Innotest Aβ_{42} values were adjusted for drift over time.[34] A positive AD biomarker profile was defined as a t-tau/Aβ_{42} ratio >0.46. In the sporadic dementias cohort, core AD biomarkers were measured locally using the aforementioned kits (for ADC and Erasmus samples) or using Luminex xMAP INNO-BIA AlzBio3 (Luminex Corp.) kits (for UPenn and Emory samples). A positive AD biomarker profile was defined as a t-tau/Aβ_{42} ratio >0.46 (ADC/Erasmus) or >0.30 (UPenn), or as an Aβ_{42}/t-tau ratio <6 (Emory). The use of these ratios is consistent with the previous paper on PEA measurements in the sporadic dementias cohort,[22] and their diagnostic accuracy to discriminate sporadic AD from other dementias and controls has been demonstrated.[35]

Proteomics analyses were performed using validated multiplex panels based on PEA technology (Olink Proteomics Inc.).[9] Briefly, target proteins are bound by unique pairs of oligonucleotide-labelled antibodies. When in close proximity to one another, the oligonucleotides hybridize to form a PCR target sequence. The resulting DNA amplicon is quantified either on an Illumina NovaSeq platform using Next Generation Sequencing (Olink Explore) or on a Fluidigm BioMark™ HD real-time PCR platform (Olink Target). Protein levels are reported on a $\log_2$ scale as normalized protein expression (NPX), which is derived from cycle threshold values and thus directly proportional to the concentration of target protein in the sample.

In the ADAD cohort, 1472 CSF and plasma proteins were measured using four Olink Explore 384-plex panels (Cardiometabolic, Inflammation, Neurology and Oncology; Olink Proteomics). All samples were randomized on one plate. Samples were prepared according to the dilution blocks shown in Supplementary Table 1. Dilution factors were provided by the manufacturer based on validation experiments to estimate the linear range of quantification in different matrices. Quality control samples and negative controls were supplied by the manufacturer. Details on assay characteristics and quality control (QC) procedures are provided on the manufacturer's website.[36] For each protein, the lower limit of detection (LOD) was defined as the sample of blanks plus 3 standard deviations. Proteins were included in further analyses if they were above the LOD in >85% of samples and passed quality control. Extreme outliers, defined as NPX values above or below a z-score of 5, were capped at a z-score of ±5.

Of the 1472 measured proteins, 814 were above the LOD in >85% of CSF samples (Supplementary Table 2). Two proteins (IL6 and CXCL8) were measured in all four Olink panels; NPXs of these replicates were strongly correlated [r_s (range): 0.77–0.98; Supplementary Fig. 1] and one replicate for each protein was selected at random for inclusion in subsequent analyses. Among the remaining 808 unique proteins measured in CSF samples of the 42 ADAD mutation carriers and controls, 21 extreme outlying NPX values (0.06%) were capped. Of the 808 proteins included in CSF analyses, 798 were detected in >85% of the 21 paired plasma samples and were included in correlative analyses between CSF and plasma.

In the sporadic dementias cohort, 979 CSF proteins were measured using the 11 Olink Target 96 panels that were available at the time (Cardiometabolic, Cardiovascular II and III, Cell regulation, Development, Immune response, Inflammation, Metabolism, Neurology, Oncology II and Organ damage; Olink Proteomics). CHI3L1 was excluded from our comparative analyses due to a presumed hook-effect in the version of the assay used in the sporadic dementias cohort.[22]

For validation purposes, we quantified the levels of NEFL, GFAP and THOP1 using ELISA techniques. NEFL and GFAP were measured with single molecule array technology (Simoa) using the N4PE kit [(502542; coefficient of variation of QCs: NEFL 5.3%, GFAP 4.7%). THOP1 was measured in triplicate using a homebrew assay developed on the Ella™ automated immunoassay system 48-Digoxigenin cartridges (lot 25519, coefficient of variation of QCs: 5.2%).[37]

Statistical analyses

All data processing and statistical analyses were performed using R version 4.0.3.

Demographic characteristics were compared between groups using Kruskal–Wallis tests for numerical data and chi-square tests for sex. Normality of NPX values was assessed using Shapiro–Wilk tests. As most (>80%) proteins were normally distributed, we used two-sided t-tests to compare CSF protein expression levels between ADAD mutation carriers and non-carriers. These comparisons were repeated after exclusion of presymptomatic mutation carriers. Fold changes were calculated based on the ratio of NPX values in mutation carriers to controls. Spearman's rank correlation was used for correlative analyses with core AD biomarkers (CSF Aβ_{42}, t-tau,

Table 1 Subject characteristics

	ADAD cohort			Sporadic dementias cohort				
	Mutation carriers[a,b]	Controls	P	Sporadic AD	Non-AD dementia		Controls	P
					CSF+	CSF−		
n	22	20	–	230	93	208	195	–
Age, years	48 (40–58)	49 (45–52)	0.668	66 (59–72)	68 (63–74)	66 (61–71)	57 (52–62)	<0.001
Disease duration if symptomatic, years[c]	2 (1–3)	–	–	3 (2–4)	3 (1–4)	3 (2–4)	–	0.574
Sex, female (%)	11 (50%)	8 (40%)	0.734	95 (41%)	39 (42%)	71 (34%)	79 (41%)	0.377
MMSE[d]	23 (17–25)	29 (28–29)	<0.001	21 (17–24)	23 (19–27)	25 (22–27)	28 (27–29)	<0.001
AD biomarker profile, n +/− (%)[e]	20/2 (91%)	0/20 (0%)	<0.001	226/3 (99%)	93/0 (100%)	0/208 (0%)	1/193 (0.01%)	<0.001
ApoE4, n +/− (%)[f]	6/14 (27%)	3/17 (15%)	0.201	133/88 (60%)	36/38 (49%)	62/120 (34%)	44/125 (26%)	<0.001
Study site, n								
ADC	22	20	–	210	51	151	194	–
UPenn	0	0	–	20	35	45	0	–
Erasmus	0	0	–	0	3	12	1	–
Emory	0	0	–	0	4	0	0	–

Numerical variables are shown as median (interquartile range). *P*-values were derived from chi-square tests and Mann–Whitney U or Kruskal–Wallis tests for categorical and numerical variables, respectively.
[a]*PSEN1* mutations: A79V (*n* = 3), A231V (*n* = 1), G384A (*n* = 1), L232F (*n* = 1), L262F (*n* = 5), Y115C (*n* = 4), G206D (*n* = 1); *APP* mutations: duplication (*n* = 4), H677R (*n* = 1), M722K (*n* = 1).
[b]Clinical diagnoses: AD (*n* = 16), MCI (*n* = 2) or cognitively healthy (*n* = 4). All subjects with MCI and AD initially presented with memory complaints (i.e. amnestic phenotype).
[c]Disease duration, defined as the difference in age at CSF collection and age at symptom onset as estimated by a caregiver, was missing in 119 subjects from the sporadic dementias cohort.
[d]MMSE missing in two subjects from the ADAD cohort and in 39 subjects from the sporadic dementias cohort (six sporadic AD, 25 non-AD dementia, four controls).
[e]A positive AD biomarker profile (CSF+) was defined based on site-specific cut-off values: t-tau/Aβ_{42} ratio >0.46 (ADC and Erasmus); t-tau/Aβ_{42} ratio >0.30 (UPenn); or Aβ_{42}/t-tau ratio >6 (Emory).
[f]At least one ApoE4 allele present.

p-tau), MMSE and proteins measured by ELISA for validation, as well as between CSF and plasma NPX values among mutation carriers. In the sporadic dementias cohort, cases and controls were not age- and sex-matched, and therefore, protein expression levels were analysed using nested linear models with age and sex as covariates.[22] To eliminate the risk of misdiagnoses among non-AD dementias, we performed additional comparative analyses between ADAD and non-AD dementias, limiting non-AD dementias to subjects with pathological or genetic confirmation. All analyses were false discovery rate (FDR)-corrected using the Benjamini–Hochberg method. The statistical significance threshold was set at FDR-corrected *q* < 0.05.

Functional enrichment analysis was performed using Metascape,[38] selecting Gene Ontology Biological Processes and Reactome as the ontology sources. All measured proteins included in the statistical analyses were used as the enrichment background. Default parameters were used for the analysis, in which terms with a *P*-value <0.01, a minimum count of three and an enrichment factor >1.5 (i.e. the ratio between observed counts and counts expected by chance) were collected and grouped into clusters based on their membership similarities. The most statistically significant term from each cluster was chosen to represent the cluster. In addition, we assessed the possible cellular origin of the differentially expressed CSF proteins by a cell type specificity assessment with the EWCE (expression weighted cell type enrichment) R package.[39] Here, the input was the cell type expression of the DroNc-seq snRNAseq data set.[40] Finally, we conducted pathway enrichment analyses with the KEGG database, inputting the differential expression and *P*-values of all measured proteins. We tailored the R package KEGGREST for the analyses, with the measured proteins as the background.[41,42] A threshold was set at a minimum count of three proteins measured and *P* < 0.05. The enrichment is presented by the median beta estimate of up- or down-regulation for the pathway.

Results

Subject characteristics are shown in Table 1. ADAD mutation carriers and controls did not differ significantly in terms of age or sex. Among the 22 mutation carriers, 18 were symptomatic and four were presymptomatic. All symptomatic mutation carriers as well as two presymptomatic carriers had a positive AD biomarker profile. Mutation types included various *PSEN1* mutations in 16 mutation carriers from 15 pedigrees, and *APP* mutations in six mutation carriers from five pedigrees (Table 1). All symptomatic mutation carriers presented with an amnestic phenotype. A detailed overview of neuropsychological test results is provided in Supplementary Table 3.

In the sporadic dementias cohort, controls were significantly younger than subjects with sporadic AD and non-AD dementia, but no differences in sex were observed. Of 230 sporadic AD subjects, 226 had a positive AD biomarker profile, two had a negative biomarker profile and biomarker data were missing in two subjects. Six sporadic AD subjects were autopsy confirmed, including the two with a negative AD biomarker profile and one with missing biomarkers. The non-AD dementias group consisted of 93 subjects with a positive AD biomarker profile (53 FTD, 40 DLB), and 208 with a negative AD biomarker profile (138 FTD, 70 DLB). Thirty-one non-AD subjects (26 FTD, 5 DLB) in the biomarker-positive group (33%), as well as 64 (56 FTD, 8 DLB) in the biomarker-negative non-AD group (31%), were confirmed by autopsy or genetics. Twenty-two DLB diagnoses were supported by FPCIT single-photon emission computed tomography (DAT-SPECT).[43]

CSF proteomics analyses

Differential CSF protein abundance in ADAD

Results for all proteins included in the analyses of ADAD mutation carriers versus controls are shown in Supplementary Table 2.

In total, 65 CSF proteins were significantly upregulated in ADAD mutation carriers compared to controls, and one protein, IL17A, was significantly downregulated ($q < 0.05$; Fig. 1A and Supplementary Table 4A). The largest fold change was seen for CHIT1 (fold change = 4.5), followed by NEFL, MAPT, TMSB10 and MMP-10 (fold change > 2). The most significantly dysregulated proteins ($q < 0.001$) included those involved in cytoskeletal structure (MAPT, NEFL), tissue remodelling (TMSB10), immunity (CHIT1, ITGAM, MIF, ITGB2), synapse function (CC2D1A, PPP3R1), vesicle transport (SDC4, NSFL1C) and energy metabolism (GLO1).

After exclusion of presymptomatic mutation carriers ($n = 4$), we identified 89 significantly upregulated proteins (Supplementary Fig. 2 and Supplementary Table 4B), including 25 proteins which were significant in the original analyses. The top 20 most significant proteins remained unchanged except for one (LACT2B replacing DTX3).

In support of the validity and replicability of our findings, we found a strong correlation between CSF proteomics and ELISA techniques for measurement of NEFL ($r_s = 0.97$, $P < 0.001$), GFAP ($r_s = 0.79$, $P < 0.001$) and THOP1 ($r_s = 0.86$, $P < 0.001$) (Supplementary Fig. 3).

Biological pathway analysis

In the functional enrichment analysis using Metascape, the most significantly enriched terms among ADAD mutation carriers included several terms related to cellular metabolism, such as small molecule catabolic processes, hydrogen peroxide metabolic process and carbohydrate metabolic processes (Fig. 1B). The terms associated with the largest number of proteins in our list included signalling by interleukins (13 proteins), regulation of neuron death (12 proteins) and small molecule catabolic process (nine proteins). The origin of the proteins based on cell type expression data was diverse, with clear clusters of predominant microglia ($n = 6$ proteins), excitatory neuron ($n = 15$ proteins), endothelial ($n = 4$ proteins) and astrocyte ($n = 2$ proteins) expressed proteins, as well as several protein clusters less clearly related to one cell type (Fig. 1C). In terms of KEGG pathway enrichment, significant enrichment of the systems neurology and immune response was found. On the deeper layer, the most significant enriched pathways were selenocompound metabolism, longevity regulation and peroxisome (Fig. 1D).

Correlation with core Alzheimer's disease biomarkers and Mini-Mental State Examination

Moderate to strong positive correlations were found for all but one of the 66 differentially expressed proteins with CSF p-tau [median (range) $r_s = 0.68$ (0.41–0.93)] and t-tau [$r_s = 0.72$ (0.41–0.97)] (Fig. 2). In addition, 33 of these proteins correlated negatively with CSF Aβ$_{42}$ [$r_s = -0.45$ (-0.64; -0.35)]. One protein, IL17A, was negatively correlated with p-tau and t-tau and not significantly correlated with Aβ$_{42}$.

Significant negative correlations with MMSE in mutation carriers were found for CC2D1A ($r_s = -0.504$, $P = 0.020$), PARK7 ($r_s = -0.446$, $P = 0.043$) and MAPT ($r_s = -0.445$, $P = 0.043$), but these were no longer significant after correction for multiple testing (Supplementary Table 5).

CSF-plasma correlations

Across all 798 proteins analysed in both CSF and plasma samples of 21 mutation carriers, a significant positive correlation was found for 11 proteins (Supplementary Table 6A). Of the 66 significantly dysregulated CSF proteins, 63 were also measured in plasma; among these, SMOC2 ($r_s = 0.522$, $P = 0.016$) and LILRB1 ($r_s = 0.514$, $P = 0.018$) correlated with their plasma counterparts, although these correlations were no longer significant after FDR-correction (Fig. 3 and Supplementary Table 6B).

Comparison with sporadic dementias

Overall, 560 proteins measured in the ADAD cohort had previously been measured in the sporadic dementias cohort, including 36 of the 66 significantly dysregulated proteins in ADAD (Supplementary Table 4A).

Among these 36 proteins, 34 (94%) were also significantly upregulated in sporadic AD (Fig. 4A). A strong correlation was found between the fold changes of these proteins in both cohorts ($r_s = 0.730$, $P < 0.001$) (Fig. 4B). Of these 36 proteins, 29 (81%) were also upregulated in non-AD dementias with suspected AD co-pathology. In contrast, in the comparison with non-AD dementias with a negative AD biomarker profile (i.e. without suspected AD co-pathology), just 4 of the 36 proteins (MMP-10, CHIT1, CCL3, PRDX1; 11%) were dysregulated (Fig. 4C). Finally, ASAH2 and IL1R1 were upregulated in ADAD but not in any of the sporadic dementia groups.

Among pathologically or genetically confirmed non-AD dementias with a positive AD biomarker profile, 13 of the 36 proteins were found to overlap with ADAD (Supplementary Fig. 4).

Discussion

We performed a systematic and detailed investigation of CSF proteome changes in ADAD mutation carriers using state-of-the-art PEA technology. Our results demonstrate large and significant changes in the CSF proteome in ADAD, with involvement of inflammatory, cytoskeletal and metabolic proteins, as well as a very large overlap in proteome changes with sporadic AD.

The remarkable similarities in CSF protein dysregulation between ADAD and sporadic AD provide convincing evidence of shared pathways in both diseases and confirm that mechanistic insights gained from ADAD CSF studies may apply to sporadic AD as well. These findings warrant replication in future proteomics studies that directly compare ADAD and sporadic AD protein profiles (i.e. generated in the same study), ideally also in paired brain tissue and blood samples.[19] The ability to identify preclinical ADAD mutation carriers, who are certain to develop the disease, uniquely offers the opportunity to study very early pathological brain changes and test drugs aimed at secondary prevention.[1,5] Our results support a framework in which early-stage biomarkers and potential therapies are identified in ADAD and replicated in sporadic AD, which could greatly expedite the journey towards effective treatment. The power of such an approach is demonstrated by the oft-cited example of statins, which were initially trialled in familial hypercholesterolaemia and are now widely used to treat the much more common sporadic form.[44]

In order to extrapolate ADAD findings to sporadic AD, some differences between both diseases should be considered. First, a fundamental difference exists in the aetiology of amyloid pathology. While mutations in *APP*, *PSEN1* and *PSEN2* directly alter APP metabolism to produce more aggregation-prone Aβ species or more total Aβ, Aβ aggregation in sporadic AD probably results from a chronic imbalance in Aβ production and clearance following a complex interaction of (epi)genetic and environmental factors.[45,46] Nonetheless, our results suggest common disease mechanisms downstream of Aβ pathology, and potential efficacy of drugs
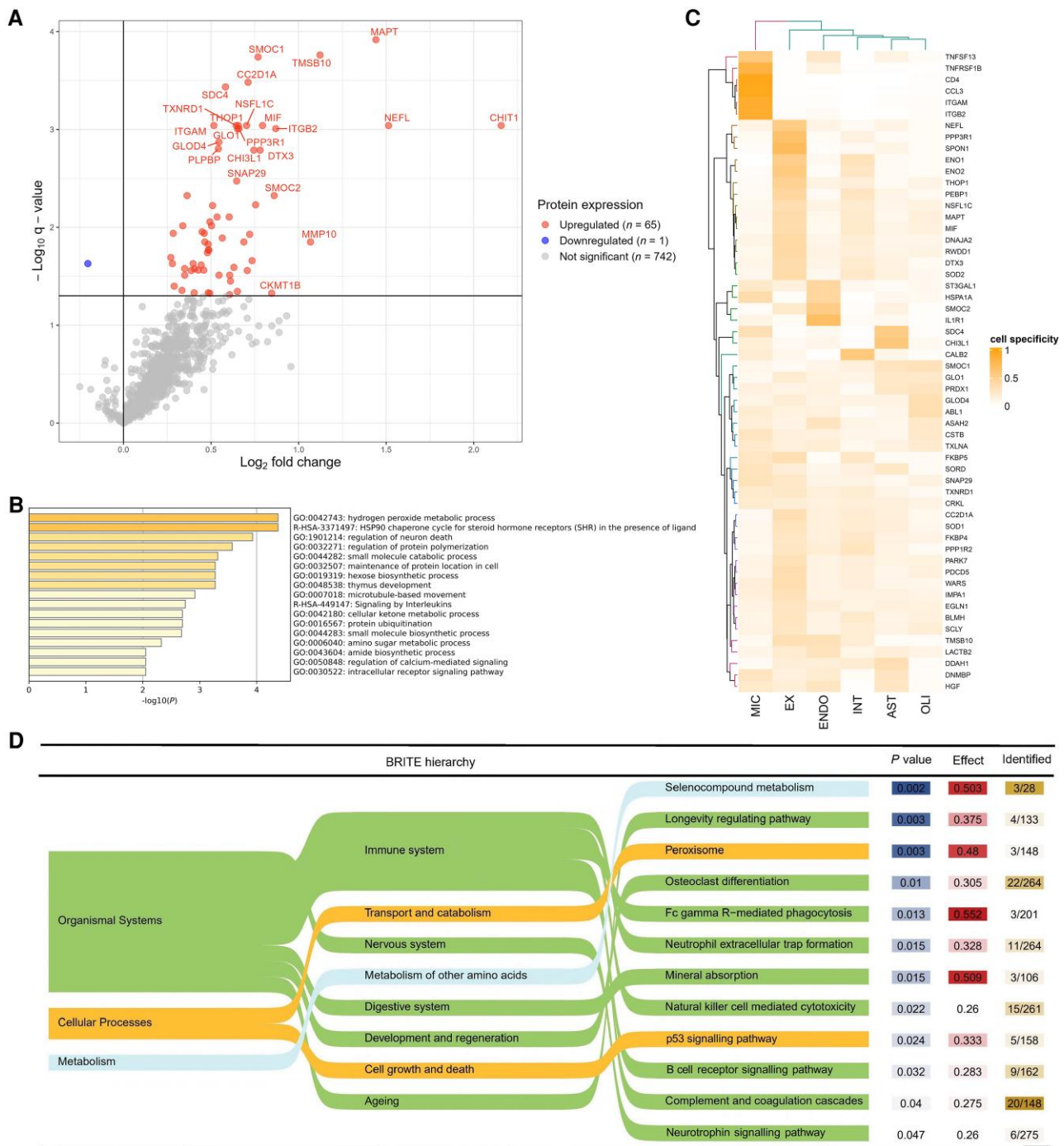
Figure 1 Differential protein abundance and associated biological pathways in ADAD mutation carriers. (A) Differential protein abundance in ADAD mutation carriers versus controls. The $\log_2$ fold change, which is equivalent to the difference in $\log_2$-transformed normalized protein expression (NPX) levels, is plotted against the $-\log_{10}$-transformed q-value. The horizontal line indicates the statistical significance threshold, set at $q < 0.05$. Significantly dysregulated proteins are shown in red (upregulated) or blue (downregulated). The top 20 proteins with the smallest q-value and the top 10 proteins with the largest fold change are labelled. **(B)** Enriched biological pathways among ADAD mutation carriers. Functional enrichment was performed using Metascape,[35] selecting Gene Ontology Biological Processes and Reactome as ontology sources. All analysed proteins were included as the enrichment background ($n = 808$). Terms with a P-value <0.01, a minimum count of three and an enrichment factor >1.5 (i.e. the ratio between observed counts and counts expected by chance) were collected and grouped into clusters based on their membership similarities. P-values were calculated based on the accumulative hypergeometric distribution. Kappa scores are used as the similarity metric when performing hierarchical clustering on the enriched terms, and subtrees with a similarity of >0.3 are considered a cluster. The most statistically significant term is chosen to represent the cluster. Stronger terms indicate more significant enrichment. **(C)** Cell-type specificity of dysregulation proteins. In this heat map, the dysregulated CSF proteins are clustered based on the proportion of cell-type expression. The columns list the cell types: microglia (MIC), excitatory neuron (EX), endothelial cells (ENDO), interneurons (INT), astrocytes (AST) or oligodendrocytes (OLI). **(D)** Enriched KEGG pathways according the BRITE hierarchy, with increasing granularity from *left* to *right*. For the analyses, the measured proteins from the background. A threshold was set at a minimum count of three proteins measured and $P < 0.05$. The enrichment is presented by the median beta estimate of up- or downregulation for the pathway, the P-value and the number of proteins detected with the current proteomics analyses relative to all proteins in the pathway.
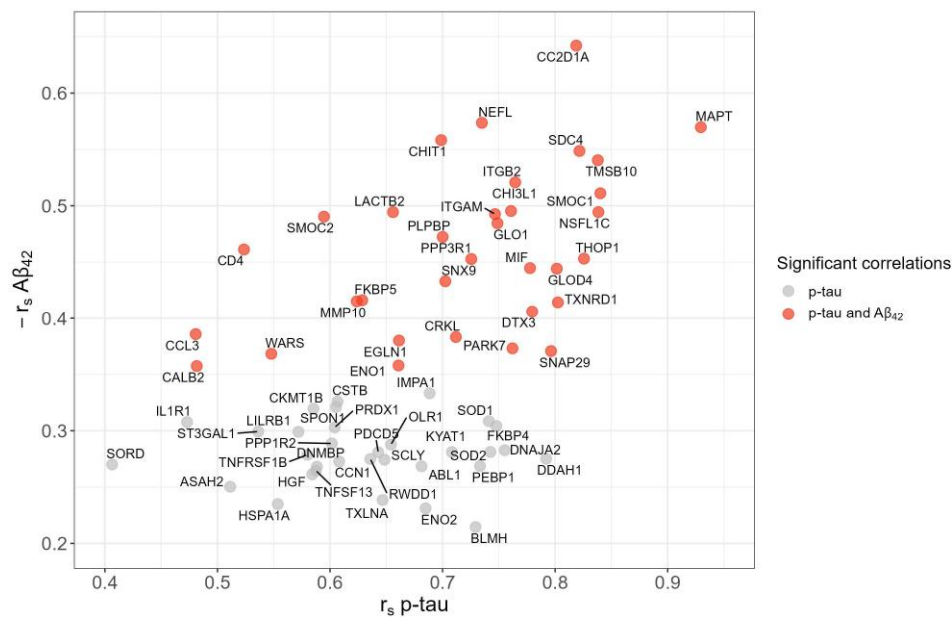
Figure 2 Correlations between differentially abundant CSF proteins and core CSF AD biomarkers. The correlation coefficient with $A\beta_{42}$ and p-tau was determined for each protein using Spearman's rho and considered significant at $q < 0.05$. All proteins shown ($n = 65$) correlated significantly with p-tau; those shown in red also correlated with $A\beta_{42}$. For visualization purposes, correlation coefficients with $A\beta_{42}$ are inverted (i.e. $-r_s$ $A\beta_{42}$). One protein, IL-17A, was negatively correlated with p-tau ($r_s = -0.38$; $q = 0.015$) and positively correlated with $A\beta_{42}$ ($r_s = 0.11$; $q = 0.48$) and is not shown here.

targeting these processes may be independent of the initial underlying aetiology. Second, symptom onset in ADAD is on average 30 years earlier than in sporadic AD,[6,47] and the effects of ageing-related comorbidities as well as the more frequent occurrence of multiple neuropathologies in sporadic AD[7,48,49] must be taken into account. Third, given the more rapid rate of decline in ADAD reported in some studies,[50] treatment effects in sporadic AD might be more subtle than expected based on ADAD studies. Fourth, mutation-specific differences in molecular biomarkers,[51,52] pathology[53] and clinical features[54,55] suggest divergent neurotoxic effects depending on the nature and position of the genetic mutation.[56,57] As such, certain ADAD variants might align less well with sporadic AD. The mutations included in the present study were diverse, precluding identification of mutation-specific proteome changes. A better understanding of the molecular consequences of each mutation might enable biochemically-driven mutation groupings and reveal which genetic subtypes match best with sporadic AD.

Many of the dysregulated proteins identified here have previously been linked to AD, providing strong support for our approach and findings. These include extensively studied proteins, such as NEFL, CHIT-1, MAPT and CHI3L1,[58-60] as well as proteins which have emerged more recently in the dementia field, such as SMOC2, MIF and MMP-10.[13,22,61-66] CSF proteins not previously linked to AD, such as GLOD4 and DTX3, require replication in independent CSF data, as well as brain tissue studies, to further examine their relevance. Here, we discuss a selection of proteins based on their relevance to and novelty in AD research.

SMOC1 and SMOC2 were among the most strongly upregulated proteins in ADAD mutation carriers (respective fold changes = 1.7 and 1.8). Although their function in the brain is unclear, the closely related SPARC protein is highly expressed in glia and implicated in microglial response to damaging stimuli.[67-69] Robust elevations in CSF SMOC1[15,16,19,21,70-72] and SMOC2[22,63] have been reported in AD across various proteomics platforms, and SMOC1 accurately

discriminates AD from controls (area under the receiver operating characteristic curve = 0.84).[70] We and others found strong correlations with CSF $A\beta_{42}$,[15] and post-mortem studies show co-localization of SMOC1 and/or SMOC2 with $A\beta$ plaques,[73,74] suggesting that these proteins may be directly related to amyloid pathology. Accordingly, CSF SMOC1 was not elevated in non-AD dementias in our study nor in other cohorts of patients with non-AD neurodegenerative diseases.[15] Further studies are required to determine the differential diagnostic potential of SMOC1 and SMOC2. The correlations between CSF and plasma SMOC2, as well as elevated levels in AD plasma reported elsewhere[15] are promising, and replication is warranted to explore its potential as a peripheral biomarker.

CSF MMP-10 was strongly upregulated in our ADAD dataset (fold change = 2.1), replicating previous findings in MCI and sporadic AD.[13,63-66] MMP-10 belongs to a family of matrix metalloproteinases (MMPs) which degrade extracellular matrix proteins. In the brain, MMPs are overexpressed by astrocytes and microglia in response to $A\beta$ accumulation and are capable of degrading $A\beta$.[75,76] In line with the MMP-10 increases in our non-AD dementias groups, elevated levels of MMPs have been reported in other neurodegenerative diseases[13,22,77,78] and therefore might reflect a non-specific inflammatory response in an effort to clear toxic protein aggregation. While its non-specific nature precludes its use as a differential diagnostic biomarker, MMP-10 was recently shown to have additional prognostic value besides core AD biomarkers to predict conversion from MCI to AD;[79] this prognostic potential is supported by the strong correlations with p-tau in our cohort ($r_s = 0.84$, $P < 0.001$). The lack of correlation with MMSE in our study, which was similarly absent for the other differentially expressed proteins, might be due to limited statistical power in the relatively small sample of 18 symptomatic mutation carriers and requires replication in larger cohorts. Taken together, our results strongly support further investigation into MMP-10 as an inflammatory biomarker in neurodegenerative diseases.
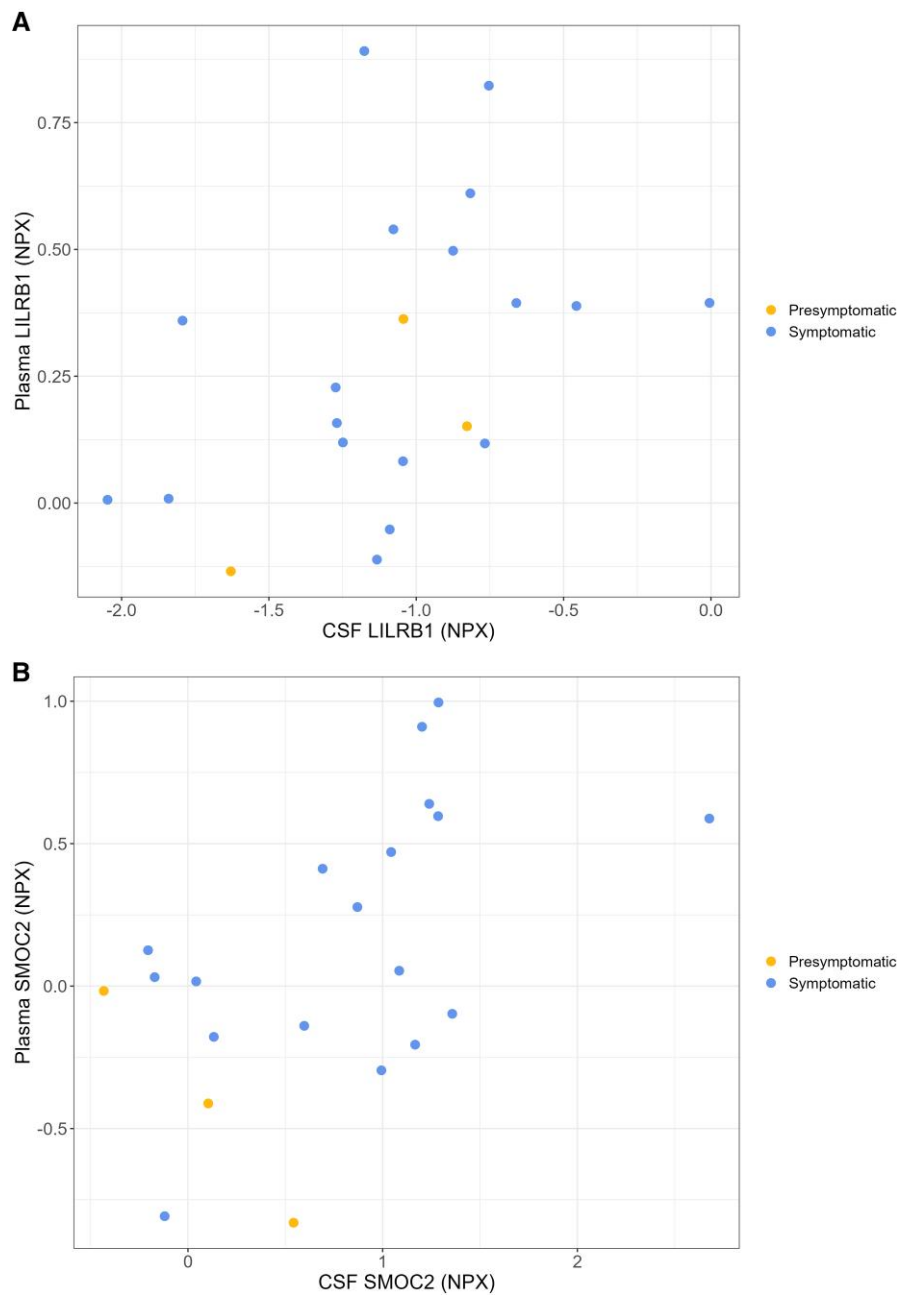
Figure 3 Correlations between CSF and plasma (A) LILRB1 and (B) SMOC2 among ADAD mutation carriers. CSF and plasma protein abundance are expressed on a $\log_2$ scale as normalized protein expression (NPX). Correlation coefficients and *P*-values are derived from Spearman's rho. Blue and yellow symbols represent symptomatic ($n = 18$) and presymptomatic mutation carriers ($n = 4$), respectively.

We also observed a large fold change for TMBS10 (fold change = 2.2) a member of the thymosin beta family, which is implicated in cell proliferation, angiogenesis and tissue repair.[80] CSF TMSB10 has only recently come to light in relation to dementia, with strong increases reported in sporadic AD.[22] Its more extensively studied paralog, TMSB4, is elevated in CSF in AD and Creutzfeldt-Jakob disease[81] and thought to be neuroprotective in various neurological diseases.[80] Interestingly, TMSB4 overexpression in mouse models with *APP/PSEN1* mutations reduced Aβ accumulation and attenuated AD phenotypes.[82] The elevated TMSB10 levels found in our study might reflect an attempt to mitigate CNS damage secondary to AD. If confirmed, thymosin upregulation might be considered as

a potential therapeutic strategy for dementia, as has been suggested for stroke and multiple sclerosis.[80]

Of note, four (MMP-10, CCL3, PRDX1, CHIT1) of the ADAD-associated proteins were upregulated in non-AD dementias in the absence of AD co-pathology. These probably reflect non-specific inflammation-related changes, corroborating elevated levels of these proteins found in other neurodegenerative diseases.[13,22,60,77,78,83-85] However, we caution against over-interpreting lack of statistically significant results in the remaining ADAD-associated proteins in these subjects, which might be due to a lack of statistical power in a heterogeneous population of FTD and DLB patients, and emphasize that we cannot draw conclusions
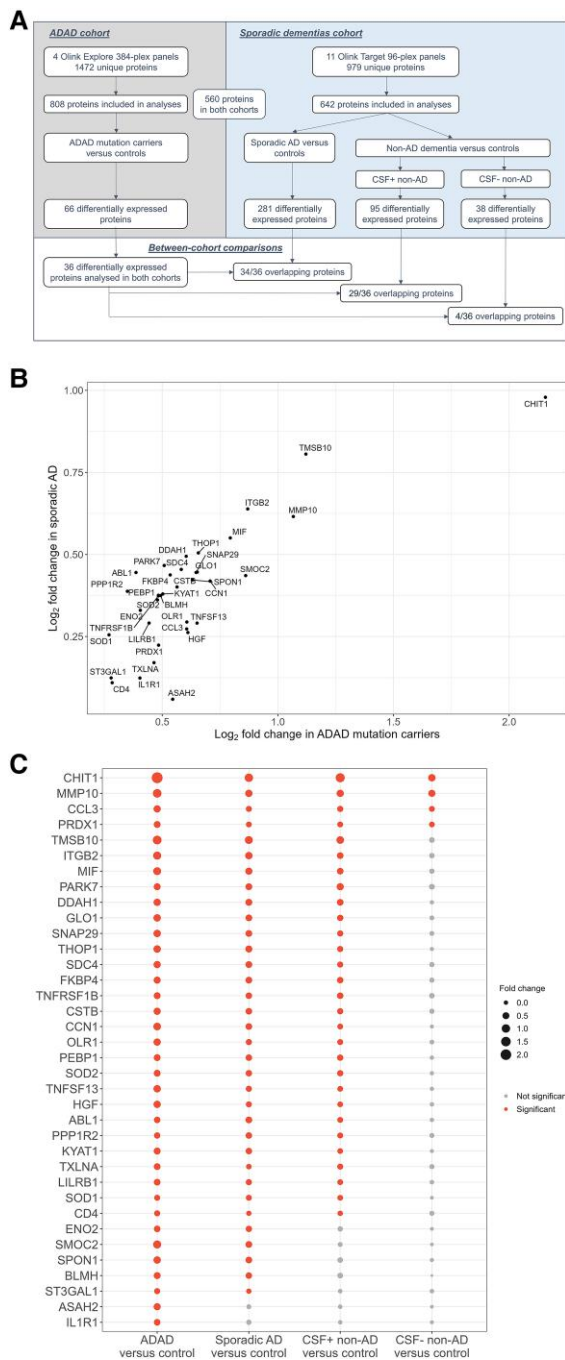
Figure 4 Comparisons of differentially abundant CSF proteins in ADAD with sporadic dementias. (**A**) Flow chart showing design of between-cohort comparisons. In total, 560 proteins were measured in both cohorts and included in the comparisons, including 36 of the 66 differentially regulated proteins among ADAD mutation carriers. CSF+ non-AD and CSF− non-AD indicate non-AD dementia patients with a positive or negative AD biomarker profile, respectively. (**B**) Scatter plot showing $\log_2$-fold changes in ADAD mutation carriers and sporadic AD patients versus controls for the proteins ($n = 34$) which were significantly upregulated in both cohorts. (**C**) Bubble chart displaying overlap of differentially abundant proteins in the various comparisons. Red circles indicate significantly dysregulated proteins; the circle size is proportional to the fold change. Of the 36 proteins upregulated in ADAD and included in the comparison study, 34 were also upregulated in sporadic AD compared to controls; 29 proteins were upregulated in both sporadic AD and CSF+ non-AD dementia, whereas just four were also upregulated among CSF− non-AD dementia. Two proteins were uniquely dysregulated in ADAD. ADAD = autosomal dominant Alzheimer's disease.

regarding the AD-specificity of proteins. In the same vein, the proteins which were upregulated in ADAD but not in sporadic AD and non-AD dementias need not necessarily be ADAD-specific: ASAH2 is implicated in lipid metabolism by its cleavage of ceramides, the metabolism of which is disrupted in (sporadic) AD.[86,87] IL1R1, a member of the interleukin-1 family, plays a central role in the pro-inflammatory response seen in sporadic AD and various other diseases.[88,89]

We identified a larger number of differentially expressed proteins after exclusion of presymptomatic mutation carriers, suggesting that certain proteins change around after disease onset or fluctuate depending on the disease stage.[17] A better understanding of the biochemical changes occurring in presymptomatic stages will be essential for identification of early biomarkers and therapeutic targets. Future studies that include enough presymptomatic carriers to analyse protein dynamics over the course of ADAD, for example by relating protein changes with estimated years to onset, will be highly insightful.

The protein dysregulation profiles identified here are broadly in line with recent proteomics studies of sporadic AD, both those using Olink technology[11,63] and other strategies such as (targeted) mass spectrometry.[90-92] We examined the biological significance of our findings using two pathway enrichment analyses, which revealed functional enrichment of terms related to immunity, energy metabolism, ageing and cell death. Our cell type expression analyses confirm a prominent role for microglia in AD protein dysregulation. The clear clusters of proteins expressed by endothelial cells is in line with previously reported dysregulation of proteins implicated in angiogenesis and blood–brain barrier function.[63,93] Together, these findings provide further evidence of the similarities between ADAD and sporadic AD on the protein level.

Strengths of this study include the well-characterized cohort of ADAD mutation carriers and age-matched controls, as well as the very extensive protein panels which cover a wide range of biological processes. The large number of dysregulated proteins identified in this study, which remained significant after multiple testing correction, demonstrates the large-scale CSF proteome disruptions occurring in ADAD, and highlights the potential of CSF proteomics to study disease pathophysiology *in vivo*, even in rare diseases with modest sample sizes. The comparison of ADAD and sporadic AD on the CSF proteome level is novel and represents an important step in furthering our understanding of the similarities and differences between the two. The PEA platform employed here guarantees excellent sensitivity by using PCR-based protein quantification; at the same time, cross-reactive binding is minimal due to its reliance on dual-recognition immunoassays, which require binding of two antibodies and hybridization of oligonucleotides to produce a signal.[9,15,94] The fact that we were able to replicate numerous previous findings underlines its reproducibility, which remains a major issue in the field of proteomics.[95,96] Finally, the vast majority of samples from both cohorts was collected in the ADC as part of the same biobanking study, and the other centres adhered to consensus protocols for CSF biobanking, facilitating homogeneity in pre-analytical protocols.

This study must be viewed in light of some limitations. First, certain proteins of interest, such as neurogranin and SNAP-25, were not included in this targeted proteomics approach. However, given the very large number of proteins included, there is still ample room to identify novel protein dysregulations. Second, the large number of statistical tests inherent to proteomics studies necessitates multiple testing correction. While we believe the FDR correction applied in this discovery study, with a 5%

threshold of false-positive results, offers an appropriate balance between the risks of false-negative versus false-positive results, the need for independent validation studies is evident. Third, our comparisons between ADAD and sporadic dementias might have been affected by the inevitable age differences between cohorts. Furthermore, the protein panels and assays used in both cohorts were not identical, and minor assay changes might have led to improved detectability of certain proteins in the ADAD cohort, which used more recently developed panels. A potential risk of overfitting exists given the use of the 560 proteins measured in both cohorts, which we mitigated by expressing the overlap in protein expression between both cohorts as a fraction of the 36 dysregulated proteins in ADAD. Future replication studies would ideally measure the same protein panels in both cohorts. Finally, we must acknowledge the risk of misdiagnoses among FTD and DLB cases, especially in those with an AD biomarker profile. However, diagnoses were made in specialized memory clinics, and almost one-third of FTD and DLB cases was either pathologically or genetically confirmed. Moreover, the analysis including only these confirmed cases revealed similar protein dysregulations, albeit with a smaller number of significant proteins (possibly due to a lack of statistical power).

In conclusion, the marked similarities in CSF protein profiles between ADAD and sporadic AD provide convincing evidence of dysregulation of the same biological processes. Although the two cannot be directly equated, these exciting results indicate that findings from ADAD studies might be extrapolated to sporadic AD, which could greatly benefit therapeutics development. In this light, further exploration of plasma protein dysregulation among ADAD mutation carriers may help identify valuable peripheral biomarkers of AD.

Data availability

The data from the sporadic dementias cohort are available at https://www.synapse.org/PRIDE_AD. At the time of writing, submission of the data from the ADAD cohort to https://www.synapse.org has been initiated and is awaiting approval.

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Competing interests

Supplementary material

Supplementary material is available at *Brain* online.

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