RESEARCH PAPER

CSF neurogranin as a neuronal damage marker in CJD: a comparative study with AD

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ABSTRACT

Objective To investigate whether cerebrospinal fluid (CSF) neurogranin concentrations are altered in sporadic Creutzfeldt-Jakob disease (CJD), comparatively with Alzheimer’s disease (AD), and associated with neuronal degeneration in brain tissue.

Methods CSF neurogranin, total tau, neurofilament light (NFL) and 14-3-3 protein were measured in neurological controls (NCs, n=64), AD (n=46) and CJD (n=81). The accuracy of neurogranin discriminating the three diagnostic groups was evaluated. Correlations between neurogranin and neurodegeneration biomarkers, demographic, genetic and clinical data were assessed. Additionally, neurogranin expression in postmortem brain tissue was studied.

Results Compared with NC, CSF neurogranin concentrations were increased in CJD (4.75 times of NC; p<0.001, area under curve (AUC), 0.96 (95% CI 0.93 to 0.99)) and AD (1.94 times of NC; p<0.01, AUC 0.73, 95% CI 0.62 to 0.82), and were able to differentiate CJD from AD (p<0.001, AUC 0.85, 95% CI 0.78 to 0.92). CSF tau was increased in CJD (41 times of NC) and in AD (4 times of NC) and AD (41 times of NC) both at p<0.01. In CJD, neurogranin positively correlated with tau (r=0.55, p<0.001) and was higher in 14-3-3-positive (p<0.05), but showed no association with NFL (r=0.08, p=0.46). CJD-MM1/MV1 cases displayed higher neurogranin levels than VV2 cases. Neurogranin was increased at early CJD disease stages and was a good prognostic marker of survival time in CJD. In brain tissue, neurogranin was detected in the cytoplasm, membrane and postsynaptic density fractions of neurons, with reduced levels in AD, and more significantly in CJD, where they correlated with synaptic and axonal markers.

Conclusions Neurogranin is a new biomarker of prion pathogenesis with diagnostic and prognostic abilities, which reflects the degree of neuronal damage in brain tissue in a CJD subtype manner.

INTRODUCTION

Neurogranin is a calmodulin-binding protein abundantly expressed in the soma and dendrites of neurons of the telencephalon involving in synaptic plasticity and long-term potentiation. Neurogranin has been suggested to be a specific cerebrospinal fluid (CSF) Alzheimer’s disease (AD) biomarker, since its concentration is increased in AD, but not in other neurodegenerative diseases (ie, frontotemporal dementia, Lewy body dementia, Parkinson’s disease, progressive supranuclear palsy, multiple system atrophy and Huntington’s disease). Although CSF neurogranin presents only moderate diagnostic value for AD, this can be improved when combined with other CSF biomarkers of AD such as tau and neurofilament light (NFL). In AD, CSF neurogranin displays strong positive correlation with other AD biomarkers such as tau and phospho tau, while weak or no correlations were detected with amyloid-beta42, a biomarker of amyloid plaques load.

A prognostic value for neurogranin in AD has been proposed, as its CSF concentration is differentially elevated in mild cognitive impairment (MCI) patients with biomarker AD signature as well as in MCI patients who progress to AD dementia compared with those who remain cognitively stable. Similarly, CSF neurogranin correlates with rate of cognitive decline in MCI and with reduction of brain volume in AD. In cognitively normal individuals, CSF neurogranin is also useful in predicting future cognitive impairment. Regrettably, neurogranin analysis in paired plasma-CSF samples indicated that the AD-specific increased CSF levels are not reproduced in plasma, discarding the potential use of blood neurogranin measurements for diagnostic or prognostic purposes.

Although extensive work has been done in AD, data are lacking regarding neurogranin levels in other diseases presenting substantial synaptic and neuronal loss. This is the case of prion diseases, one of whose fundamental characteristics is synaptic degeneration and disorganisation, which leads to neuronal loss and spongiform changes. Indeed, over a 30% reduction in the relative synaptic index has been reported in prion disease-affected brains compared with controls. Similarly to AD, synaptic loss occurs at early stages of prion diseases, and it is suggested that synaptic pathology is initiated at the synaptic spine. Experiments conducted in prion disease mouse models revealed that axon terminal degeneration and synaptic loss precede neuronal death and are associated with the onset of clinical symptomatology.

Sporadic Creutzfeldt-Jakob disease (CJD) is the most prevalent human prion disease characterised by rapidly progressive dementia and short disease duration. The combination of genotype at codon 129 (methionine or valine) and pathogenic prion protein (PrPSc type 1 or 2 based on the size of protease resistant PrP fragments) gives rise to different CJD subtypes with characteristic disease phenotype and neuropathological
features. Thus, synaptic and neuronal damage, neuroinflammation, deposition of PrPSc and lesion profile occur in a well-defined regional-specific and subtype-specific manner. The most prevalent subtypes are CJDMM1/CJDMM1 (60%–70% of the cases) with predominant cortical affection and, CJD VV2 (16% of the cases), with prominent cerebellar affection. Several pathological mechanisms are suggested to contribute to CJD synaptic pathology, including the accumulation of the abnormal form of prion protein in synaptic structures.

In the present study, we quantified CSF neurogranin in CJD and AD cases in order to comparatively unveil its diagnostic and prognostic potential. We also characterised the presence of neurogranin in CJD and AD brains to investigate the underlying pathological conditions in the central nervous system that may lead to the observed disease-specific CSF signatures.

METHODS
Antibodies
The monoclonal neurogranin antibody Ng2 was produced using KLH-conjugated peptide Ng52–75 as immunogen, as described previously and was used (1:400) for immunohistochemistry (IHC). The neurogranin antibody Ng36 was generated using the same protocol, but with KLH-conjugated peptide Ng63–75 as immunogen and was used for western blot (1:6000). Antibodies against sodium-potassium adenosine triphosphatase (ATPaseNa/Kβ, Affinity-MA3-930;1:2000), glyceraldehyde3-phosphate dehydrogenase (GAPDH, Abcam ab9485;1:2500), postsynaptic density protein 95 (PSD-95, Thermo-Fisher-MA3-930;1:1000), synaptophysin (SYNP, Novocasera-NCL-L-SYNAP-299;1:40000), total tau (Sigma-T5530;1:500) and beta-actin (β-actin, Sigma-A5316;1:30000) were used in the western blot experiments.

Patients and CSF sampling
Neurological controls (NC) were composed of patients diagnosed with a neuropsychiatric disease non-associated with a primarily neurodegenerative disease, and were diagnosed according to acknowledged standard neurological clinical and paraclinical findings based on the 10th revision of the International Statistical Classification of Diseases definitions. NCs include the following diagnoses: alcohol abuse, astrocytoma, bipolar disorder, cerebral lymphoma, cerebral vasculitis, depression, epilepsy, Graves’ disease, acute or chronic headache, acute hypoxia, ischaemic stroke, menigitis, multiple infarct, pain syndromes, paraneoplasia, paranoid psychosis, peripheral polyneuropathy, psychosis, schizophrenia, vascular encephalopathy, vasculitis and vertigo. AD was diagnosed according to the National Institute on Aging-Alzheimer’s Association workgroups criteria. CJD was diagnosed according to consensus criteria. 60 definite and 21 probable CJD cases were included. All CSF samples were collected at the Clinical Dementia Center and the National Reference Center for CJD Surveillance in the Department of Neurology of the University Medical Center of Göttingen, Germany.

Lumbar punctures (LPs) were performed for diagnostic purposes at the first evaluation. For disease stage, samples were stratified in three categories according to whether CSF was collected in the first (early) (time of LP to disease onset/total duration of the disease <0.33), second (middle) (0.33–0.66) or third (last) (>0.66) stage of the disease. Disease duration was recorded as the time (in months) from symptom onset to the death of the patient.

Brain samples
Brain tissue was obtained from the Institute of Neuropathology HUB-ICO-IDIBELL-Biobank following the guidelines of Spanish legislation on this matter (Real Decreto de Biobancos 1716/2011). Control cases had not suffered from neurologic or psychiatric diseases, infections of the nervous system, brain neoplasms or systemic and central immune diseases, and did not have abnormalities in the neuropathological examination. Neurofibrillary tangles stages were categorised according to Braak et al modified for paraffin sections. CJD cases underwent neuropathological diagnosis according to established neuropathological criteria. Information about brain cases used in this study is detailed in online supplementary table 1. CSF was not available for study in any of the postmortem brain series.

CSF analyses
Neurogranin and NFL were quantified using two in-house ELISA as described before. Tau was quantified using the ELISA kit INNOTESThTAU-Ag (Fujirebio Europe, Ghent, Belgium). CSF was analysed for the presence of 14-3-3 protein by Western blot according to established CJD diagnostic protocol. The analysts were blinded to clinical data.

Immunohistochemistry
Dewaxed sections, 4 μm thick, were processed for IHC and incubated at 4°C overnight with one of the primary antibodies and then incubated with R.T.U. Biotinylated Universal Antibody (Vector,BP1400) for 30 min at room temperature followed by R.T.U. HRP-Streptavidin (Vector, SA-5704). The peroxidase reaction was visualised with diaminobenzidine and hydrogen peroxide.

Brain homogenates, subcellular fractionation and western blot
The purification of PSD fractions from human postmortem brain tissue was performed as published before. Brain homogenates and fractions were mixed with Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) sample buffer, boiled and subjected to 8%/15% SDS-PAGE. Gels were transferred onto nitrocellulose membranes and probed for specific immunodetection with chemiluminescence (ECL-Amersham) using the indicated antibodies. Densitometries were carried out with the ImageJ software and for brain homogenates values were normalised using β-actin or GAPDH levels. Since neurogranin was expressed in all subcellular fractions, difference among NC, AD and CJD cases was determined in the input. Brain homogenates were mixed with NuPAGE (Thermo-Fisher) LDS buffer and reducing agent, boiled and subjected to electrophoresis in NuPAGE Bis-Tris 4%/12% gels (Thermo-Fisher). Proteins were transferred to polyvinylidene difluoride membranes and immunodetection was performed as mention above. Densitometries were determined with the ImageJ software and were normalised using β-actin levels.

Statistical tests
According to distributional features, Mann-Whitney U tests or unpaired t-tests were used to compare two groups of samples; Kruskal-Wallis test followed by Dunn’s post hoc tests or analysis of variance test followed by Tukey’s post hoc tests was applied for multiple comparisons. To assess the diagnostic accuracy of neurogranin in the discrimination of the diagnostic groups, receiver operating characteristic (ROC) curve analyses were performed.
with AD (p<0.001) (figure 1A) in agreement with previous studies.34 35

**p<0.01, ***p<0.001. aD, alzheimer’s disease; aUc, area under curve; csF, cerebrospinal fluid; cJD, creutzfeldt-Jakob disease; NC, neurological control; NFL, neurofilament light (NFL) (mean±SD) are indicated. (B) Neurogranin concentrations in NC, AD and CJD. Neurogranin was significantly different in NC versus AD (p<0.01), NC versus CJD (p<0.001) and AD versus CJD (p<0.001) comparisons. Statistical significance derived from a multicomparison analysis for tau, NFL and neurogranin among the diagnostic groups is indicated. Kruskal-Wallis test followed by Dunn’s post-test (correction for multiple testing) was applied. (C) Diagnostic accuracy of CSF neurogranin in the discrimination of NC, AD and CJD groups. AUC with SE and 95% CI derived from receiver operating characteristic curves for the comparisons between pairs of diagnostic groups is shown. **p<0.01, ***p<0.001. AD, Alzheimer’s disease; AUC, area under curve; CSF, cerebrospinal fluid; CJD, Creutzfeldt-Jakob disease; NC, neurological control; NFL, neurofilament light.

**Figure 1** Analysis of CSF neurogranin levels in the differential diagnosis of AD and CJD. (A) Demographic and biomarker characteristics of the CSF cases used in the present study. Number of cases, sex (F: female, M: male), age, semiquantitative analysis of 14-3-3 protein (POS: positive, neg: negative) and quantitative analysis of neurogranin, total tau and neurofilament light (NFL) (mean±SD) are indicated. (B) Neurogranin concentrations in NC, AD and CJD. Neurogranin was significantly different in NC versus AD (p<0.01), NC versus CJD (p<0.001) and AD versus CJD (p<0.001) comparisons. Statistical significance derived from a multicomparison analysis for tau, NFL and neurogranin among the diagnostic groups is indicated. Kruskal-Wallis test followed by Dunn’s post-test (correction for multiple testing) was applied. (C) Diagnostic accuracy of CSF neurogranin in the discrimination of NC, AD and CJD groups. AUC with SE and 95% CI derived from receiver operating characteristic curves for the comparisons between pairs of diagnostic groups is shown. **p<0.01, ***p<0.001. AD, Alzheimer’s disease; AUC, area under curve; CSF, cerebrospinal fluid; CJD, Creutzfeldt-Jakob disease; NC, neurological control; NFL, neurofilament light.

**RESULTS**

**CSF neurogranin in AD and CJD**

The study population included NC (n=64), AD (n=46) and CJD (n=81) cases. CSF NFL showed a mild increase in AD (1.3 times of NC; p<0.05) and a marked increase in CJD (4.3 times of NC; p<0.001). CSF tau showed a moderate increase in AD (3.1 times of NC; p<0.001) while levels in CJD were very markedly (41 times) higher than in NC (p<0.001). Additionally, increased tau and NFL concentrations were detected in CJD compared
Neurogranin concentrations did not correlate with sex distribution in CJD cases. Spearman’s rank correlation and unpaired t-test analysis were used, respectively. (C) Neurogranin concentrations in CJD stratified by prion protein gene (PRNP) codon 129 polymorphism (M=Methionine, V=Valine, MM, n=38, MV: n=13, VV: n=14). Kruskal-Wallis test followed by Dunn’s post-test (correction for multiple testing) was applied (**p<0.05 for MM vs VV and MV vs VV comparisons). (D) Neurogranin concentrations in scJD MM1/MV1 (n=15) and VV2 (n=9) subtypes. Unpaired t-test analysis was applied (**p<0.01 for MM1/VV1 vs VV2 comparison). scJD, sporadic Creutzfeldt-Jakob disease. (n=8) cases, representing the two most prevalent CJD subtypes, were studied. Due to their low number, other subtypes were not included in the analysis. Neurogranin concentrations were significantly higher in CJD MM1/MV1 (718±306 pg/mL) compared with CJD VV2 (373±160 pg/mL) (p<0.01) (figure 2D).

Correlations between neurogranin, surrogate prion biomarkers and clinical data

In CJD, CSF neurogranin showed a good correlation with tau (r=0.55, p=0.001), but did not correlate with NFL (r=0.08, p=0.46) (figure 3A). Additionally, tau and NFL displayed a positive but weak correlation (r=0.26, p=0.01), in agreement with previous reports. 34 CJD cases displaying positive 14-3-3 protein were detected in the western blot test (p<0.05) (figure 3B). No statistical differences between disease stages were detected. Kruskal-Wallis test followed by Dunn’s post-test (correction for multiple testing) was applied. (E) Association between neurogranin concentrations and disease duration (months) in CJD patients using a fractional polynomial approach based on a linear regression model. Disease duration can be modelled as a function of neurogranin values based on the formula: neurogranin (in g/mL)=533+1/(47*[survival time in months-1.6])−28*[survival time in months-0.6]. cJD, Creutzfeldt-Jakob disease; NFL, neurofilament light.

To study a potential association between neurogranin levels at the time of LP and the timeliness of the disease in CJD patients, samples were stratified in early, middle and late stages. Neurogranin concentrations were not significantly different between early (n=9, 510±292 pg/mL), middle (n=26, 576±294 pg/mL) or late (n=28, 635±319 pg/mL) disease stages (figure 3C).

Next, we assessed the potential role of neurogranin as a biochemical marker of disease survival in 63 CJD cases where disease duration was available, and compared it with the performance of tau and NFL. When allowing for non-linear associations between biomarker levels and disease duration, neurogranin was able to explain more of the variability in disease duration (R2=0.19) than tau (R2=0.10) and NFL (R2=0.07). All three biomarkers showed a log-linear decrease with increasing disease duration (figure 3E for neurogranin). For neurogranin, the association with survival time can be modelled using a linear combination of the terms: neurogranin (in g/mL)=533+1/(47*[survival time in months-1.6])−28*[survival time in months-0.6]; it showed a good ability as a prognostic marker, represented by Somers’ D value of 0.32; Harrell’s C value of 0.66 and a Brier score at 12 months of 0.09. For tau and NFL, similar values were achieved (tau: Somers’ D=0.27, Brier score=0.11; NFL: Somers’ D=0.16, Brier score=0.09). In AD, total disease duration was available in 32 cases, in which neurogranin values were also associated with disease (as well via a log-linear decline, R2=0.32).

Neurogranin expression in brain tissue

In human brain tissue of control cases, neurogranin was highly expressed in the neuronal soma of the cerebral cortex (n=13) and hippocampus (n=6), but absent in the white matter (n=13) and cerebellum (n=8) (figure 4A). To further study neurogranin subcellular levels, different brain fractions from control cases (n=4) were purified. Neurogranin was detected in the cytoplasmic (41%±5%), membrane (32%±4%) and PSD (27%±2%) fractions. As control proteins for each fraction, we
used PSD-95, ATPase Na/Kβ (plasma membrane) and synaptophysin (presynaptic) for membrane fraction and GAPDH (cytoplasm) (figure 4B).

Neuronal neurogranin levels were analysed in the cerebral cortex (control, n=10, AD, n=10, CJD, n=9) and hippocampus (control, n=6, AD, n=7, CJD, n=5) (figure 5A). A multiple-comparative test analysis of neurogranin expression from immunohistochemical analysis revealed a significant decrease in CJD (p<0.001) and AD (p<0.001) compared with controls in both brain regions (figure 5B). Additionally, neurogranin immunostaining in CJD was significantly lower than in AD in both brain regions (p<0.01 in cerebral cortex and p<0.05 in hippocampus). No statistical differences were detected in neurogranin levels between Braak stages IV (n=3), V (n=4) and VI (n=3), indicating that alterations in neurogranin expression were not an end-stage feature on AD pathology (figure 5A).

Reduction of neurogranin levels in the frontal cortex of CJD MM1 (n=10) and VV2 (n=10) cases compared with controls (n=8) was validated by western blot analysis and accompanied by decreased levels of PSD-95, presynaptic (synaptophysin) and axonal (tau) markers (figure 6A and B). Compared with controls, and similar to PSD-95, synaptophysin and tau, decreased neurogranin levels were more severe in CJD MM1 (p<0.001) than VV2 cases (p<0.05) (figure 6B). Neurogranin in CJD (n=20) correlated significantly with tau and PSD-95 (p<0.001) and with synaptophysin (p=0.01). All four proteins presented close correlations with each other (figure 6C).

Neurogranin levels by means of western blot analysis in the frontal cortex region of AD cases (n=18) were also reduced significantly compared with controls (n=23, p<0.01). Moderate decreases in synaptic proteins PSD-95 (p<0.01) and synaptophysin (p<0.01) were detected, while tau levels were not altered (figure 7A,B). Neurogranin in AD (n=18) significantly correlated with synaptophysin (p<0.001) and PSD-95 (p<0.05) but not with tau (p>0.05). An additional correlation was detected between PSD-95 and synaptophysin (p=0.01) (figure 7C). No significant associations between age, sex, postmortem time delay and neurogranin levels measured by western blot were found in controls, CJD and AD cases.

**DISCUSSION**

In this study, we demonstrated that CSF neurogranin is increased in CJD compared with NC (4.75-fold change) and AD (2.5-fold change), reaching good diagnostic accuracies in the discrimination of CJD from AD (AUC 0.85, 95% CI 0.78 to 0.92). The increased CSF neurogranin concentrations detected in CJD compared with AD is in line with the lower neurogranin levels detected in the cerebral cortex and hippocampus of CJD cases, and with the well-known higher neuronal damage present in CJD compared with AD.
Neurodegeneration

Figure 6  Neurogranin expression in CJD and association with synaptic and axonal markers. (A) Western blot analysis of PSD-95, tau, synaptophysin, neurogranin and β-actin in the frontal cortex of control, sCJD MM1 and sCJD VV2 cases. A representative image (4 controls, 5 CJD MM1 and 5 CJD VV2) is shown. (B) Quantification of the western blot analysis from the complete cohort of cases analysed, which included: controls; n=8, CJD MM1; n=10 and CJD VV2; n=10. ANOVA test followed by Tukey’s post hoc was applied. PSD-95, tau, synaptophysin and neurogranin levels were reduced in CJD cases compared with controls. (C) Correlation analysis of neurogranin with tau, synaptophysin and PSD-95 in CJD cases (n=20) (left panel) and correlation values (r, 95% CI and p value) for each comparison between pair of proteins (right panel). *p<0.05, **p<0.01, ***p<0.001. ANOVA, analysis of variance; CJD, Creutzfeldt-Jakob disease; PSD, postsynaptic density; AU, Arbitrary Units.

Figure 7  Neurogranin levels in AD and association with synaptic and axonal markers. Western blot analysis of PSD-95, tau, synaptophysin, neurogranin and β-actin in the frontal cortex of control and AD cases. A representative image (4 controls and 4 AD) is shown. (B) Quantification of the western blot analysis from the complete cohort of cases analysed (controls; n=23, AD; n=18). ANOVA test followed by Tukey’s post hoc was applied. PSD-95, synaptophysin and neurogranin expression were reduced in AD cases compared with controls. (C) Correlation analysis of neurogranin with tau, synaptophysin and PSD-95 in AD cases (n=18) (left panel) and correlation values (r, 95% CI and p value) for each comparison between pair of proteins (right panel). *p<0.05, **p<0.01. ANOVA, analysis of variance; AD, Alzheimer’s disease; PSD, postsynaptic density.

In CJD, CSF neurogranin concentrations at early disease stages were not different from those detected at middle and late stages, indicating that synaptic damage is an early event in CJD, similar to what previously has been found for AD.6 Indeed, the observation that neurogranin levels in AD brain tissue were not different between early and late Braak stages further supporting that synaptic loss, as measured by neurogranin, is not a late-stage pathological event. In this regard, it is well known that synaptic damage is an early event in AD.38

In our study population, CSF neurogranin correlated neither with age nor with sex in any of the diagnostic groups but we detected differences in CJD cases regarding codon 129 PRNP polymorphism and subtype with potential clinical implications. First, neurogranin concentrations were significantly higher in CJD MM and MV compared with VV cases, in contrast to tau, which shows higher concentrations in MM and VV, compared with MV cases.39 Since codon 129 PRNP data are premortem available, the combined analysis of tau and neurogranin could lead to specific codon 129 PRPN polymorphism-dependent cut-offs enhancing the discriminatory value of single biomarker measurements. Second, CJD MM1/MV1 cases, two subtypes with similar clinopathological phenotype, displayed higher CSF neurogranin concentrations than VV2. As described before39 and in the present study, synaptic and neuroaxonal damage is higher in CJD MM1/MV1 than in VV2 in cortical regions, where neurogranin is highly expressed. Thus, it is tempting to speculate that CSF neurogranin levels reflect the neuropathological heterogeneity of CJD prion subtypes regarding synaptic and neuronal loss. In this regard, biomarkers, such as neurogranin, able to recapitulate the heterogeneity of CJD pathology, may turn into valuable markers for disease diagnosis, prognosis and for, monitoring potential therapeutic approaches and inclusion of patient populations in clinical trials. Limitations of this study were the low number of CJD cases with subtype available and the absence of CSF-brain paired cases. Thus, further analysis including less prevalent subtypes and paired cases should be carried out to determine the complete neurogranin profile in the spectrum of CJD cases and its association with neuropathological correlates.
Recently, the presence of increased neurogranin processing peptides and decreased full-length protein has been reported in AD brain tissue. These observations suggest that neurogranin processing in AD may reflect both synaptic and axonal damage. Since neurogranin was associated with tau and amyloid pathology, it would be interesting to study whether a similar proteolytic pattern is observed in CJD, where neurogranin levels are altered in brain and CSF tissue without the presence of AD pathological hallmarks.

In total, this study evaluates for the first time the diagnostic and prognostic value of CSF neurogranin in CJD in comparison to AD. Additionally, we show a striking correlation between brain and CSF findings regarding different diseases (CJD vs AD) and CJD subtypes (MM1/MV1 vs VV2). This strongly supports the usefulness of comparative analysis between brain and biological fluids to comprehensively understand the molecular mechanisms underlying neurodegenerative dementias and the associate value of their study as diagnostic and prognostic markers for these conditions.

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Contributors IZ, IFA and FL designed the study. KB, DDL, HZ, IFA and FL performed experiments. KB, DDL, HZ, AV-P, AK, MS, IFA and FL analysed data and interpreted the results. EV provided reagents and technical expertise. FL wrote the manuscript draft. All authors critically revised the manuscript and approved its content before submission.

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Competing interests KB has served as a consultant or at advisory boards for Alzheimer, CogRx, Biogen, Novartis, and Roche Diagnostics, unrelated to this work. HZ has served at scientific advisory boards for Eli Lilly, Roche Diagnostics, Samumed, CogRx and Wave and has received travel support from Teva. KB and HZ are cofounders of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg. The other authors report no conflicts of interest related to the present study.

Ethics approval The study was conducted according to the revised Declaration of Helsinki and Good Clinical Practice guidelines, and approved by local Ethics committees (Reference numbers 11/11/193, 9/06/08, Universitaetsmedizin Göttingen, Germany).

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Data availability statement All data relevant to the study are included in the article or uploaded as online supplementary information.
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