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Cerebrospinal Fluid Amyloid β_{40} Is Decreased in Cerebral Amyloid Angiopathy

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Abstract

Cerebral amyloid angiopathy is caused by deposition of the amyloid β protein in the cerebral vasculature. In analogy to previous observations in Alzheimer disease, we hypothesized that analysis of amyloid β_{40} and β_{42} proteins in the cerebrospinal fluid might serve as a molecular biomarker. We observed strongly decreased cerebrospinal fluid amyloid β_{40} ($p < 0.01$ vs controls or Alzheimer disease) and amyloid β_{42} concentrations ($p < 0.001$ vs controls and $p < 0.05$ vs Alzheimer disease) in cerebral amyloid angiopathy patients. The combination of amyloid β_{42} and total tau discriminated cerebral amyloid angiopathy from controls, with an area under the receiver operator curve of 0.98. Our data are consistent with neuropathological evidence that amyloid β_{40} as well as amyloid β_{42} protein are selectively trapped in the cerebral vasculature from interstitial fluid drainage pathways that otherwise transport amyloid β proteins toward the cerebrospinal fluid.

The amyloid β -peptide ($A\beta$) not only accumulates in the brain parenchyma as senile plaques in Alzheimer disease (AD), but also in brain vessels as cerebral amyloid angiopathy (CAA). In contrast to the parenchymal depositions, CAA contains both $A\beta_{40}$ and $A\beta_{42}$,^{1,2} with $A\beta_{40}$ as the major isoform.³ Progression of CAA is associated with increased $A\beta_{40}$ accumulation.⁴

The cerebrospinal fluid (CSF) concentration of $A\beta_{42}$ is significantly decreased in AD patients⁵ and in patients with mild cognitive impairment that progress into clinical AD after several years⁶ compared with controls, whereas the $A\beta_{40}$ concentration remains unaltered.

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Furthermore, the increased CSF total tau (t-tau) and phosphorylated tau (p-tau) concentrations in AD⁵ are likely related to the pathological accumulation of these proteins in neurofibrillary tangles.

CAA can occur in the setting of AD or as an isolated finding. Patients with primary CAA clinically present with multiple large or small hemorrhages restricted to lobar brain regions⁷; these hemorrhages may be directly related to cognitive decline.⁸ In addition, autosomal dominantly inherited mutations in the amyloid precursor protein (APP) may cause familial CAA, such as the E693Q mutation causing hereditary cerebral hemorrhage with amyloidosis of the Dutch type (HCHWAD)⁹ or the D694N mutation causing CAA in an Iowa family.¹⁰

In this study, we examined if we could identify a specific pattern of A β and tau protein concentrations in CSF of CAA patients. Our a priori hypothesis was that CAA without dementia might be characterized by a CSF pattern, distinct from either controls or AD, of decreased A β ₄₀ with normal or only marginally elevated tau protein. We discuss our findings in the context of the pathophysiology of CAA as described in literature.

Patients and CSF Analysis

We included 72 AD patients (probable diagnosis according to the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association criteria), 58 control subjects, and 17 patients with cerebral amyloid angiopathy (CAA; diagnosed according to the Boston criteria⁷) in this study. Lumbar punctures were performed after informed consent was obtained from the patients themselves or from the patients' legal representatives. CSF was analyzed for A β ₄₀, A β ₄₂, t-tau, and p-tau₁₈₁ by enzyme-linked immunosorbent assay.¹¹ Unpaired *t* tests for comparison between two groups was used. Chi-square test was used to study gender distribution in the groups. Receiver operator characteristic (ROC) analysis was used to evaluate the prognostic ability of the individual biochemical variables to discriminate between CAA and either controls or AD. Forward linear regression was performed using SPSS (SPSS Inc., Chicago, IL). ROC analysis was also performed for the equation resulting from multivariate logistic regression. More detailed information on the patients included in this study and the CSF and statistical analyses can be found in the supplementary text available online.

Results

Age, gender, and an overview of the CSF results are provided in the Table and Figures 1 and 2. The age distribution was similar in the CAA and control groups, whereas AD patients were significantly older than CAA patients ($p = 0.008$; $t = 2.71$). CSF A β ₄₂ was decreased in both AD ($p < 0.0001$; $t = 11.7$) and CAA ($p < 0.0001$; $t = 7.48$) patients compared with control subjects and was even lower in the CAA group compared with the AD group ($p = 0.039$; $t = 2.10$). The mean A β ₄₂ concentration in the CAA group was 42.4% of the controls; in the AD group this was 51.6% of control values. In contrast, however, CSF A β ₄₀ concentrations were decreased only in the CAA group compared with the AD ($p = 0.0063$; $t = 2.80$) and control ($p = 0.0012$; $t = 3.40$) groups (CAA: 72.7% of control; AD: 92.8% of control). Both CSF t-tau ($p = 0.002$; $t = 3.19$) and p-tau₁₈₁ ($p = 0.0013$; $t = 3.33$) concentrations were significantly higher in the AD patients compared with the CAA group, although the concentrations in the latter group ($p = 0.0009$; $t = 3.46$) were also higher than in controls (CAA: t-tau, 184% of control; p-tau₁₈₁, 138% of control; AD: t-tau, 332% of control; p-tau₁₈₁, 230% of control). The A β ₄₀/A β ₄₂ ratio was clearly elevated in both the AD ($p < 0.0001$; $t = 10.2$) and CAA ($p < .0001$; $t = 5.98$) groups as compared with the control group (CAA: 192% of control; AD: 182% of control) and did not differentiate between CAA and AD ($p = 0.57$; $t = 0.57$). The results were essentially unchanged when the

two familial CAA cases were omitted from the analyses and were also unaffected by controlling for subject age.

ROC analysis for optimal differentiation of CAA from controls yielded the following results for sensitivity (Se), specificity (Sp), and area under the curves (AUC) for $A\beta_{40}$ (Se: 87.5%; Sp: 47.1%; AUC: 0.76), $A\beta_{42}$ (Se: 86.2%; Sp: 94.1%; AUC: 0.96), t-tau (Se: 96.5%; Sp: 52.9%; AUC: 0.73), and p-tau₁₈₁ (Se: 77.6%; Sp: 58.8%; AUC: 0.67). When $A\beta_{42}$ and t-tau (Fig 2B) were selected in a forward linear regression procedure ($y = -4.264 + 0.014 \times A\beta_{42} - 0.009 \times t\text{-tau}$), ROC analysis resulted in an AUC of 0.98. ROC analysis for optimal differentiation of CAA from AD yielded the following results for $A\beta_{40}$ (Se: 88.2%; Sp: 59.7%; AUC: 0.74), $A\beta_{42}$ (Se: 58.8%; Sp: 86.1%; AUC: 0.68), t-tau (Se: 76.5%; Sp: 76.4%; AUC: 0.80), and p-tau₁₈₁ (Se: 88.2%; Sp: 56.5%; AUC: 0.79). When $A\beta_{42}$ and p-tau₁₈₁ were selected in a forward linear regression procedure ($y = -3.586 + 0.037 \times p\text{-tau}_{181} + 0.005 \times A\beta_{42}$), ROC analysis resulted in an AUC of 0.82.

Discussion

The major findings of our study are the decreased CSF $A\beta_{40}$ and $A\beta_{42}$ concentrations in CSF of CAA patients relative to controls and AD. Our results are particularly striking given the known overlap between CAA and AD pathologies,¹² such that the majority of subjects diagnosed with AD have some degree of cerebrovascular amyloid, and the majority of those diagnosed with CAA have some senile plaque pathology, but not to such an extent that a clinical diagnosis of dementia was applicable. The fact that significant differences could nonetheless be identified suggests that the predominance of one pathology relative to the other is strong enough to generate distinct CSF profiles.

The observation of low CSF $A\beta_{40}$ and $A\beta_{42}$ possibly relates to the specific mechanisms of $A\beta$ deposition that are involved in the development of CAA. Neurons are the main source of cerebral $A\beta$, and it has been proposed that $A\beta$ secreted by neurons is dragged along interstitial fluid drainage pathways along the cerebral vasculature toward the subarachnoid space, where it is collected in the CSF.¹³ Indeed, in transgenic mice models in which neuronally expressed APP leads to excessive production of $A\beta$, abundant CAA may develop, suggesting a nonvascular source of $A\beta$.¹⁴ The deposition of $A\beta$ in the vasculature is counterbalanced by its transport out of the brain by passage across the blood-brain barrier, for example, by interaction with specific $A\beta$ receptors such as the low-density lipoprotein receptor related protein-1, which mediate the transendothelial transport of $A\beta$ and degradation by proteases such as neprilysin, angiotensin-converting enzyme, or insulin-degrading enzyme.¹⁵ The balance between $A\beta$ production, clearance from the brain by transport across the blood-brain barrier or proteolytic degradation, and retention within the brain in the form of senile plaques or CAA determines what amounts of soluble $A\beta$ will finally reach the CSF. Alterations in this dynamic equilibrium of production, clearance, and accumulation may lead to altered $A\beta$ concentrations in the CSF. Thus, the decreased $A\beta_{40}$ and $A\beta_{42}$ concentration in CSF of CAA patients is the result of a net reduction in both $A\beta$ peptides eluted with interstitial fluid toward the CSF. Very likely, the most important cause is the selective trapping of $A\beta_{40}$ and $A\beta_{42}$ isoforms in the cerebral vasculature. These data are in line with neuropathological data of the deposition of both $A\beta_{40}$ and $A\beta_{42}$ proteins in CAA,^{1,2} whereas the predominant deposition of $A\beta_{42}$ in diffuse senile plaques in AD leads to selective reduction in CSF levels of this protein in AD.⁵

In AD CSF t-tau and p-tau₁₈₁ concentrations are clearly elevated; this has been related to the formation of neurofibrillary tangles and might be a nonspecific consequence of neural damage. In contrast, however, neurofibrillary tangles are not among the pathological hallmarks of CAA patients.^{16,17} CSF t-tau and p-tau₁₈₁ concentrations were increased in

CAA patients, but not to the same degree as in AD. A plausible explanation for this observation is the presence of some accompanying AD pathology even in these nondemented CAA subjects.

Our studies suggest that CSF analysis may be helpful in the identification of CAA patients, but the diagnostic accuracy to differentiate CAA from AD is limited so far. CSF analysis appears to be more useful for differentiating CAA from non-AD controls, especially the analysis of A β ₄₂ in combination with t-tau. This observation raises the possibility that CSF testing could be useful in clinical situations where the diagnosis of CAA based on magnetic resonance imaging (MRI) findings alone is not clear-cut. This possibility assumes that potential mimics of CAA (such as hypertensive vasculopathy or multiple cavernous hemangiomas) will show similar CSF A β and tau profiles to that seen in our elderly control subjects, an assumption that will need to be assessed in future analyses. Along with this limitation to the current study, we also note that our AD subjects did not undergo T2*-weighted gradient-echo MRI, and therefore could not be excluded from having the CAA-related microbleeds seen in a substantial proportion of AD patients.¹⁸ This source of error makes the observed differences in CSF profile between CAA and AD more striking, as any contamination of the AD group with advanced CAA would be expected to bias against significant differences.

Together with recent studies using the A β -binding agent Pittsburgh Compound B,¹⁹ these results suggest that biomarkers might be used to identify cerebrovascular A β . MRI has been the primary noninvasive diagnostic method for CAA,⁷ but it detects only the secondary consequences of CAA such as microbleeding and white matter hyperintensities, not the vascular amyloid itself. Future studies will determine whether CSF can be used as a quantitative measure of CAA severity, and thus potentially a predictor of the effects of CAA on vascular function and small vessel brain injury.²⁰

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig 1.

Scatter plots of amyloid β -peptide ($A\beta$)₄₂, $A\beta$ ₄₀, total tau, phosphorylated tau₁₈₁, and $A\beta$ ₄₀/ $A\beta$ ₄₂ in the control, cerebral amyloid angiopathy (CAA) and Alzheimer disease (AD) groups. The CAA patient with the Iowa mutation is indicated by a short arrow, the CAA patient with the Dutch mutation by a long arrow. Mean concentration is indicated by a horizontal line. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (t tests).

Fig 2. Combination of amyloid β -peptide ($A\beta_{40}$ and total tau (t-tau) analysis (A) or $A\beta_{42}$ and t-tau analysis (B) in the cerebral amyloid angiopathy (open circles) and control groups (*closed squares*).

Table

Overview of Gender and Age Distribution in the Patient Groups and Results of CSF t-tau, p-tau₁₈₁, A β ₄₂, and A β ₄₀ Analyses

	Gender (M/F; n)	Age (yr) ^a	A β ₄₂ (pg/ml) ^a	A β ₄₀ (pg/ml) ^a	A β ₄₀ / A β ₄₂ ^a	t-tau (pg/ml) ^a	p-tau ₁₈₁ (pg/ml) ^a
CAA	11/6	62.8 ± 11.9 (n = 17)	355 ± 146 (n = 17)	2912 ± 977 (n = 17)	9.43 ± 4.95 (n = 17)	395 ± 292 (n = 17)	66.3 ± 30.2 (n = 17)
AD	34/38 ^b	69.4 ± 8.3 (n = 72) ^c	433 ± 135 (n = 72) ^d	3713 ± 1081 (n = 72) ^c	8.95 ± 2.57 (n = 72) ^b	712 ± 384 (n = 72) ^c	110 ± 51.8 (n = 69) ^c
Controls	28/30 ^b	61.0 ± 8.7 (n = 58) ^b	838 ± 253 (n = 58) ^e	4003 ± 1185 (n = 48) ^c	4.91 ± 1.13 (n = 48) ^e	215 ± 78.3 (n = 58) ^e	47.9 ± 14.8 (n = 58) ^e

Tests for statistical significance are shown for CAA vs AD and for CAA vs controls. Gender distribution was tested by chi-square analysis; other comparisons by t-tests.

CSF = cerebrospinal fluid; t-tau = total tau; p-tau = phosphorylated tau; A β = amyloid β -peptide; M = male; F = female; CAA = cerebral amyloid angiopathy; AD = Alzheimer disease.

^aMean ± standard deviation is shown.

^bNot significantly different.

^c $p < 0.01$.

^d $p < 0.05$.

^e $p < 0.001$.