Original Research Article

β -Amyloid (1–42) Levels in Cerebrospinal Fluid and Cerebral Atrophy in Mild Cognitive Impairment and Alzheimer's Disease

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Key Words

Aging-associated cognitive decline • Biomarkers • Cerebrospinal fluid • Hippocampus • Mild cognitive impairment • MRI • Voxel-based morphometry

Abstract

Background: Recent studies consistently reported Alzheimer's disease (AD) and, to a lower extent, mild cognitive impairment (MCI) to be accompanied by reduced cerebrospinal fluid (CSF) levels of β -amyloid. However, how these changes are related to brain morphological alterations is so far only partly understood. **Methods:** CSF levels of β -amyloid (1–42) were examined with respect to cerebral atrophy in 23 subjects with MCI, 16 patients with mild-to-moderate Alzheimer's disease (AD) and 15 age-matched controls by using magnetic resonance imaging and voxel-based morphometry (VBM). **Results:** When contrasted with the controls, β -amyloid (1–42) levels were significantly lower ($p < 0.05$) in patients with MCI and even more so in the AD patients. This effect was significantly associated with reduced gray matter densities in both the right and left hippocampal head based on the results of a VBM analysis across the entire sample. **Conclusion:** Our finding confirms the results of previous studies and suggests that both the decrease in β -amyloid (1–42) and the development of hippocampal atrophy coincide in the disease process.

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Introduction

It is generally accepted that the aggregation of β -amyloid (1–42) and the formation of senile plaques in Alzheimer's disease (AD) correspond with decreased cerebrospinal fluid (CSF) levels of β -amyloid (1–42) in patients with mild cognitive impairment (MCI) and AD compared with healthy controls [1]. Therefore, decreased β -amyloid (1–42) levels confer an increased risk for progression from MCI to AD in longitudinal studies [2]. According to neuropathological studies, β -amyloid deposits are not evenly distributed throughout the brain but rather follow a characteristic pattern during the clinical course of AD [3, 4]. Unlike the distribution of neurofibrillary tangles, which were primarily observed in allocortical regions of the medial temporal lobe according to the model of Braak and Braak [5], the initial deposition of β -amyloid plaques is exclusively found in neocortical areas, i.e. frontal, temporal and parietal cortical fields, respectively. However, neuropathological studies in the field did not consider a number of important clinical parameters, such as education or severity of dementia. Using magnetic resonance imaging (MRI), clinical studies found decreased CSF levels of β -amyloid (1–42) in patients with mild-to-moderate AD to be significantly correlated with temporal lobe [6] and hippocampal atrophy [7], respectively. Significant correlations between β -amyloid (1–40) and cerebral changes did not arise. Hence, one may hypothesize that the decrease in β -amyloid (1–42) corresponds to the development of hippocampal atrophy in the disease process.

In a previous MRI study, we investigated CSF levels of the microtubule-associated tau protein (total tau protein) and of a hyperphosphorylated isoform [phospho-tau (181)] with respect to cerebral morphology in 39 patients with MCI or AD. Voxel-based morphometry (VBM) was used for image analyses, which allowed for user-independent detection of structural alterations throughout the entire brain. Elevated CSF levels of both biomarkers were associated with reduced gray matter density in temporal, parietal and frontal regions. In the MCI patients, increased CSF levels of phospho-tau (181) protein, but not total tau protein, were associated with pronounced atrophy in the right hippocampus [8]. Longitudinal studies of MCI patients [2, 9] yielded an increased risk of progression to AD with higher CSF tau protein concentrations. From a clinical perspective, these associations are likely to reflect two sides of the same medal since hippocampal atrophy refers to declarative memory deficits [10–12] which typically exacerbate with progression from MCI to AD as one of the core symptoms of the disease.

In the present MRI study, we investigated the potential relationship between levels of CSF β -amyloid (1–42) and gray matter changes in patients with MCI or mild-to-moderate AD and healthy controls. We studied the same group of patients examined in our previous study [8]. Groups were carefully matched for age and education. Using the protocol of our previous study, VBM was applied to consider gray matter changes in any part of the brain [8]. Decreased CSF β -amyloid (1–42) concentrations were expected to be associated with hippocampal atrophy indicating a coincidence of the respective changes in the disease process.

Patients and Methods

Patients

A total of 54 patients were included in our previous and in our current study: 23 patients with MCI defined according to the concept of aging-associated cognitive decline (AACD) [13–15], 16 patients with probable AD according to NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and

Related Disorders Association) [16] and 15 otherwise healthy controls. Of the 54 patients, only 8 MCI patients were available for follow-up examinations.

The AACD concept considers deficits in various neurocognitive domains, such as memory and learning, attention and concentration, thinking, language and visuospatial function. AACD subjects were identified based on different criteria such as their performance on a standardized test of cognition involving at least 1 of the aforementioned domains with a score of at least 1 unit of standard deviation below the age-adjusted norm; an exclusion of dementia and any medical, neurologic or psychiatric disorders associated with cognitive deterioration, as determined by patient history and/or clinical examination, and normal activities of daily living.

All participants were recruited consecutively at the Section of Geriatric Psychiatry at the University of Heidelberg, Germany. The Institutional Review Board of the University approved the study protocol. Written informed consent was obtained from the participants after the planned procedures had been fully explained by a geriatric psychiatrist.

Clinical Examination

The clinical examination included ascertainment of personal medical history, family history and detailed physical, neurological and neuropsychological examinations. None of the participants had a lifetime history of neurologic or severe medical illness, head injury or substance abuse. Global cognitive deficits were assessed using the Mini Mental State Examination [17] and cognitive performance was investigated based on an extensive neuropsychological test battery as described elsewhere [18].

CSF Analysis

All CSF samples (250 μ l in polypropylene tubes) were obtained by lumbar puncture between 10 and 12 a.m. as part of the routine diagnostic procedure. The maximum time period between MRI and lumbar puncture was 6 days. Immediately after lumbar puncture, samples were frozen in liquid nitrogen without centrifugation and stored at -80°C . Concentrations of β -amyloid (1–42) were measured using an enzyme-linked immunosorbent assay [ELISA Innostest β -amyloid (1–42) Ag kit; Innogenetics, Ghent, Belgium], as described elsewhere [19]. For the discrimination of incipient AD from controls, sensitivity and specificity of CSF β -amyloid (1–42) were determined as 86 and 90%, respectively [1].

MRI Acquisition

MRI data were obtained at the German Cancer Research Center with a 1.5-tesla Magnetom Symphony scanner (Siemens Medical Solutions). To exclude secondary causes of dementia and ischemic changes, we performed a 2-dimensional T_2 -weighted fast spin echo (TR = 4,500 ms, TE = 90 ms) sequence in axial orientation. For structural analysis, a T_1 -weighted 3-dimensional magnetization prepared rapid gradient echo sequence was performed with the following parameters: 126 coronar slices, image matrix 256×256 , voxel size $0.98 \times 0.98 \times 1.8$ mm, TR = 10 ms, TE = 4 ms.

In addition, vascular changes in the T_2 -weighted sequences were quantified according to the European Task Force on Age-Related White Matter Changes rating scale [20] and interpreted in relation to the anamnestic risk factors. Amyloid angiopathic changes were evaluated on the gradient echo sequences. Patients showing pronounced microvascular changes (age-related white matter changes of grade 2 or higher) were excluded from further evaluation.

Voxel-Based Morphometry

SPM2 software (www.fil.ion.ucl.ac.uk/spm) was used for VBM. Initially, all structural images were carefully screened for artifacts, the origin was placed on the anterior commis-

sure and the images were reoriented manually to approximate the anterior and posterior commissure to the horizontal plane.

For pre-processing of imaging data, the VBM protocol proposed by Good et al. [21] was applied to minimize the probability of misclassifications within the tissue segmentation. Briefly, this method comprised the following steps:

- creation of a customized group and tissue-specific template;
- segmentation of MRI (in native space) into tissue classes, followed by a series of additional automated morphological operations to remove unconnected non-brain voxels from the segments;
- normalization of the gray and white matter images to the group-specific templates;
- reapplication of calculated normalization parameters to the structural images and reslicing them to a voxel size of $1 \times 1 \times 1 \text{ mm}^3$;
- resegmentation of normalized structural images [owing to local volume effects (growing or shrinking) in nonlinear spatial normalization, a voxel-wise multiplication with the Jacobian determinant, derived from normalization parameters, was performed to preserve the volume of a particular volume], and
- smoothing of tissue segments with a 10-mm full-width at half-maximum Gaussian kernel [22].

Statistical Analysis

SPSS for Windows version 14 (SPSS Inc.) was used for statistical analysis of demographic and clinical data. Values of $p < 0.05$ were considered to be significant. Regional correlations were assessed between local gray matter density values and β -amyloid (1–42) in CSF using voxel-wise regression analysis. The resulting T-map was thresholded for a significance level of $p < 0.001$ uncorrected and a spatial extent of 200 voxels. To avoid false-positive findings caused by potential confounders known to affect brain morphology, ‘effects of no interest gray matter maps’ were modeled using regressors for age, sex and level of education. By creating mask images, we were able to exclude the regions related to these nuisance variables from the analysis.

In a second VBM analysis, potential associations between CSF levels of β -amyloid (1–42) and gray matter density within the AACD group were tested separately. To improve statistical power in this relatively small sample, the AACD group was divided into those subjects with normal and those with reduced β -amyloid (1–42) levels, respectively, and hereafter a two-sample t test as implemented in SPM2 software was applied. AACD subjects were assigned to the corresponding groups after determination of values for β -amyloid (1–42) levels by the 10th percentile of the control group (International Federation of Clinical Chemistry, 1987). According to a cutoff value of 904 pg/ml, 12 AACD subjects had normal and 11 AACD subjects had reduced β -amyloid (1–42) CSF levels.

To address an effect of AD diagnosis as the explanation for the observed correlation, we examined gray matter differences between controls and patients with AD using a t test as implemented in SPM2 software.

Results

Demographic characteristics and CSF β -amyloid (1–42) levels are summarized in table 1. While the diagnostic groups showed only minor, non-significant differences with respect to age, gender and level of education, mean MMSE scores differed significantly with the MCI patients ranking between the AD patients and the controls. Similarly, mean CSF levels of β -amyloid (1–42) were lowest in the AD patients followed by the MCI patients and the controls ($p < 0.01$).

Table 1. Demographics and β -amyloid (1–42) levels in healthy controls, AACD and AD (cf. [8])

Variable	Controls (1) (n = 15)	AACD (2) (n = 23)	AD (3) (n = 16)	F value	d.f.	p value	Duncan test (5%)
Female/male ratio	9/6	13/10	9/7				NS
Age, years	68.9 $\pm$ 6.7	69.4 $\pm$ 5.4	68.6 $\pm$ 6.9	0.09	2, 51	0.91	1 = 2 = 3
Education, years	10.13 $\pm$ 1.81	10.17 $\pm$ 1.78	10.38 $\pm$ 1.93	0.08	2, 51	0.92	1 = 2 = 3
MMSE	29.20 $\pm$ 0.78	26.04 $\pm$ 1.69	20.13 $\pm$ 2.96	83.92	2, 51	<0.001	1 > 2 > 3
A β_{1-42} , pg/ml	1,086.60 $\pm$ 134.29	937.83 $\pm$ 220.39	791.81 $\pm$ 233.29	7.76	2, 51	<0.01	1 > 2 > 3

Means $\pm$ SD. A β = β -Amyloid; d.f. = degrees of freedom; MMSE = Mini Mental State Examination; NS = non-significant (χ^2 test).

Fig. 1. Associations between CSF β -amyloid (1–42) concentrations and gray matter densities (across the entire sample).

VBM analyses across the entire sample revealed significant associations between lower CSF β -amyloid (1–42) concentrations and reduced gray matter densities in both the right and left hippocampal head (fig. 1; table 2). No significant correlations were found within single diagnostic groups.

Discussion

The present study yielded two major findings: (i) a confirmation that CSF levels of β -amyloid (1–42) decrease with transition from normal aging to MCI and even more so with progression from MCI to manifest AD, and (ii) an indication that this process corresponds to progressive hippocampal atrophy rather than to changes in other cerebral sites.

The decrease in β -amyloid (1–42) in AD was already described in the early 1990s [6] and assigned to the binding of the peptide to senile plaques. Dilution effects were considered as an additional explanation, because brain atrophy in AD is accompanied by an enlargement

Table 2. Anatomical structures showing significant positive correlation between gray matter density and β -amyloid (1–42) concentration in patients with AD, subjects with AACD and healthy controls

Anatomical structure	Cluster size (voxel)	T value	Peak coordinates (x, y, z)
Left hippocampal head	1,581	4.67	–28, –12, –16
Right hippocampal head	311	4.02	28, 8, 16

Height threshold $p < 0.001$, uncorrected; extent threshold = 200 voxels.

of internal and external CSF spaces. However, only one of the structural neuroimaging studies [6, 7, 23–25] conducted so far yielded significant correlations between β -amyloid (1–42) and the volume of the CSF spaces.

Our finding of a significant association of decreased CSF β -amyloid (1–42) levels and hippocampal atrophy is in accordance with the longitudinal study of Herukka et al. [23] who investigated the association of CSF biomarkers with medial temporal lobe atrophy and the prognostic value of these measures to predict AD in a group of 21 MCI patients. Already at baseline, the 8 patients in whom symptoms converted from MCI to manifest AD showed lower CSF levels of β -amyloid (1–42) and higher tau and phospho-tau (181) protein levels than patients with stable MCI. These changes corresponded to more pronounced atrophic changes in all cerebral sites considered. While the significant associations between tau and phospho-tau (181) levels and hippocampal and left entorhinal cortex volumes could be demonstrated across the entire group, the respective correlations of β -amyloid (1–42) reached significance for the left hippocampal volume in the progressive group only.

In a large multicenter study, Schuff et al. [24] investigated rates of hippocampal loss, determined by successive MRI scans, with respect to CSF β -amyloid (1–42), tau and phospho-tau (181) levels in 112 cognitively normal elderly controls, 226 MCI patients and 96 AD patients. The MCI and AD groups showed significant hippocampal volume losses over 6 months, which even accelerated towards the 1-year follow-up. In the MCI patients, increased rates of hippocampal volume loss were associated with lower CSF levels of β -amyloid (1–42). Similarly, De Leon et al. [26] observed a significant association between β -amyloid (1–42) CSF levels, phospho-tau (231) CSF levels and hippocampal atrophy in their longitudinal study of 7 patients with MCI. The prognostic values of the respective variables was underlined by Bouwman et al. [27] who found a 4-fold higher risk for MCI patients with both medial temporal lobe atrophy and abnormal CSF values to develop manifest AD than those without the respective changes.

On the basis of the ADNI (AD Neuroimaging Initiative) database, Fjell et al. [25] investigated CSF levels of β -amyloid (1–42), tau and phospho-tau (181) protein as well as brain morphometry in patients with MCI or AD and healthy controls over the course of 1 (n = 205) and 2 years (n = 176), respectively. CSF biomarker levels did not account for differences in brain morphometry at baseline between the diagnostic groups. In the MCI group, CSF biomarker levels were significantly associated with longitudinal atrophy rates in several regions, including the hippocampus, amygdala, parahippocampal gyrus, entorhinal cortex, inferior and medial temporal cortex as well as increased volumes of the lateral ventricles. Interestingly, even MCI patients with levels of β -amyloid (1–42) comparable with controls showed more atrophy than the controls. The authors concluded that morphometric changes in MCI and AD are not secondary to CSF biomarker changes and that the two types of biomarkers reflect complementary processes.

Fig. 2. Atrophic brain changes (AD vs. controls); for further details please refer to the discussion section.

The studies cited above support the association between lower CSF levels of β -amyloid (1–42) and hippocampal atrophy, which was originally described by our group [7] in 32 patients in all stages of AD using manual segmentation. Complementing these cross-sectional findings, decreased β -amyloid (1–42) levels may also herald an increase in atrophic changes in the clinical course. As emphasized by Fjell et al. [25], β -amyloid (1–42) levels do not only predict hippocampal atrophy but also atrophic changes of all medial temporal substructures, including an enlargement of the adjacent CSF spaces in MCI.

In a previous study, we investigated the cerebral correlates of elevated total tau and phospho-tau (181) protein CSF levels in the same sample using an identical VBM protocol [8]. The respective changes did not only refer to hippocampal atrophy but also involved the parahippocampal gyrus, the medial and superior temporal cortex, the left inferior parietal cortex, and the left orbital gyrus cortex. According to Fjell et al. [25], the different patterns of cerebral correlates of β -amyloid (1–42), total tau and phospho-tau (181) protein levels, respectively, corroborate the hypothesis that the two types of biomarkers yield complementary information.

Major limitations of our study were the modest sample size and the cross-sectional design. Age, gender and educational level have to be considered as potential confounding factors. However, the correlations obtained between β -amyloid (1–42) and density reductions in MRI were corrected for these variables. Noteworthy, correlations between CSF measures and brain morphology did not remain significant after correction for multiple comparisons. However, our findings are clearly in accordance with previous independent neuroimaging studies. Since the hippocampus has been shown to be particularly involved in AD pathology, it is unlikely that the present results are false positive, especially since they are confined to the medial temporal lobe. As shown in figure 2, atrophic brain changes were not restricted to the hippocampus but also involved bilateral temporal, parietal and other neocortical areas typically related to AD pathology.

In summary, this study revealed that patients with AD and – to a lesser extent – patients with MCI show significantly lower CSF levels of β -amyloid (1–42) than healthy controls. In these patients, lower CSF β -amyloid (1–42) levels are significantly associated with hippocampal atrophy rather than with changes in other cerebral sites.

Acknowledgment

This study was supported in part by the European LipiDiDiet project.

Disclosure Statement

There is no conflict of interest.

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