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Original Research Article

Conformation-Dependent Oligomers in Cerebrospinal Fluid of Presymptomatic Familial Alzheimer's Disease Mutation Carriers

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Key Words
Presymptomatic · Alzheimer's disease · Oligomer · Aβ · Aβ42 · Cerebrospinal fluid · Presenilin-1 · Amyloid precursor protein · Conformation

Abstract
Background/Aims: Oligomerization of amyloid beta (Aβ) is a hypothesized step in the formation of plaques in Alzheimer's disease (AD) but has been difficult to demonstrate in vivo in humans. As persons destined to develop familial AD (FAD) due to fully penetrant autosomal dominant mutations are essentially certain to develop the disease, they provide the opportunity to identify oligomers during the presymptomatic stage of the disease. ***Methods:*** We measured levels of $A\beta_{42}$ using a conventional immunoassay and prefibrillar, fibrillar, and annular protofibrillar oligomers using polyclonal conformation-dependent antibodies in the cerebrospinal fluid (CSF) of 7 persons at risk for inheriting FAD mutations. Levels of oligomers were compared between FAD mutation carriers and noncarriers. ***Results:*** Compared to 2 noncarriers, annular protofibrillar oligomers were elevated, prefibrillar and fibrillar oligomers trended towards elevation and $A\beta_{42}$ monomer trended towards being decreased in 5 FAD mutation carriers. ***Conclusion:*** Our data provide evidence for an identifiable elevation of CSF oligomers during the presymptomatic phase of FAD.

Introduction

The 'amyloid hypothesis' of the pathogenesis of Alzheimer's disease (AD) posits that an increased production or a decreased degradation of derivatives of the amyloid precursor protein (APP) initiate a cascade of events that cause the illness [1]. This hypothesis is based in

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part on the observation that causative autosomal dominant familial AD (FAD) mutations cause absolute or relative increases in the amount of the 42-amino acid length version of the peptide produced by cleavage of APP ($A\beta_{42}$) [2]. Also, the amyloid plaques in the brain that define the illness are largely composed of derivatives of APP, and the deposition of $A\beta_{42}$ is an early event in their formation [3]. However, there is an imperfect association between the degree of amyloid deposition and patient clinical status, and intervention studies have suggested that it may be possible to decrease plaque pathology without significant impact on the disease course [4]. There has been an increasing focus on soluble forms of Aβ as being more crucial to the development of AD than the plaques per se, with various forms of Aβ oligomers being identified and characterized. Such oligomers have been demonstrated to exert toxic effects on synapses independently of plaque formation [5].

Despite the pivotal role posited for Aβ in the cause of AD, measurements of monomeric $A\beta_{42}$ in the cerebrospinal fluid (CSF) of persons with AD [6] or destined to develop it [7] have found it to be decreased relative to controls. It has been hypothesized that this decrement represents a shift in equilibrium from the monomeric form to oligomeric or insoluble forms in the brain, but evidence for this is indirect. A recent study found decreased $A\beta_{42}$ concurrent with elevated Aβ oligomers in the CSF in a population with late-onset AD using an enzyme-linked immunosorbent assay (ELISA) [8].

As the pathogenic process of AD begins years before overt symptoms in persons carrying FAD mutations [7, 9], we hypothesized that Aβ oligomers would be elevated in the CSF of asymptomatic FAD mutation carriers at a time when $A\beta_{42}$ measured using conventional techniques would be decreased. In the current study, we measured oligomers using conformation-dependent antibodies and $A\beta_{42}$ using standard ELISA techniques in 7 asymptomatic persons at risk for developing FAD due to *PSEN1* and *APP* mutations.

Materials and Methods

The study population consisted of the initial 7 subjects who underwent lumbar punctures in an ongoing study of clinical, imaging, and biochemical changes occurring during the presymptomatic phase of FAD. Among the 7 subjects, 2 were cousins at risk for the L235V *PSEN1* mutation [10], 4 were siblings at risk for the A431E *PSEN1* mutation [11], and 1 was at risk for the V717I *APP* mutation [12]. The subjects underwent comprehensive cognitive assessments including the Clinical Dementia Rating (CDR) [13] scale blind to their genetic status as previously described [14]. All study procedures were performed in accordance with the Helsinki Declaration of 1975 and were approved by the UCLA Institutional Review Board; all subjects provided written informed consent.

Blood was drawn and DNA extracted using standard techniques. The presence of the A431E and L235V substitutions in *PSEN1* was assessed using RFLP analyses, and the presence of the V717I substitution in *APP* was assessed with direct sequencing. CSF was obtained and $A\beta_{42}$ levels determined using Luminex Reagents and X-MAP technology as previously described [15].

CSF samples were analyzed using dot-blot assays employing polyclonal A11 (anti-prefibrillar oligomer), OC (anti-fibrillar oligomer) and αAPF (anti-annular protofibril) antibodies as previously described [16–18]. The total protein concentration was determined using the BCA Protein Assay kit (Pierce 23223 and 23224). The samples were diluted with PBS pH 7.4 so that all samples had equal amounts of total protein. Diluted CSF samples were spotted onto nitrocellulose membrane BA-83 (Whatman 10 402 495) at 0.9–1.6 μg in 2 μl and allowed to air dry. Blots were then incubated in 10% milk in Low-Tween-TBS (20 mM Tris, 137 mM NaCl, 0.01% Tween 20, pH 7.6) for 1 h at room temperature. After three 5-min washes

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Table 1. Age and CSF $A\beta_{42}$ and oligomer levels in FAD mutation carriers and noncarriers (mean ± SD)

	Carriers (n = 5)	Noncarriers (n = 2)	p
Age, years	33 ± 6.8	30.5 ± 2.1	0.65
Years younger than family-specific age of dementia diagnosis	14.4 ± 6.3	13.0 ± 2.8	0.79
CSF $A\beta_{42}$, pg/ml	260.0 ± 144.0	415.6 ± 80.7	0.22
CSF A11 reactivity	38.4 ± 18.6	22.5 ± 4.6	0.31
CSF OC reactivity	13.8 ± 7.1	3.3 ± 1.9	0.11
CSF αAPF reactivity	70.2 ± 10.0	44.8 ± 6.1	0.02

in Low-Tween-TBS, the blots were incubated overnight at 4°C in primary antibody solution (A11 1:2,000, αAPF 1:1,000, and OC 1:10,000) with 5% milk in Low-Tween-TBS, 0.02% NaN_3. After three 5-min washes, the blots were incubated in goat-α-rabbit HRP-conjugated antibody (Jackson ImmunoResearch 305-035-0045, 1:12,000), 5% milk in Low-Tween-TBS for 1 h at room temperature. Following the final three 5 min washes, the blots were incubated in ECL reagent (Amersham, RPN2106) for 1 min and exposed to film (Denville, E-3012). Oligomer concentration was quantified using densitometry as previously described [19].

The age and $A\beta_{42}$, A11, OC, and αAPF levels were compared between mutation carriers and noncarriers using Student's t tests.

Results

Five subjects were mutation carriers (specific mutations not revealed secondary to subject confidentiality) and 2 were noncarriers. All subjects were asymptomatic (CDR scores = 0). The subjects did not differ in absolute age or age relative to the typical age of dementia diagnosis in their families (table 1). Levels of annular protofibrils were significantly elevated in mutation carriers relative to noncarriers (70.2 vs. 4.8, p = 0.02). Though levels of the other oligomers were not significantly different, all tended to be higher in mutation carries, whereas $A\beta_{42}$ levels tended to be lower (table 1; fig. 1).

Discussion

We present initial evidence for elevated oligomers measured using conformation-dependent antibodies in the CSF of asymptomatic persons inheriting FAD mutations. Levels of $A\beta_{42}$ measured using conventional immunoassays showed a trend in the opposite direction as has been well described, including in the current population [15, 20]. Though the higher level of oligomers was not statistically significant except in the case of annular protofibrils, the consistent trend across antibodies in the context of a trend for decreased $A\beta_{42}$ suggests the possibility of oligomerization of Aβ into a form not detectable by standard immunoassays for $A\beta_{42}$. These results are consistent with those of a recent report of elevated levels of annular protofibrils in the brain of AD subjects [21].

Though our conclusions are limited by the small number of subjects, our findings are consistent with the presence of an identifiable intermediate state between $A\beta_{42}$ monomers and the deposition of $A\beta_{42}$ in insoluble plaques during the presymptomatic phase of FAD.

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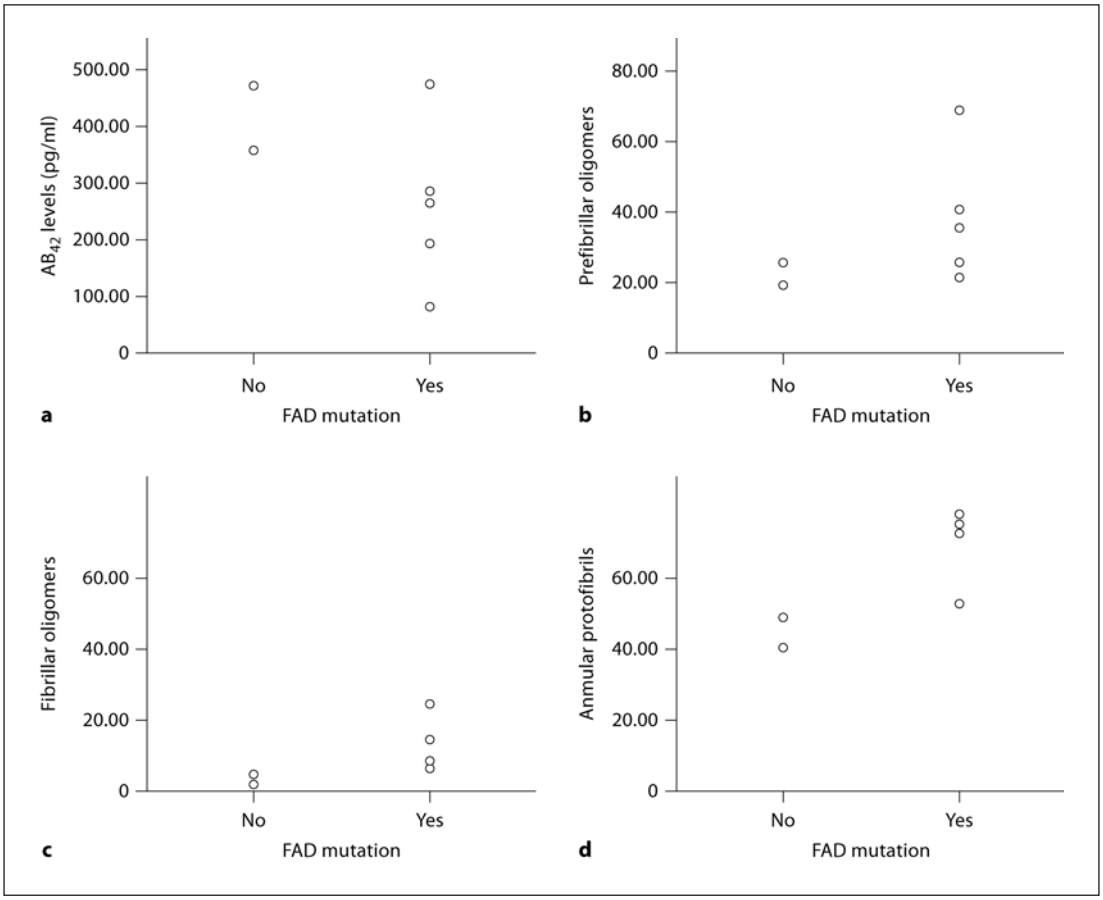

Fig. 1. CSF $A\beta_{42}$ levels measured by ELISA (pg/ml; **a**) and prefibrillar (**b**), fibrillar (**c**), and annular protofibrillar (**d**) levels (arbitrary units) in 5 FAD mutation carriers and 2 noncarriers. Annular protofibrillar levels are statistically higher in FAD mutation carriers (p = 0.02).

Our findings are consistent with the previous observation that at least a part of the decrement of CSF $A\beta_{42}$ levels seen in AD patients is eliminated when $A\beta_{42}$ levels are measured in denaturing conditions that decrease oligomerization [22]. These results should be confirmed in larger groups of presymptomatic persons with FAD mutations as well as of those destined to develop late-onset AD using monoclonal conformation-dependent antibodies [23] and other techniques for measuring oligomers [8].

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