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Comparison of Aβ levels in the brain of familial and sporadic Alzheimer's disease

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ABSTRACT

Mutations in presenilin (PS) and amyloid precursor protein (APP) genes are a predominant cause for early-onset familial Alzheimer disease (AD). Although these mutations are rare, they have in the past decades advanced our understanding of the underlying molecular mechanisms of AD. In the present study, Aβ levels were measured in cortical regions of APPsw and PS1 (M146V) mutation carriers, sporadic AD (SAD) and age-matched non-demented individuals. We found similar levels of soluble Aβ42, insoluble and soluble Aβ40 in both APPsw mutation carriers and SAD. However, lower levels of insoluble Aβ42 were detected in the frontal and temporal cortex of APPsw brain. In PS1 brain, insoluble Aβ40 and Aβ42 levels were significantly lower in all four cortical regions compared with SAD, whilst levels of Aβ40 were lower in frontal and occipital cortex compared with APPsw brain. The insoluble Aβ42/40 ratio was similar in SAD and APPsw but significantly higher in PS1 mutation carriers. Our results indicate that the pattern of Aβ deposition in PS1 mutation carriers differs from that in both APPsw and SAD, whereas the pattern in APPsw mutation carriers is more similar to that in SAD.

1. Introduction

Intracellular neurofibrillary tangles and extracellular senile plaques mainly composed of different amyloid-β (Aβ) species are the major pathological hallmarks of Alzheimer's disease (AD) (Selkoe, 2004). Aβ is generated from the amyloid precursor protein (APP) by enzymatic cleavage involving β-secretase and the γ-secretase complex, which includes presenilin activities (Evin and Weidemann, 2002; Selkoe and Schenk, 2003; Marks and Berg, 2008; Pákáski and Kálmán, 2008). In addition to the central role of Aβ and hyperphosphorylated tau protein as causative factors for the disease, accumulating evidence indicate that dysregulation of calcium homeostasis may be a key factor in AD pathogenesis (Bojarski et al., 2008). Although the majority of AD cases (>90%) typically occur after the age of 60–65 years, a smaller proportion of cases correspond to early-onset (<60 years) familial AD (FAD). Autosomal-dominant forms of FAD result from mutations in one of three genes: APP on chromosome 21, presenilin 1 (PS1) on chromosome 14 and presenilin 2 (PS2) on chromosome 1. Mutations in the PS1 gene are the most frequent cause of early-onset FAD, and more than 160 mutations in 355 families have been reported, whereas AD families carrying APP mutations are less frequent (www.molgen.ua.ac.be/ADMutations). Mutations in these three genes share a common feature of abnormal processing of APP and an earlier disease onset with an average age of onset at 50 years for APP mutations, 45 years for PS1 mutations and 52 years for PS2 mutations (Gómez-Isla et al., 1999). Except for the age of onset and family history no pathological features that distinguish FAD from sporadic AD (SAD) have been reported (Ray et al., 1998; Menédez, 2004). Both SAD and FAD share specific neuropathologic features, including neuritic plaques, neurofibrillary tangles and neuropil threads (Lippa et al., 1996). A variant form of AD with spastic paraparesis is characterized by large "cotton wool" plaques and is caused by genomic deletion of PS1 exon 9 (Crook et al., 1998; Verkkoniemi et al., 2000). The plaques are immunoreactive for Aβ but lack congophilic dense cores. In some studies clinical differences between FAD and SAD have been found, such as unusual behavioural and psychiatric changes, seizures and myoclonus (Haltia et al., 1994; Lleó et al., 2004; Menédez, 2004).

Neuropathological characterization of the cortex of several cases with PS1 mutations revealed a predominant detection of Aβ42 (Mann et al., 1996a, 2001). Similar increases in Aβ42 levels have been observed in plasma and conditioned medium from skin fibroblasts from subjects carrying PS1 mutations (Scheuner et al., 1996), as well as in cells stably transfected with mutated PS1 and in the brain of transgenic mice expressing mutant PS1 (Borchelt et al.,

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Table 1
Demographics of AD patients and non-demented controls.

Group	Gender, female/male	Apo E genotype	Age of onset (years)	Age at death (years)	Disease duration (years)
PS1					
1	F	3/4	37	47	10
2	F	3/4	39	51	11
3	F	3/3	37	43	5
Mean ± S.E.			38 ± 1*	47 ± 2*	9 ± 2
APPsw					
1	M	4/4	45	56	11
2	F	3/3	50	62	12
3	M	2/3	61	66	5
4	M	2/3	56	68	12
5	M	3/3	53	62	9
Mean ± S.E.			53 ± 3	63 ± 2	10 ± 1
Sporadic AD	8F/1M	4/4(3), 3/4(2), 3/3(3), 3/2(1)	75.3[a]	84 ± 3	8.3[b]
Controls	3F/3M	3/3(5), 4/3(1)		46 ± 1	
Controls	5F/2M	3/3(4), 4/3(3)		68 ± 12	
Controls	4F/1M	3/3(4), 3/2(1)		88 ± 2	

[a] From Engelborghs et al. (2003), 504 patients.
[b] From Gómez-Isla et al. (1999), 51 patients.
* Significantly different from APPsw mutation carriers ($P < 0.01$).

1996; Duff et al., 1996; Citron et al., 1997). PS1 mutations have been reported to increase the Aβ42/40 ratio (Murayama et al., 1999; Takeda et al., 2004; Duering et al., 2005). Yet, recent studies on mutant PS1 expressing cells suggest that the increased Aβ42/40 ratio may reflect a reduction of Aβ40 rather than an increased production of Aβ42 (Bentahir et al., 2006; Shimojo et al., 2007). The increased proportion of Aβ42, which is more prone than Aβ40 to aggregate (Jarrett et al., 1993; McGowan et al., 2005), is thought to initiate the disease process. Mutations in the APP gene either cause increased production of both Aβ40 and Aβ42 or just Aβ42 alone (Citron et al., 1992; Mann et al., 1996b; Tamaoka et al., 1998).

Up to date, no detailed comparisons of Aβ levels in rare dominant mutation carriers with common late-onset AD have been performed even though several neuropathological descriptions of FAD cases have been published. However, the neuropathological observations have been based on a small numbers of patients or on individual cases carrying different PS1 or APP mutations. Differences in the levels of Aβ peptides and their regional distribution in FAD and SAD cases may provide an understanding of the molecular mechanisms by which Aβ is deposited in these forms of the disease and may also be an important consideration in future Aβ vaccination therapies. This study is the first to report the distribution and levels of soluble and insoluble Aβ species in cortical regions of FAD and SAD brain. Our studies were conducted using postmortem brain tissue obtained from five relatives who carried the Swedish APP double mutation (KM670/671NL) (Axelman et al., 1994; Lannfelt et al., 1994) and three PS1 M146V mutation carriers from a Finnish/Swedish family (Haltia et al., 1994; Clark et al., 1995). In the Swedish APP 670/671 and PS1 M146V mutation families, a history of AD has been traced through eight and four generations, respectively.

2. Materials and methods

2.1. Postmortem human brain tissues

Frontal, temporal, parietal and occipital cortices from five FAD cases with the Swedish APP670/671 double mutation and three cases with the PS1 M146V mutation were obtained from the Huddinge Brain Bank, Karolinska University Hospital Huddinge, Sweden. Neuropathological examination in autopsy brain of APPsw and PS1 mutation carriers confirmed the clinical diagnosis of AD in these families, and a large number of neuritic plaques were detected throughout the cortex (Lannfelt et al., 1994; Mann et al., 1996a; Marutle et al., 1999; Bogdanovic et al., 2002). Brain tissue from nine sporadic AD cases and 18 control individuals were obtained from the Netherlands Brain Bank. Autopsies were performed on donors from whom written informed consent was obtained either from the donor or from next of kin. The clinical diagnosis of dementia was performed according to the NINCDS-ADRDA criteria (McKhann et al., 1984) and all subjects were confirmed with AD and met the established CERAD criteria. The control individuals had no known history or symptoms of neurological or psychiatric disorders. The individual case histories of the FAD subjects are listed in Table 1. Samples from each of the four cortical regions were obtained from most cases.

2.2. Measurements of Aβ levels

Brain tissue (150–200 mg) was homogenized in 7 volumes of 20 mM Tris–HCl, pH 8.5 including protease inhibitors (Complete, Roche Diagnostics) and then centrifuged at 100,000 × *g* at 4 °C for 1 h. Soluble fractions of Aβ40 and Aβ42 were measured in the supernatant. For quantification of Aβ in the insoluble fraction, the pellet was extracted with 10 volumes of 5.0 M guanidine–HCl in 20 mM Tris–HCl, pH 8.0 and then diluted 1:10 with phospho-buffered saline containing 0.5% bovine serum albumin, 0.05% Tween 20 and protease inhibitor (standard buffer) and centrifuged at 13,100 × *g* for 25 min at 4 °C. This guanidine–HCl-extractable fraction is hereafter referred to as insoluble Aβ. The supernatants were further diluted with standard buffer plus 0.1 M guanidine–HCl, before assays in order to analyze all samples in the linear range of the ELISA. The levels of Aβ40 and Aβ42 were analyzed by colorimetric sandwich ELISA kits according to the manufacturer's instructions (Signal Select™ Human β Amyloid 1-40 and 1-42 kit, respectively, BioSource International). The C-terminal-specific ELISA uses a monoclonal capture antibody directed against the first 16 amino acid residues of the N-terminal region of human Aβ and 2 other antibodies specific for Aβ1-40 and Aβ1-42. Since the epitope is close to the truncated end of the N-terminal (starting at residue 11) there may be some capturing of truncated Aβ in addition to intact full-length Aβ. Hence, hereafter Aβ40 and Aβ42 indicate full-length forms and possible truncated Aβ forms. The Aβ40 and Aβ42 levels were calculated by comparison with a standard curve of synthetic human Aβ1-40 and Aβ1-42. For each Aβ species quantified, all samples from the three AD groups and controls were run in the same ELISA. The Aβ levels were expressed as pg/mg tissue, calculated from the original brain weight measurement before homogenization.

2.3. Data analysis

For group comparisons, data are given as mean values ± S.E. and analyzed by one-factor ANOVA. The relationship between Aβ40 and Aβ42 and between Aβ and age were based on Spearman's rank order correlation coefficient.

3. Results

3.1. Distribution of insoluble (guanidine-extractable) and soluble Aβ40 and Aβ42 in familial and sporadic AD cases

In both SAD cases and patients carrying the APPsw mutation the highest levels of insoluble Aβ40 were found in the frontal cortex followed by occipital cortex having just slightly lower Aβ40 levels (Fig. 1A, and Table 2). There was no significant difference in mean Aβ40 levels between the four cortical regions in SAD, but in APPsw brain the level in temporal cortex was significantly lower