Acta Neuropathol (1999) 97 : 82–84

SHORT ORIGINAL COMMUNICATION

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A high incidence of apolipoprotein E ε4 allele in middle-aged non-demented subjects with cerebral amyloid β protein deposits

Received: 16 March 1998 / Revised, accepted: 29 June 1998

Abstract We examined the apolipoprotein E (ApoE) genotypes of 19 middle-aged non-demented subjects with cerebral amyloid β protein (Aβ) deposits, and compared the results with those of 16 patients with sporadic Alzheimer's disease (AD) and those of 34 age-matched controls. The frequency of the ApoE ε4 allele was higher (P = 0.0256) in these 19 subjects (0.211) than in controls (0.059), and was close to that in AD patients (0.281). This result suggests that middle-aged non-demented subjects with cerebral Aβ deposits are at high risk of developing AD, and that the diffuse Aβ deposits in these cases represent an early stage of AD pathology. We speculate that in the majority of late-onset sporadic AD patients, cerebral Aβ deposition commences when these patients are in their forties or fifties, and that the pathological process progresses gradually, taking 20 to 30 years for clinical manifestation of dementia.

Key words Apolipoprotein E · Alzheimer's disease · Amyloid β protein · Middle-age

Introduction

Deposition of amyloid β protein (Aβ) and neurofibrillary tangles (NFTs) are the neuropathological hallmarks of Alzheimer's disease (AD). These pathological changes in the brains of AD patients are believed to progress gradually, taking a long time to cause clinical symptoms of dementia. It is important to identify the initial changes and when the processes start, to develop an appropriate therapeutic strategy. In Down's syndrome (DS), the disease process commences with cerebral Aβ deposition in the form of diffuse plaques, when patients are in their twenties or thirties [10, 11]. In normal aging, the diffuse plaques first appear in some subjects in their forties [9, 14] or fifties [3]. However, it remains unclear whether such cases represent a preclinical stage of AD, since diffuse plaques are also found in a significant number of elderly non-demented subjects. To address this point, we studied the apolipoprotein E (ApoE) allele frequency in middle-aged non-demented subjects who had cerebral Aβ deposits, but had no infarctions or NFTs in the cerebral neocortex.

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Materials and methods

We examined autopsied brains from 177 unselected subjects between the ages of 40 and 59 years, from the brain bank at Tokyo Institute of Psychiatry. Neither dementia nor neurological complications were documented in these subjects from a retrospective examination. The brains were fixed in 10% formalin and cut in the coronal plane. Paraffin-embedded hemisphere sections (10 μm thick) including the hippocampal region were stained by methenamine-silver staining. The temporal, parietal, cingulate, insular and parahippocampal cortices and hippocampus in these sections were examined for senile plaques. Deposition of Aβ was further confirmed by immunohistochemical staining with a rabbit antibody (E50) against Aβ 17–31 [6]. Moreover, plaques were examined immunohistochemically with end-specific anti-Aβ 40 and anti-Aβ 42 antibodies, which recognized the 40 and 42 ends of Aβ, respectively [4]. The presence of NFTs was investigated using the modified Gallyas-Braak method in the same cerebral regions. We excluded cases with neocortical NFTs since such cases might represent early stages of AD.

The ApoE allele frequency of selected Aβ-positive patients was compared with that of 34 age-matched controls (mean 51.9 ± 6.2 years, range 41–59 years) and 16 sporadic AD subjects (mean 79.4 ± 7.6 years, range 65–89 years). To determine the ApoE allele genotype, genomic DNA was extracted from formalin-fixed paraffin-embedded, or frozen brain tissues. Polymerase chain reaction (PCR) and *Hha*I digestion was carried out as described previously

Table 1 The prevalence of amyloid β protein (Aβ) deposits in middle-aged non-demented subjects

Age (years)	*n*	Aβ deposits
40–49	61	5/61 (8.1%)
50–59	116	20/116 (17.2%)
Total	177	25/177 (14.1%)

Table 2 Allele frequency of apolipoprotein E

Allele	Present cases (*n* = 38)	Controls (*n* = 68)	Sporadic AD (*n* = 32)
ε4	0.211*	0.059	0.281
ε3	0.711	0.882	0.719
ε2	0.079	0.059	0

* $P = 0.0256$ vs controls; chi-square test

[5]. Differences in the frequency of ApoE genotype between groups were evaluated for significance using the χ^2 test.

Results

By methenamine-silver staining, senile plaques in the cerebral cortex were found in 25 of a total of 177 subjects (14.1%). Of the 25 positive subjects, 5 were in their forties (8.1%) and 20 in their fifties (17.2%) (Table 1). These findings were comparable with those previously reported by Sugihara et al. [14]. Of the 25 cases 6 were excluded because of the presence of cerebral infarctions or neocortical NFTs. The mean age at the time of death of the remaining 19 positive cases was 54.2 ± 5.2, with a range of 43–59 years. According to the three-grade staging of Aβ deposits by Braak and Braak [1], 9 cases were classified as stage A and 10 as stage B. Plaques were found to be predominantly of the diffuse type in 17 of the 19 cases. The degree of staining of plaques with anti-Aβ 42 was comparable to that with E50, and stronger than that with anti-Aβ 40 in all cases (data not shown).

ApoE allele frequencies in these 19 subjects as well as in controls and sporadic AD subjects are summarized in Table 2. The frequency of the ApoE ε4 allele was higher ($P = 0.0256$) in our 19 cases (0.211) than in controls (0.059), and was close to that in sporadic AD patients (0.281).

In the parahippocampal cortex, we found a few NFTs in 7 of the 19 cases with cerebral Aβ deposits. The number of NFTs in these cases indicated stage I–II according to Braak and Braak [1]. The remaining 12 cases had virtually no NFTs even in this region. There was no significant difference between the cases with NFTs and those without NFTs in the frequency of ApoE ε4 allele.

Discussion

Many investigators consider that the pathological changes of AD progress gradually and asymptomatically for a long period before they reach a certain degree of severity beyond the tolerance of each patient, and clinical symptoms of dementia become apparent. It is difficult, however, to determine the time of onset of these processes in AD brains. In DS, Aβ deposits begin when the patients are in their twenties or thirties [10, 11], and dementia becomes evident when patients in their fifties [7]. Thus, it takes 20–30 years from the beginning of AD changes until the clinical manifestation of dementia in DS cases [7]. However, it is not certain whether the same processes and time span apply to ordinary AD. Although some neurologically normal, non-demented subjects have been reported to have cerebral Aβ deposits in middle age [3, 9, 14], there is no evidence that they would have developed AD had they lived longer. It has been established that the incidence of the ApoE ε4 allele is much higher in the population of AD patients than in the age-matched control population [12]. In our cases with Aβ deposits, the ApoE ε4 allele frequency was unusually high and was comparable to that in AD cases. It seems reasonable to speculate that the subjects in this group are at high risk of developing AD and that the diffuse Aβ deposits in these cases represent an early stage of AD pathology. The results of this study suggest that in late-onset sporadic AD, the pathological process in the preclinical stage is similar to that in DS, taking 20–30 years for clinical manifestation of dementia.

Another implication of our study is that the ApoE ε4 allele appears to contribute to the increased Aβ deposits in the brain. This is consistent with previous reports which demonstrated that ApoE4 promoted Aβ fibril formation in vitro [8] and increased Aβ deposition in vivo [13].

Braak et al. [2] have argued that NFTs precede the deposition of Aβ since they are present in the parahippocampal cortex of a number of non-demented subjects without Aβ deposits. In our series, however, 12 of 19 cases had virtually no NFTs even in the parahippocampal cortex, and 4 of 12 NFT-free cases had at least one ApoE ε4 allele. Thus, the parahippocampal NFTs and the neocortical Aβ deposits seem to occur independently in the pathological process of AD rather than one being a cause of the other. Clearly, the ApoE allele frequency of NFT-bearing, non-demented subjects must be the subject of future investigations.

Acknowledgement It is great pleasure to express our gratitude to Dr. H. Shiraishi, MD, Professor of Psychiatry, Institute of Clinical Medicine, University of Tsukuba, for his advice and encouragement.

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