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Apolipoprotein E mRNA in the brains of patients with Alzheimer's disease

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Abstract

To clarify the role of apolipoprotein E (apoE) in the pathophysiology of Alzheimer's disease (AD), we used an RT-PCR method to investigate apoE and glial fibrillary acidic protein (GFAP) mRNA expression in the brain. ApoE mRNA was significantly more abundant in AD (0.379 ± 0.191, mean $\pm$ SD) than in control (0.125 ± 0.073, $p < 0.05$) brain tissue, but in AD it was decreased in relation to the apoE-ϵ4 gene dosage. The GFAP mRNA content also was greater in AD (5.96 ± 2.94) than in control (3.80 ± 2.78) tissue, and in AD showed an increase relative to the apoE-ϵ4 gene dosage. AD patients who had long survival times showed high expression of apoE and low expression of GFAP. These results suggest that apoE suppresses the progression of AD, including gliosis, in the brain.

Keywords: Alzheimer's disease; Apolipoprotein E; Glial fibrillary acidic protein; PCR

1. Introduction

Alzheimer's disease (AD) is a common cause of dementia in late adulthood. Most cases are considered sporadic, although familial cases (FAD) are well documented. Some cases of early-onset FAD are associated with mutations in the Alzheimer amyloid precursor protein (APP) gene (Goate et al., 1991) or in an unidentified gene on chromosome 14 (Schellenberg et al., 1992). Possible linkage of late-onset FAD to a locus on chromosome 19 has been reported (Pericak-Vance et al., 1991). The apolipoprotein E (apoE) gene has been mapped at that locus as well as a candidate gene associated with late-onset FAD. ApoE has three alleles, ϵ2, ϵ3 and ϵ4, and studies of apoE allelic variation have shown increased frequency of ϵ4 in both late-onset FAD (Strittmatter et al., 1993a) and sporadic AD (Saunders et al., 1993; Rebeck et al., 1993), suggesting that an apoE-ϵ4 gene dose is a major risk factor in AD.

We investigated the expression of apoE mRNA in AD brains to gain understanding of the role of apoE in the pathophysiology of AD. Results showed that apoE expression is increased in AD brains, especially in patients with long survival times, which suggests that apoE may suppress AD progression.

2. Materials and methods

2.1. Cases

The 9 cases of AD studied were all sporadic, female, and autopsy-confirmed. The patients' ages at onset and survival time, respectively, ranged from 48 to 85 years and from 4 to 23 years (Table 1). Senile dementia of the Alzheimer type was interpreted as being late-onset AD. Frozen brain tissue (temporal cortex) was obtained for all 9 patients. The controls were fresh brain tissue (frontal cortex) resected surgically from 2 patients with glioma (a 39-year-old man and a 32-year-old woman) and frozen brain tissue (temporal cortex) from one patient with gastric cancer (a 69-year-old woman).

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2.2. Neuropathological studies

The densities of the neuritic plaques and neurofibrillary tangles (NFTs) in the hippocampus or temporal cortex were assessed qualitatively by silver impregnation methods (Bodian or Hirano staining) in 5 of the patients with AD.

2.3. ApoE genotyping

DNA was extracted from the frozen brain tissue by digestion with 200 μg/ml proteinase K in lysis buffer (50 mM Tris-HCl (pH 8.0), 100 mM EDTA and 0.5% SDS) at 55°C overnight. ApoE genotyping was done by PCR and restriction analysis as described elsewhere (Wenham et al., 1991).

2.4. Reverse-transcriptase polymerase chain reaction (RT-PCR)

Total RNA was extracted from the frozen brain tissue by the acid guanidium thiocyanate/phenol/chloroform method (Chomczynski and Sacchi, 1987). cDNA was synthesized with a first-strand cDNA synthesis kit (Pharmacia), 1 μg of total RNA, and random primer (6-mer) in a 15-μl reaction mixture at 37°C for 60 min. Reverse transcription was terminated by heating the reaction mixture at 94°C for 5 min.

The ApoE primers were

pA1 TCAGCTCCCAGGTCACCCAG (3172–3191 nt, Das et al., 1985)

pA2 GCGCCGCCTGCAGCTCCTTG (3899–3880 nt)

The glial fibrillary acidic protein (GFAP) primers were

pG1 GAGGAAGATCCACGAGGAG (603–621 nt, Reeves et al., 1989)

pG2 GAGGTCTGGCTTGGCCACGT (696–677 nt)

The β-actin primers were

pB1 TCTACAATGAGCTGCGTGTG (399–418 nt, Nakajima-Iijima et al., 1985)

pB2 TACATGGCTGGGGTGTTGAA (969–950 nt)

The PCR reaction mixture, which contained 1 μl of the above cDNA reaction product, 100 nM of each pair of primers, 200 μM dNTP, 33 nM [α-^{32}P]dCTP (3000 Ci/mmol, Amersham), and 0.25 unit of AmpliTaq (Perkin Elmer Cetus, CT) in a final volume of 10 μl, was subjected to 25 cycles of amplification (denaturation at 94°C for 30 sec, annealing at 55°C for 1 min and extension at 72°C for 30 sec) then extension at 72°C for 10 min. The amplified product from each mRNA was confirmed by DNA sequencing after subcloning with a TA cloning kit. Each sample was electrophoresed on a 10% native polyacrylamide gel, after which the radioactivity of the amplified band was counted with an Image Analyzer Fujix BAS2000A (Fuji Film, Japan).

2.5. Statistical analysis

The ratio of the radioactivity of the amplified product from the apoE or GFAP mRNA to that from the β-actin mRNA was calculated to normalize the individual mRNA contents in the brain samples. Student's *t*-test was used to compare the values between the AD and control groups. The correlations between the mRNA contents in the brain and survival time (from onset until death) also were assessed in the AD group.

3. Results

3.1. ApoE4 allele frequency and neuropathology

The respective allele frequencies of ε2, ε3 and ε4 were 0.0%, 72.2% and 27.8% for the AD samples, and 4 of the 8 patients whose age at onset was more than 60 years had the ε4 allele (Table 1). The survival

Table 1
Summary of AD characteristics

Patient	Age at onset (yrs)	Survival time (yrs)	ApoE genotype	NFT/plaque *	
				Hippocampus	Temporal cortex
1	48	19	3/3	n.e.	n.e.
2	60	23	3/3	n.e.	n.e.
3	63	12	3/4	n.e.	n.e.
4	72	7	3/4	n.e.	n.e.
5	85	17	3/3	++/+	−/−
6	75	8	3/3	±/+	−/−
7	78	8	3/3	++/+	−/+
8	72	7	3/4	++/++	±/+
9	75	4	4/4	++/+	+/++

* Densities of NFT and plaque in the hippocampus and temporal cortex: ++, abundant; +, moderate; ±, sparse and −, none. n.e., not evaluated.

period was 7.5 ± 3.3 years (mean ± SD) for AD with the ε4 allele and 16.8 ± 6.3 years for AD without it; significantly shorter for the former ($p < 0.05$). Age at onset did not differ between the two AD groups. Neuropathological studies showed numerous senile plaques and NFTs in the hippocampus of all the AD brains. Evaluation of 5 AD cases using silver impregnation methods showed that the plaque and NFT densities were higher in patients with the ε4 allele than in those without it and that the difference was more marked in the temporal cortex than in the hippocampus (Table 1, Fig. 1).

3.2. Quantification of mRNAs by RT-PCR

Because the RNA from the frozen brain samples was not suitable for northern blot analysis, RT-PCR was used to quantify the mRNAs. β-Actin was used as the internal control for the quantitation of PCR. The primers for the RT-PCR of each mRNA are located in exons 3 and 4 of the corresponding gene, and RT-PCR should generate a fragment of 147 bp (apoE), 94 bp (GFAP) or 130 bp (β-actin). RT-PCR conditions first were examined to obtain an accurate estimate of the amounts of mRNA. Calculations made every 5 cycles showed that amplification by PCR was exponential between 20 and 35 cycles (data not shown). When the amplification cycle number was fixed at 25, the amplification rates of the individual mRNAs were equivalent between 0.5 and 4 μg of RNA (Fig. 2). Therefore, subsequent RT-PCR was done with 1 μg of total RNA for 25 cycles. Under these conditions, the content of apoE or GFAP mRNA in the sample could be normalized as a ratio of the β-actin mRNA present.

3.3. Expression of apoE and GFAP mRNAs in brains

The normalized apoE mRNA contents in the brain ranged from 0.078 to 0.210 (0.125 ± 0.073, mean ± S.D.) for the control group and from 0.114 to 0.759 (0.379 ± 0.191) for the AD group; significantly higher in the AD group ($p < 0.01$) (Fig. 3A). The content range was 0.274–0.759 (0.451 ± 0.188) for AD with ε3/ε3; 0.114–0.456 (0.334 ± 0.193) for AD with ε3/ε4; but 0.161 for AD with ε4/ε4. Although these differences were not statistically significant, apoE expression was decreased by an apoE-ε4 gene dosage.

The normalized GFAP mRNA content range was 1.87–6.99 (3.80 ± 2.78) for the control group and 3.03–12.7 (5.96 ± 2.94) for the AD group (Fig. 3B), being more abundant in the latter but without significance. The range was 3.03–5.27 (4.39 ± 0.94) for AD with ε3/ε3; 4.18–7.79 (6.35 ± 1.9) for AD with ε3/ε4; but 12.7 for AD with ε4/ε4, indicative that GFAP expression was increased in AD with the ε4 allele.

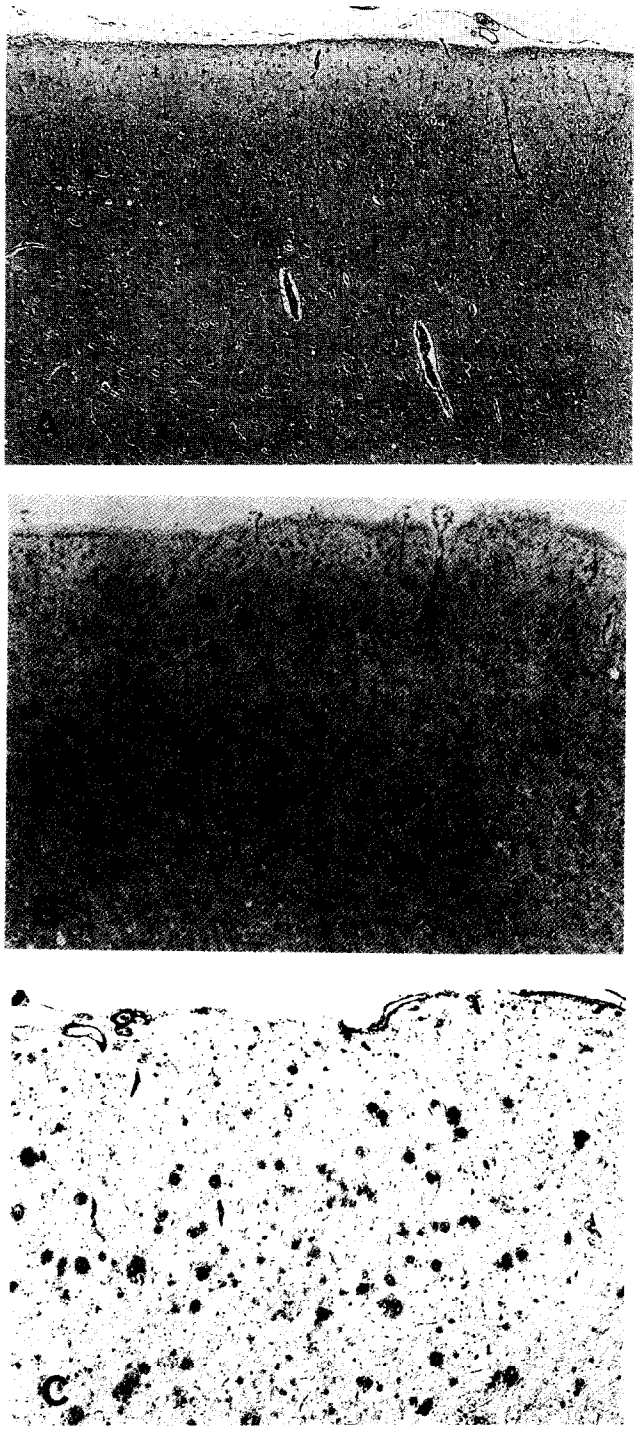

Fig. 1. Neuropathology of the temporal cortex. A: case 5, AD with ε3/ε3. Neither senile plaques nor NFTs are present. Bodian staining, ×45. B: case 8, AD with ε3/ε4. Numerous senile plaques and a small number of NFTs are present. Bodian staining, ×45. C: case 9, AD with ε4/ε4. Numerous senile plaques and NFTs are present. Hirano staining, ×45.

The correlative coefficient of the apoE and GFAP mRNA contents in the brain was −0.57 in the AD group, but was not statistically significant (Fig. 4).

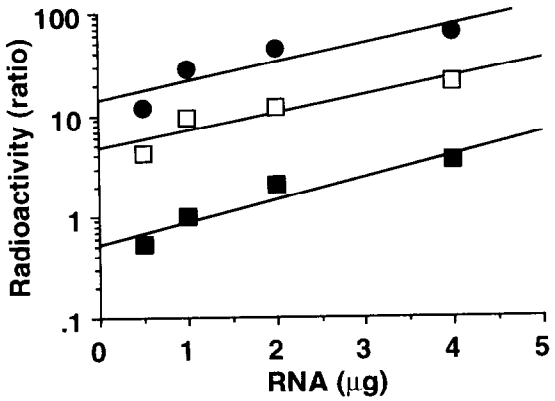

Fig. 2. Quantification of mRNAs (apoE, GFAP and β-actin) in the brain done with the RT-PCR technique. Radioactivity incorporated into the amplified fragments is plotted versus the amount of total RNA used in the reaction. The PCR cycle was fixed at 25. The standard curves that express the amplification rates of the mRNAs are parallel, indicative of equivalent rates. Closed squares: apoE, closed circles: GFAP, open squares: β-actin.

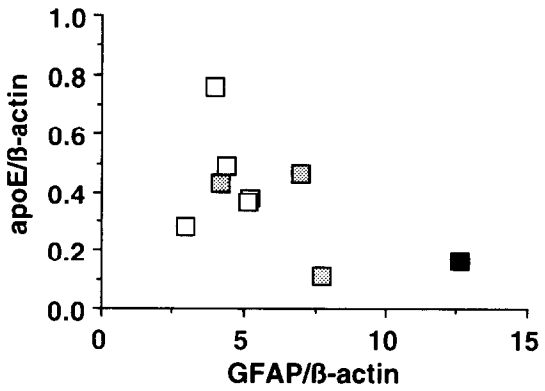

Fig. 4. Relationship between GFAP and apoE mRNA expression in AD. Open squares: ε3/ε3, stippled squares: ε3/ε4, closed square: ε4/ε4.

The respective correlative coefficients for age at onset and the apoE or GFAP mRNA content, respectively, were −0.779 ($p < 0.01$) and 0.124 (not significant) (Fig. 5A and B). The respective correlative coefficients for survival time and apoE or GFAP mRNA content were 0.621 ($p < 0.05$) and −0.612 ($p < 0.05$) (Fig. 5C and D).

4. Discussion

Our results are limited by the small numbers of cases but could provide a deeper insight in the role of apoE in the AD pathophysiology. ApoE-ε4 frequency was increased in our AD cases as compared with the value reported for the Japanese population, 9.3% (Noguchi and Murakami, 1993). This is consistent with previous reports (Saunders et al., 1993; Rebeck et al., 1993; Noguchi and Murakami, 1993). The gene dosage of the ε4 allele reduces the age of AD onset (Corder et al., 1993). Our studies did not confirm this but did indicate that the presence of the ε4 allele reduced survival time; i.e. it accelerated the progress of the illness. Neuropathological studies also showed that the extent of plaque and NFT formation is more intense in AD with the ε4 allele, which is consistent with previous reports (Rebeck et al., 1993; Schmechel et al., 1993). Thus, our results support the contention that the presence of apoE-ε4 is a risk factor for late-onset AD.

The apoE mRNA in the brain was increased in the AD patients, which is consistent with a previous report by Diedrich et al., 1991, who reported that apoE mRNA increased in astrocytes parallel with the increase in GFAP mRNA. Also our results showed that although GFAP mRNA increased in AD, the correlationship

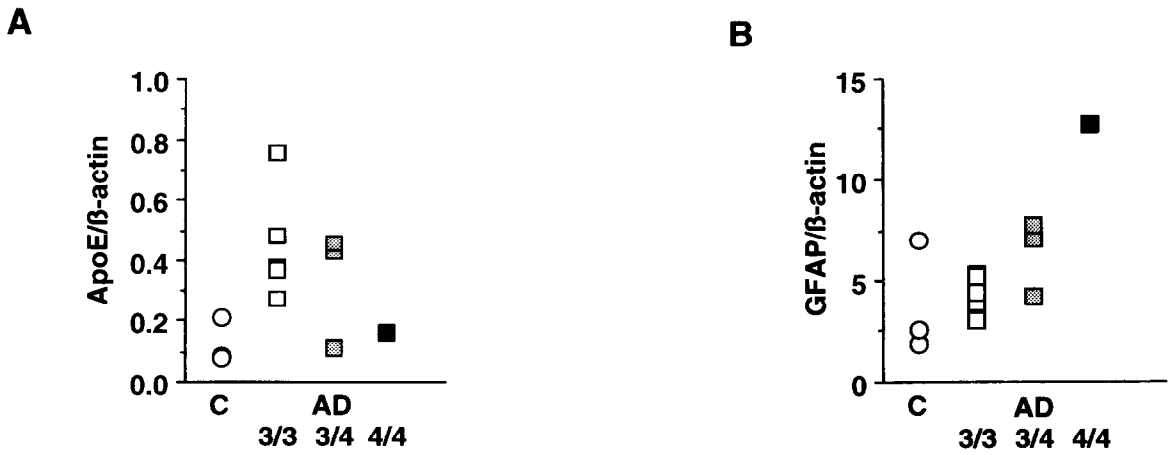

Fig. 3. mRNA expression in the brain as estimated by RT-PCR. The mRNA content is normalized in reference to β-actin mRNA. C: control; numbers below the abscissa are the apoE genotypes. A: apoE mRNA is significantly more abundant in the entire AD group than in the control group. * $p < 0.01$, Student's t-test. ApoE expression is lower in AD with the ε4 allele. B: GFAP mRNA is more abundant in the entire AD group than in the control group, but without significance. GFAP expression is higher in AD with the ε4 allele.

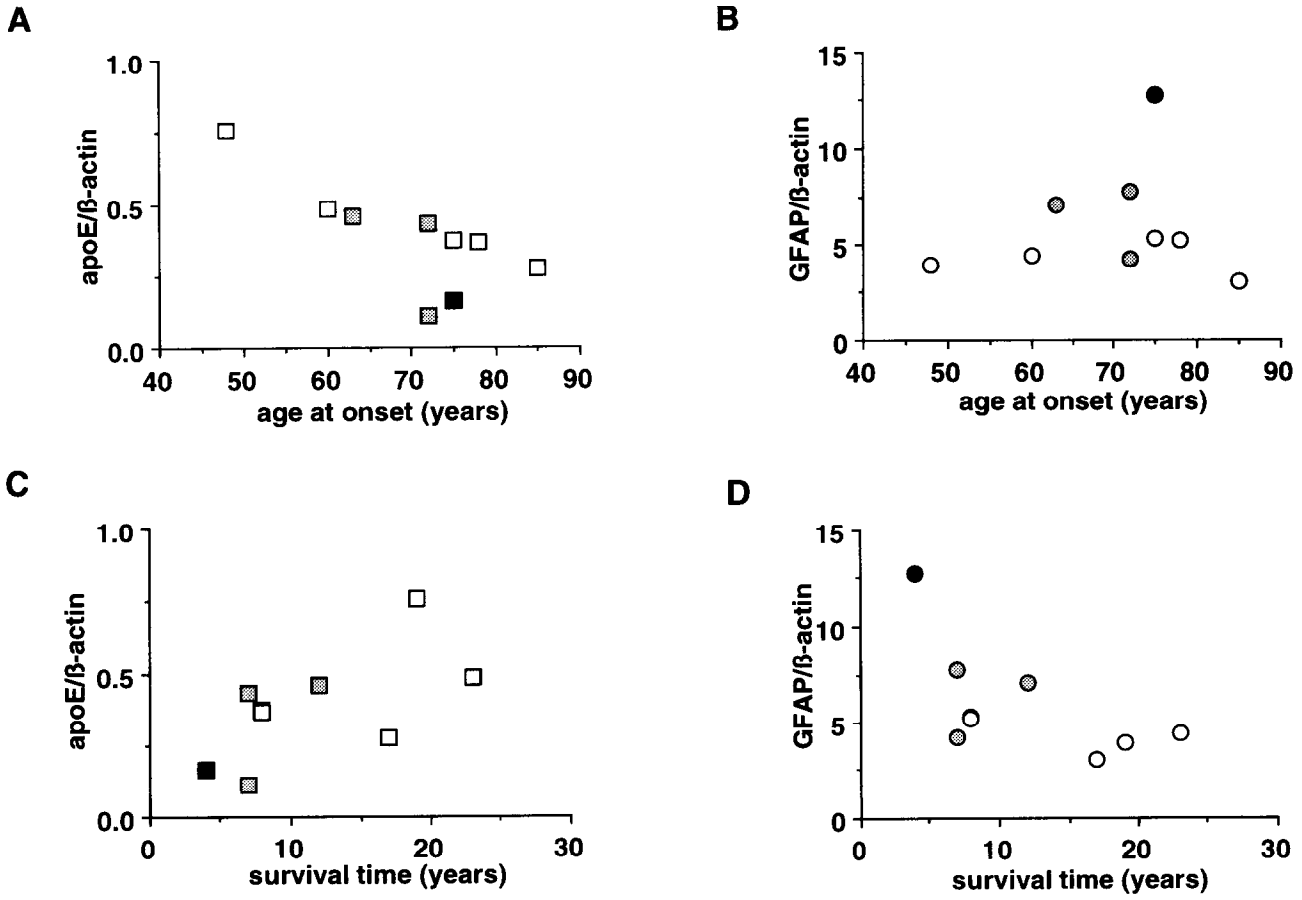

Fig. 5. Relationship between age at onset or survival time and mRNA expression in AD. Open squares or circles: $\epsilon 3/\epsilon 3$, stippled squares or circles: $\epsilon 3/\epsilon 4$, closed square or circle: $\epsilon 4/\epsilon 4$. A: ApoE mRNA content versus age at onset. B: GFAP mRNA content versus age at onset. C: ApoE mRNA content versus survival time. D: GFAP mRNA content versus survival time.

between the two mRNAs was negative, which indicates that the increase in apoE expression is not merely a reflection of gliosis. ApoE mRNA expression paralleled survival time in our study, which suggests that apoE may protect against the progression of AD. ApoE is present in senile plaques in AD (Namba et al., 1991; Kida et al., 1994). It binds to a synthetic amyloid β peptide (Strittmatter et al., 1993b), which is indicative of a relationship between apoE and amyloid deposition in the brain. The more intense amyloid deposition seen in AD with the $\epsilon 4$ allele suggests that apoE-$\epsilon 4$ may have low protective potency against AD pathogenesis. Further studies are necessary to confirm these speculations.

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