sporadic or FAD. Furthermore, no unique pattern of duplication was observed at these loci in four clinically demented DS patients (mean age ± SD, 61 ± 5.4 years), compared to four nondemented DS patients (59 ± 5.4 years).

It is conceivable that in AD, somatic cells may suffer nondisjunction or some other event that would randomly duplicate either copy of chromosome 21. Under these circumstances, the signal intensities of the two allelic fragments for a given RFLP in DNA from large numbers of cells might be expected to be approximately equal, although both would be increased relative to normal. To rule out this possibility, we assessed the summed signal intensities of both alleles at the AP locus relative to the hybridization signal of a control locus on chromosome 4, D4S10 (Fig. 1) (*13*). Again, the analysis (Figs. 1 and 2) revealed no significant difference between DNA from AD and normal individuals ($P > 0.05$) but readily yielded evidence of increased dosage in DS relative to AD ($P < 0.0001$) and normal ($P < 0.0001$) (*14*).

Since gene duplication is only one of several mechanisms by which putative inherited defects on chromosome 21 might cause sporadic AD, we also examined the possibility of allelic association between sporadic AD and RFLPs at each of the genetic loci discussed above. Allelic association, which often results from linkage disequilibrium, provides a potential strategy to determine the proximity of polymorphic DNA segments to the sites of disease-producing mutations. In the case of sporadic AD, the demonstration of a significant allelic association at one of the chromosome 21 loci, especially the AP gene, would provide a persuasive argument for both a genetic etiology in sporadic AD and a primary role for this region of the chromosome in the pathogenesis of AD. This approach has been used to identify a candidate gene for cystic fibrosis (*15*). However, when the frequency of individual alleles in the 63 AD patients, all of whom were Caucasian, was compared to the frequency of the same alleles in the normal Caucasian population, no significant difference was observed for any of the chromosome 21 loci ($P > 0.10$ in all cases). The lack of an allelic association for RFLPs at the AP and *SOD*I loci with AD does not support the view that either of these genes plays a role in predisposition to the disorder. In the case of the anonymous markers linked to FAD, the lack of allelic association with AD is not surprising, because these markers are not located precisely at the site of the FAD mutation and are not in apparent linkage disequilibrium with FAD (*3*).

The absence of either gene duplication or allelic association, together with the deposition of amyloid in normal aged brain and the observation that FAD is not tightly linked to RFLPs in either the AP gene or the *SOD1* gene (*2, 10*), indicates that neither gene is the site of the primary defect causing AD. It remains unclear if the appearance of amyloid in AD brain is merely a by-product of neuronal death or if this protein plays an active but secondary role in the pathogenesis of the disorder.

REFERENCES AND NOTES

1. R. Katzman, *N. Engl. J. Med.* **314**, 964 (1986); W. A. Rocca *et al.*, *Ann. Neurol.* **19**, 415 (1986); R. Katzman, *Arch. Neurol. (Chicago)* **33**, 217 (1976).
2. L. L. Heston, A. R. Mastri, V. E. Anderson, J. White, *Arch. Gen. Psychiatry* **38**, 1085 (1981); A. Heyman *et al.*, *Ann. Neurol.* **14**, 507 (1983); C. Oliver and A. J. Holland, *Psychol. Med.* 16, 307 (1986); G. A. Chase, M. F. Folstein, J. C. Breitner, T. H. Beaty, S. G. Self, *Am. J. Epidemiol.* 177, 590 (1983); R. C. Mohs, J. C. Breitner, J. M. Silverman, K. L. Davis, *Arch. Gen. Psychiatry* **44**, 405 (1987).
3. P. H. St George-Hyslop *et al.*, *Science* **235**, 885 (1987).
4. J.-M. Delabar *et al.*, *ibid.*, p. 1390.
5. M. Schweber *et al.*, *Neurology* **37**, 222 (1987).
6. G. McKhann *et al.*, *ibid.* **34**, 939 (1984); Z. S. Khachaturian, *Arch. Neurol. (Chicago)* **42**, 1097 (1985).
7. Informed consent was obtained from all living individuals based on a reviewed and approved protocol.
8. J. F. Gusella *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **51**, 359 (1986); B. R. Seizinger *et al.*, *Cell*, in press.
9. D. R. Crapper-McLachlan *et al.*, *Ann. Neurol.* **15**, 329 (1984).
10. R. E. Tanzi *et al.*, *Science* **235**, 880 (1987); R. E. Tanzi *et al.*, *Nature (London)*, in press.
11. G. D. Stewart, P. Harris, J. Galt, M. A. Ferguson-Smith, *Nucleic Acids Res.* **13**, 4125 (1985).
12. N. Sacchi *et al.*, in preparation.
13. J. F. Gusella *et al.*, *Nature (London)* **306**, 234 (1983); J. F. Gusella *et al.*, *Science* **225**, 1320 (1984); J. F. Gusella *et al.*, *Nature (London)* **318**, 75 (1985).
14. Multivariate analysis was performed across disease and tissue type. The Normal, AD, and DS groups had relative absorbances (±SE) of 1.00 ± 0.04, 1.04 ± 0.02, and 1.55 ± 0.05, respectively. The calculated statistical power of the analysis was sufficient to detect an intergroup difference between AD and normal of 24% with an 80% probability ($\alpha = 0.05$).
15. X. Estivill *et al.*, *Nature (London)* **326**, 840 (1987).
16. P. C. Watkins *et al.*, *Nucleic Acids Res.* **13**, 6075 (1985).
17. R. Hallewell *et al.*, *ibid.*, p. 2017.
18. Brain specimens were obtained from the Massachusetts AD Research Center Brain Bank and from the National Neurological Research Bank, which is supported by grants from National Institute of Neurological and Communicative Disorders and Stroke, the National Institute of Mental Health, the National Multiple Sclerosis Society, the Veterans Administration, the Hereditary Disease Foundation, and the CEP program.
19. X. O. Breakefield *et al.*, *J. Neurogenet.* **3**, 159 (1986).

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The Amyloid β Protein Gene Is Not Duplicated in Brains from Patients with Alzheimer's Disease

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Complementary DNAs (cDNAs) encoding portions of the amyloid β protein were used to investigate possible amyloid gene duplication in sporadic Alzheimer's disease. A strategy employing two Eco RI restriction fragment length polymorphisms (RFLPs) detected by the amyloid cDNAs was used. RFLPs allow the detection of a 2:1 gene dosage in the DNA of any individual who is heterozygous for a particular RFLP. The amyloid gene regions homologous to the cDNAs used were not duplicated in the DNA from brains of individuals with sporadic Alzheimer's disease. Similar results were also obtained with a strategy employing a test for 3:2 gene dosage.

THE AMYLOID β PROTEIN (AP) IS A 4.2-kD peptide present in amyloid plaques, neurofibrillary tangles, and cerebrovascular deposits in the brains of individuals affected with Alzheimer's disease (AD) (*1, 2*) and in older patients with Down syndrome (DS) (trisomy 21) (*2*). Complementary DNAs (cDNAs) encoding AP have been isolated and mapped to the chromosomal region 21q11.2–21q21 (*3,*

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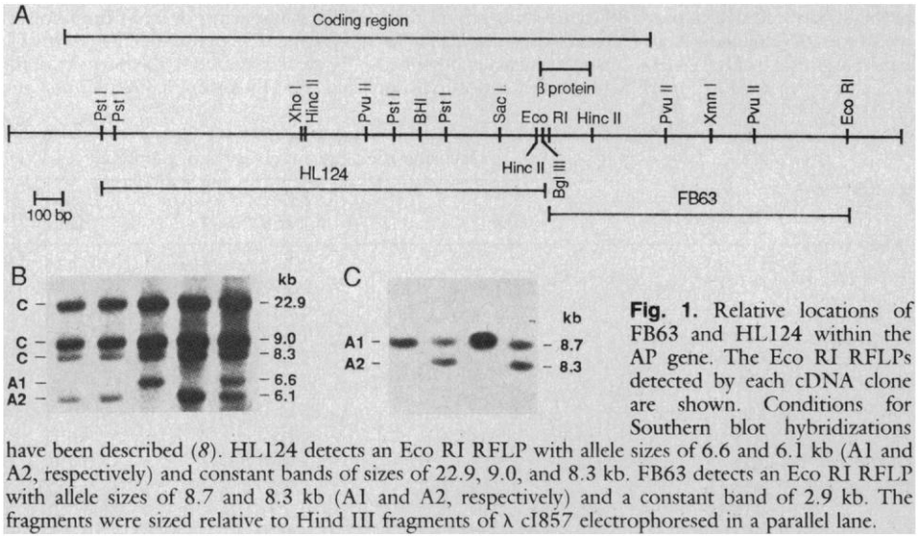

Fig. 1. Relative locations of FB63 and HL124 within the AP gene. The Eco RI RFLPs detected by each cDNA clone are shown. Conditions for Southern blot hybridizations have been described (*8*). HL124 detects an Eco RI RFLP with allele sizes of 6.6 and 6.1 kb (A1 and A2, respectively) and constant bands of sizes of 22.9, 9.0, and 8.3 kb. FB63 detects an Eco RI RFLP with allele sizes of 8.7 and 8.3 kb (A1 and A2, respectively) and a constant band of 2.9 kb. The fragments were sized relative to Hind III fragments of λ cI857 electrophoresed in a parallel lane.

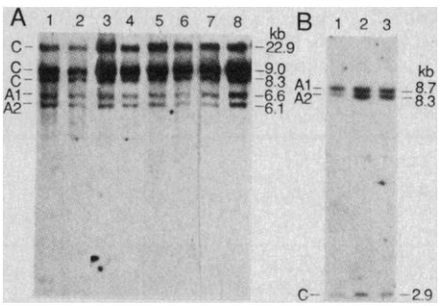

Fig. 2. Hybridization of HL124 and FB63 to Eco RI–digested DNAs from AD, DS, and control (UA) individuals heterozygous for the Eco RI RFLPs detected by these cDNAs. Postmortem brain regions from which DNA was isolated included Brodman areas 4, 17, and 24 and the cerebellum. DNA was extracted according to standard protocol (*9*) in the presence of 25 m*M* EDTA to minimize deoxyribonuclease activity. (**A**) Hybridization of HL124 to DNAs from AD (lanes 1 to 4) and control (lanes 5 to 8) individuals. Lane 1, AD 15; lane 2, AD 18; lane 3, AD 28; lane 4, AD 31; lane 5, UA 36; lane 6, UA 37; lane 7, UA 52; lane 8, UA 61. (**B**) Hybridization of FB63 to DNAs from DS 1 (lane 1), AD 17 (lane 2), and UA 6 (lane 3). This figure depicts a composite of lanes taken from several different autoradiograms.

4). Increased expression of the AP gene occurs in fetal DS brain relative to normal (*3*) and is probably the result of a 50% increase in AP gene dosage due to the presence of a third copy of chromosome 21. Thus, the abundance of amyloid plaques and deposits observed in AD brain might also be due to increased AP gene dosage. A duplication of the AP gene in leukocyte DNA from three patients with sporadic AD has been reported (*5*). This observation was based on a densitometer-detected 3:2 increase in the signal intensity of an 8.6-kb Eco RI band in three AD DNA samples when the DNAs were hybridized with a 1.1-kb cDNA encoding the 3′ end of the AP gene.

We have investigated possible AP gene duplication with both simple quantitative blotting and an alternate strategy employing restriction fragment length polymorphisms (RFLPs) detected by the AP cDNAs. RFLPs allowed us to detect a 2:1 gene dosage in the DNA of any individual who is heterozygous for a particular RFLP. Two Eco RI RFLPs are detected at the AP gene locus (Fig. 1). An infrequent Eco RI RFLP is revealed by the clone FB63, a 1.05-kb fetal brain cDNA representing the 3′ end of the AP gene. The alleles comprising this RFLP are 8.7- and 8.3-kb bands with frequencies of 0.96 and 0.04, respectively, in a panel of random unaffected individuals (n = 70). A more frequent Eco RI RFLP is detected by a 1.6-kb cDNA, HL124, which was isolated from an HL60 promyelocytic leukemia cell line cDNA library and contains the coding region of the AP gene immediately 5′ to FB63. The alleles constituting this RFLP are 6.6 and 6.1 kb in size with frequencies of 0.37 and 0.63 (n = 70), respectively.

DNA was extracted from frozen tissue from various brain regions of 35 individuals who had died of AD. All samples were confirmed upon autopsy to display the neuropathological hallmarks of AD (senile amyloid plaques, neurofibrillary tangles, and cerebrovascular amyloid). Thirty control brain samples were obtained from autopsied individuals who survived past the age of 65 without displaying senile dementia and whose brains showed no histopathological evidence of AD beyond that expected for their age group. DNA from the 65 brain samples was digested with Eco RI and separated by agarose gel electrophoresis for subsequent hybridization to the AP cDNAs, FB63 and HL124 (Fig. 2). Of the 35 AD DNAs, only one was heterozygous for the RFLP detected by FB63, while 4 of the 30 control DNAs displayed both allelic bands. When hybridized with HL124, ten of the AD DNA samples and nine of the control samples were heterozygous for the RFLP.

Eight of the heterozygous AD DNA samples and seven of the unaffected controls were then chosen for further analysis. By restricting analysis to only those individuals who were heterozygous for one of the two Eco RI RFLPs, we could compare directly the intensity of hybridization signal on each allele of the AP gene within each individual. If the AP gene on one of the number 21 chromosomes were duplicated, it would be reflected in a doubling of the intensity of one of the RFLP allelic bands with respect to the other. We also examined a DNA sample from a DS patient with a trisomy 21 karyotype (Fig. 2B).

We measured by densitometry the signal intensities of the two allelic bands in nine AD samples and ten control DNA samples heterozygous for either of the two Eco RI RFLPs detected by FB63 and HL124 (Table 1). Densitometric measurements of FB63 hybridization to control DNA sam-

Table 1. Relative hybridization of (**A**) FB63 or (**B**) HL124 to polymorphic Eco RI digest fragments of DNA from normal (UA), sporadic Alzheimer's disease (AD), or Down syndrome (DS) individuals. Values given are estimates of the ratios of the radioactivity exposures, necessary for the observed relative autoradiographic densities (*10*) for the 8.7-kb versus the 8.3-kb band (A) or the 6.6-kb versus the 6.1-kb band (B) recognized by the relevant probes. A few individual data points represent the average of two or three readings; others reflect one scan. Calculated for the UA and AD data in (B), from all samples for which scanning densitometry was possible, are the mean ± SEM values.

(**A**)	Sample	Hybridization ratio (8.7-kb: 8.3-kb band)	Mean
	UA 6	1.132	
	UA 37	0.989	1.061
	AD 17	1.065	
	DS 1	2.067	
(**B**)	Sample	Hybridization ratio (6.6-kb: 6.1-kb band)	Mean ± SEM
	UA 36	1.073	
	UA 37	1.400	
	UA 46	1.581	
	UA 52	1.613	
	UA 56	1.645	
	UA 58	1.448	
	UA 61	1.561	1.474 ± 0.075
	AD 11	1.589	
	AD 15	1.641	
	AD 18	1.230	
	AD 19	1.773	
	AD 20	1.501	
	AD 25	1.563	
	AD 28	1.331	
	AD 31	1.120	1.468 ± 0.078

Table 2. Hybridization of (**A**) FB63 or (**B**) HL124 probes, relative to that of probe p9Tau (9-kb band) to Hind III digest fragments of DNA from normal (UA), sporadic Alzheimer's disease (AD), or Down syndrome (DS) individuals. In (A), data are given for the 3.7-kb fragment recognized by FB63. In (B), data are given for the 4.3-kb, 3.0-kb, or 2.5-kb fragments recognized by HL124. All values, estimated as described (*10*), are normalized to the intensity of the 9.0-kb Hind III band to which p9Tau hybridizes. In (A) the mean ± SEM for the hybridization ratios in normal and AD DNA were 0.399 ± 0.033 and 0.423 ± 0.023, respectively.

(A)	Sample	Hybridization ratio 3.7-kb (FB63):9-kb(Tau)	(B)	Sample	Hybridization ratio (versus 9-kb Tau band)		
					4.3-kb band	3.0-kb band	2.5-kb band
	UA 36	0.342		UA 36	1.182	1.015	0.444
	UA 37	0.396		UA 37	1.464	1.373	0.384
	UA 52	0.402		UA 52	1.871	1.518	0.462
	UA 58	0.282		UA 58	2.255	1.657	0.607
	UA 61	0.506		UA 61	1.991	1.531	0.552
	UA 62	0.468		UA 62	1.625	1.337	0.441
				Mean ± SEM	1.731 ± 0.158	1.405 ± 0.091	0.482 ± 0.033
	AD 11	0.371		AD 11	1.105	1.039	0.501
	AD 15	0.406		AD 15	1.118	1.160	0.290
	AD 18	0.447		AD 18	1.219	1.246	0.221
	AD 19	0.390		AD 19	1.749	1.281	0.323
	AD 28	0.501		AD 28	1.927	1.909	0.569
				Mean ± SEM	1.424 ± 0.173	1.327 ± 0.151	0.381 ± 0.066
	DS 1	0.695		DS 1	2.819	3.296	0.608

ples from two unaffected individuals revealed an average ratio of 1.06:1 for the intensity of the signal of the 8.7-kb band compared to the 8.3-kb band (Table 1A). The DS control DNA sample, however, yielded a ratio that was approximately double this value (2.07:1), due to a third copy of the AP gene on the extra chromosome 21. The 8.7-kb:8.3-kb band ratio in the DNA sample from the sporadic AD patient that was heterozygous for the FB63 RFLP was 1.07:1. This suggests that the 3′ region of the AP gene homologous to FB63 is not duplicated in this AD DNA sample.

The average ratio of the 6.6-kb and 6.1-kb bands (detected by HL124) from seven DNA samples from unaffected controls was 1.47:1. The ratio is not unity, probably because HL124 shares more sequence with the 6.6-kb fragment than with the 6.1-kb fragment. The average ratio of the 6.6-kb:6.1-kb band intensities yielded by hybridization of HL124 to eight DNA samples from individuals with sporadic AD was 1.47:1. Thus, AP allelic duplication of the 5′ portion of the gene homologous to HL124 does not appear to exist in these eight sporadic AD brain DNA samples.

This method does not eliminate the possibility of AP gene duplication on both number 21 chromosomes. Therefore, we also investigated the possibility of ≥3:2 AP gene dosage in the AD DNA samples by comparing the amounts of DNA in genomic bands detected by the AP cDNAs and a control probe from a chromosome other than chromosome 21. The control probe used was p9TAU, a cDNA encoding the microtubule-associated protein tau, that has been mapped to chromosome 17 (*6*). A Southern

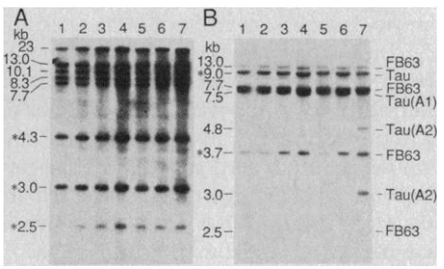

Fig. 3. Hybridization of HL124 (**A**) and FB63 plus p9TAU (**B**) to the following Hind III–digested DNAs: Lane 1, AD 19; lane 2, UA 37; lane 3, AD 28; lane 4, UA 61; lane 5, UA 36; lane 6, AD 18; lane 7, DS 1. After its simultaneous hybridization with FB63 and p9TAU, the filter was stripped in 0.01× standard sodium citrate at 65°C for 2 hours with one change of wash buffer before its hybridization with HL124. Asterisks have been positioned next to the genomic bands compared in the densitometry experiments. Lane 7 in (B) reveals an RFLP detected by p9TAU.

blot containing Hind III–digested DNA from sporadic AD patients, unaffected controls, and a DS control was simultaneously hybridized to FB63 and p9TAU (Fig. 3B). The average ratio of FB63(3.7 kb) and p9TAU(9.0 kb) band intensities derived from values obtained with six unaffected DNA samples was 1:2.5 (Table 2A). A similar average ratio (1:2.4) was obtained from five AD DNA samples. The DS DNA sample, however, yielded a ratio of 1:1.44. These data indicate that, although the DS sample demonstrates a clear duplication of the AP gene, neither the six unaffected nor the five AD samples show any evidence of increased AP gene dosage relative to the DS control. The same Southern blot was rehybridized with HL124. Three of the Hind III bands detected by HL124, with sizes of 4.3, 3.0, and 2.5 kb, were chosen for comparison to the 9.0-kb p9TAU fragment. When the signal strength of each AP Hind III fragment was compared to that of the 9.0-kb p9TAU Hind III band, only the DS DNA sample demonstrated increased AP gene dosage relative to the control probe (Table 2B). The values obtained for the unaffected samples were within the same range as those yielded by the AD DNAs and displayed no duplication of the AP gene when compared to the DS control sample.

Thus, our data indicate that neither the 5′ nor the 3′ portions of the AP gene are duplicated in DNA from brain tissue of patients with sporadic AD. The absence of AP gene duplication in leukocytes of familial AD patients has been observed with the 3′ FB63 cDNA (*7*). Together, these data cast doubt on the hypothesis that a duplication of the AP gene underlies AD.

Other mechanisms could lead to overexpression of AP. These may include promoter defects, abnormal production of inducers or suppressors of the AP gene, aberrant microenvironmental cues leading to tissue-specific enhancement of AP synthesis, or abnormal post-translational modifications of the precursor AP molecule.

REFERENCES AND NOTES

1. G. G. Glenner, in *Biological Aspects of Alzheimer's Disease*, R. Katzman, Ed. (Banbury Report 15, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1983), pp. 137–144; ——— and C. W. Wong, *Biochem. Biophys. Res. Commun.* **120**, 885 (1984).
2. G. G. Glenner and C. W. Wong, *Biochem. Biophys. Res. Commun.* **122**, 1131 (1984); C. L. Masters *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4245 (1985).
3. R. E. Tanzi *et al.*, *Science* **235**, 880 (1987).
4. D. Goldgaber, M. I. Lerman, O. W. McBride, U. Saffiotti, D. C. Gajdusek, *ibid.*, p. 887; J. Kang *et al.*,

Nature (London) **325**, 733 (1987); N. K. Robakis *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 4190 (1987).
5. J.-M. Delabar *et al.*, *Science* **235**, 1390 (1987).
6. R. L. Neve, P. Harris, K. Kosik, T. A. Donlon, *Mol. Brain Res.* **1**, 271 (1986).
7. P. H. St George-Hyslop *et al.*, *Science* **238**, 664 (1987).
8. R. L. Neve *et al.*, *Gene (Amsterdam)* **49**, 361 (1986).
9. T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982), pp. 280–281.
10. Radioactive signals from blots causing different autoradiogram exposures were estimated with the LKB Ultroscan XL soft laser scanning densitometer. Areas under optical density peaks over a 0.8-mm-wide path in the center of an autoradiographic band were measured. First, the dose-response curve of the film as detected by the densitometer was determined by measuring the areas due to 1.5-fold increments of ^{32}P-labeled DNA spotted onto small rectangular Whatman 3-mm filter strips. The fractional increase in area per fractional increase in radioactivity was determined at each average optical density between two bands. The inverse of this, varying from 0.80 to 1.15 over a range of 0.5 to 2.5 optical density units, was used to convert area ratios into radioactivity ratios.
11. We thank J. Amos and G. Stewart for providing DS DNAs and S. Orkin for the HL60 cDNA library. Supported by NIH grants HD18658 (R.L.N. and S.A.L.) and MH/NS 31862 (E.D.B.) and by the Howard Hughes Medical Institute (S.A.L.). This work was done during R.L.N.'s tenure as a National Down Syndrome Society Scholar.

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Gene Dosage of the Amyloid β Precursor Protein in Alzheimer's Disease

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The progressive deposition in the human brain of amyloid filaments composed of the amyloid β protein is a principal feature of Alzheimer's disease (AD). Densitometric analysis of Southern blots probed with a complementary DNA for the amyloid protein has been carried out to determine the relative dosage of this gene in genomic DNA of 14 patients with AD, 12 aged normal subjects, and 10 patients with trisomy 21 (Down syndrome). Whereas patients in the last group showed the expected 1.5-fold increase in dosage of this gene, none of the patients with AD had a gene dosage higher than that of the normal controls. These results do not support the hypothesis that the genetic defect in AD involves duplication of a segment of chromosome 21 containing the amyloid gene. Alternative mechanisms for the brain-specific increase in amyloid protein deposition in AD should be considered.

THE DEPOSITION OF EXTRACELLULAR amyloid filaments within clusters of degenerating neurites (neuritic plaques) and in cerebral and meningeal blood vessels is a constant accompaniment of Alzheimer's disease (AD) (*1*). The major proteinaceous component of this brain amyloid is a 4- to 5-kD hydrophobic protein designated the amyloid β protein (β-AP) (*2–5*). The recent isolation of complementary DNA (cDNA) clones for the β-AP precursor (*6–9*) has shown that it is a fragment of a larger precursor and that its gene is on the long arm of human chromosome 21. Patients with trisomy 21 [Down syndrome (DS)] almost invariably develop amyloid deposits in the brain at a premature age—perhaps due in part to increased dosage of the β-AP gene.

Because vascular and plaque amyloid deposits in AD are morphologically and biochemically similar if not identical to those in DS (*1, 10, 11*), an increased gene dosage for the β-AP precursor could also be operative in AD (*12*). In view of the potential importance of this issue for understanding amyloidogenesis in AD and for diagnosing subjects at risk, we isolated cDNAs for the β-AP precursor and determined its relative gene dosage in genomic (leukocyte) DNA from 14 patients with AD, 10 patients with DS, and 12 normal aged humans. Our results do not support recent reports (*13, 14*) that a subsegment of chromosome 21 containing the amyloid gene is duplicated in AD.

A λgt11 cDNA library derived from normal adult human brain (*15*) was screened with a 29-base oligonucleotide corresponding to bases 1849 to 1877 of the full-length cDNA clone for the β-AP precursor reported by Kang *et al.* (*7*). Of seven clones isolated, the largest clone (β-6) contained a 1.6-kb insert, which, by partial sequencing, corresponded to nucleotides 849 to 2451 of the sequence of Kang *et al.* Digestion of β-6 with Eco RI produced two fragments of ~0.9 and ~0.7 kb (*6, 7*). The 0.7-kb fragment encodes the carboxyl terminal of the precursor and contains an additional 366 bases of 3′ untranslated region; this fragment coincides to a large extent with the probe used in the AD gene dosimetry study of Delabar *et al.* (*13*). The 0.9-kb fragment encodes a major portion of the precursor protein that is on the amino-terminal side of the amyloid peptide. Each Eco RI fragment was purified and used independently to probe Southern blots. As a control probe not localized to chromosome 21, we used a cDNA for the human microtubule-associated protein 2 (MAP 2) (*16*). This gene is on chromosome 2 and is not linked to the familial AD defect in four pedigrees with autosomal dominant AD (*17*).

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Table 1. Results of the relative gene dosage of the β-AP in normal controls (C), Alzheimer's disease (AD), and Down syndrome (DS) shown for each Southern blot used in this study. The relative gene dosage was determined as described in Fig. 2. Each value represents the mean relative gene dosage (± SD) derived from *n* samples in a particular diagnostic category (C, AD, or DS) examined on that blot. *n* is defined in Fig. 2. *N* is the total number of DNA samples for diagnostic category run on blot.

Patient groups		Blot number																
		1	3	5	7	8	9	11	12	13	16	17	18	23	28	30	31	32
C	Mean	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	SD	0.1	0.2	0.2	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.2	0.2	0.0	0.2	0.1	0.1	0.2
	n/N	6/6	8/8	6/6	6/6	5/5	4/6	5/6	4/6	5/5	5/5	4/6	5/5	3/5	3/5	4/5	4/6	5/6
AD	Mean	1.0	0.9	0.9	1.1	1.0	1.1	1.0	1.0	1.0	0.9	0.9	1.0	1.0	0.9	1.0	1.0	0.9
	SD	0.1	0.1	0.1	0.2	0.2	0.3	0.2	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.2	0.2	0.3
	n/N	8/8	8/8	5/5	6/8	8/8	8/9	7/9	8/8	2/2	6/7	5/6	6/7	5/7	5/6	4/6	4/4	3/5
DS	Mean	1.5	1.5	1.6	1.5	1.6	1.6	1.6	1.5	1.3	1.6	1.5	1.6	1.4	1.4	1.5	1.6	1.5
	SD	0.1	0.4	0.5	0.4	0.6	0.2	0.2	0.2		0.2	0.0	0.1	0.2	0.2	0.3	0.1	0.3
	n/N	3/3	3/4	3/5	3/3	2/3	2/3	3/3	3/3	1/1	6/7	2/6	6/6	5/5	3/5	4/5	5/7	5/6