of these categories and the amyloid in AD and DS may be formed from a precursor synthesized in neurons as well as in other cells such as microglia and brain macrophages. Overexpression of this gene or altered posttranslational modification of its product in response to environmental stress, as a result of a genetic defect or increased gene dosage (*26*), or a combination of the above might lead to accumulation of the truncated amyloid polypeptide in the brain and to the observed neuropathological changes in AD, DS, Guamanian amyotrophic lateral sclerosis parkinsonism dementia, and aging. Thus a protein synthesized in neurons produces neurofibrillary tangles; that synthesized in microglial cells and brain macrophages is extruded from the cells and forms the extracellular amyloid of amyloid plaques and vascular amyloid deposits. Dying neurons and glial cells could also contribute to the extracellular deposits.

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Amyloid β Protein Gene: cDNA, mRNA Distribution, and Genetic Linkage Near the Alzheimer Locus

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The amyloid β protein has been identified as an important component of both cerebrovascular amyloid and amyloid plaques of Alzheimer's disease and Down syndrome. A complementary DNA for the β protein suggests that it derives from a larger protein expressed in a variety of tissues. Overexpression of the gene in brain tissue from fetuses with Down syndrome (trisomy 21) can be explained by dosage since the locus encoding the β protein maps to chromosome 21. Regional localization of this gene by both physical and genetic mapping places it in the vicinity of the genetic defect causing the inherited form of Alzheimer's disease.

ALZHEIMER'S DISEASE (AD) IS A progressive neurodegenerative disorder characterized by gradual loss of memory, reasoning, orientation, and judgment (*1*). AD generally occurs as a sporadic disorder of unknown cause. A proportion of cases, which have been termed familial Alzheimer's disease (FAD), are caused by a genetic defect that is transmitted in an autosomal dominant fashion (*2*). Although the nature of this defect is not known, it has been mapped to chromosome 21 by genetic linkage analysis (*3*). Aside from a generally earlier age of onset, the symptoms and pathology of FAD are similar to that of the apparently noninherited form of AD (*2*).

One of the hallmarks of AD is the presence of numerous neuritic plaques in postmortem brain tissue that are revealed by neuropathological examination (*4*). The degree of intellectual impairment in patients appears to be correlated with the frequency of neuritic plaques in the cortex (*5*). The mature neuritic plaque consists of degenerating axons and neurites surrounding an amyloid plaque core (APC) composed of 5- to 10-nm filaments (*6*) that stain with Congo red (*7*). Similar filaments are also found outside of plaques as independent bundles in the cortical neuropil and in the walls of meningeal and intracortical blood vessels [termed cerebrovascular amyloid (CA)] (*8*). A 4.2-kD polypeptide, called the β protein (due to its partial β-pleated sheet structure), has been isolated from both CA and APC. An identical 28–amino acid sequence (with the exception of a Glu to Gln substitution) has been obtained from the CA and APC β proteins, suggesting a possible common ori-

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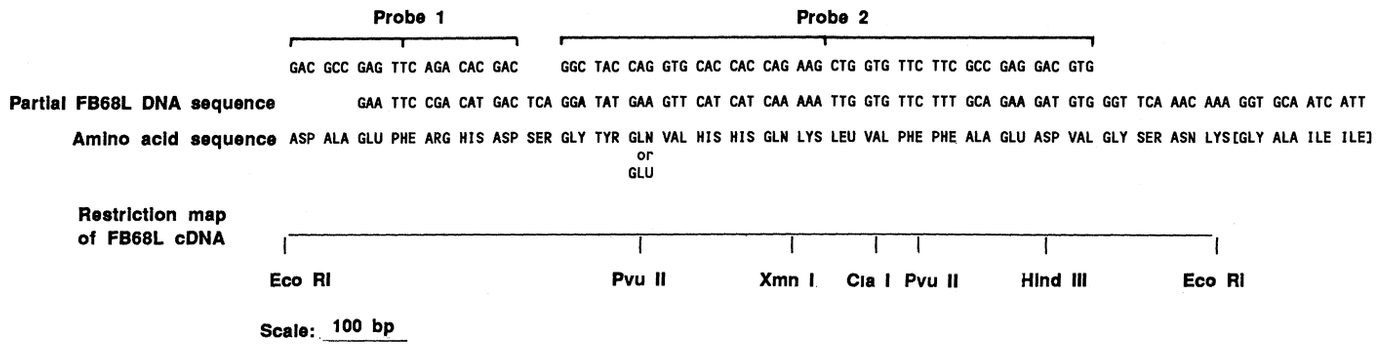

Fig. 1. Sequence analysis and restriction map of FB68L cDNA. The oligonucleotide probes 1 and 2 are shown above a partial sequence of FB68L (beginning at the left-hand Eco RI site on the restriction map) as determined by the method of Maxam and Gilbert (*25*). All hybridizations with these oligonucleotides were performed in 6× SSC (0.15*M* sodium chloride, 0.15*M* citrate) at 42°C and washed under the same conditions. The alternative amino acids at position 11 are based on the observation of Gln in CA of AD, but Glu in CA of DS and APC of both disorders (*9, 10*). Bracketed amino acids are predicted from the cDNA sequence but no amino acid sequence is available for comparison.

gin for both types of amyloid (*9, 10*). The brains of aged individuals with Down syndrome (DS) (trisomy 21) also have both APC and CA that contain a β protein with the same stretch of 28 amino acids (*10*); this similarity indicates that a common mechanism may underlie the formation of amyloid in AD and DS.

In order to investigate the molecular basis for amyloid deposition in AD and DS, we have isolated two complementary DNAs (cDNAs) (FB68L and FB63), which encode the amino acid sequence of amyloid β protein from human fetal brain. To obtain these cDNA clones for the amyloid β protein, we synthesized two unique oligonucleotide probes corresponding to the first 24 amino acids of the sequence. These "best guess" sequences were constructed from a human codon usage table (Fig. 1). The 21-base oligonucleotide based on amino acids 1 to 7 and the 48-base oligonucleotide based on amino acids 9 to 24 were first used to probe genomic Southern blots containing DNA (from five unrelated individuals) that had been digested with 35 different restriction enzymes. The rationale for this approach was that any genomic restriction fragments hybridizing to both nonoverlapping oligonucleotide probes were likely to derive from the correct locus. This set of common fragments detected by both oligonucleotide probes could provide a pattern against which candidate cDNA clones could be matched to identify putative cDNAs encoding the β protein. Among a mixture of fragments detected by the probes, common fragments could be discerned with 15 enzymes.

We then screened 1 million clones from a cDNA library (*11*) derived from fetal brain (20 to 22 weeks gestation) in the vector λgt11 (*12*) by simultaneous hybridization with both oligonucleotide probes. Four of the six positive plaques were purified, digested with restriction enzymes, electrophoresed on agarose gels, and transferred to nylon membrane. The filters were hybridized separately with the two oligonucleotides; fragments that hybridized with both probes were subcloned into the plasmid pUC18 (*13*). When these fragments were used to probe genomic Southern blots none of the four detected a primary pattern of bands corresponding to that we expected for the β protein gene. However, one clone, FB5, hybridized weakly to a minor set of fragments that corresponded to the expected pattern. Northern blot analysis demonstrated that FB5 detected a 7.5-kb messenger RNA (mRNA) that was in all tissues tested, but was most abundant in liver. Additional cDNA clones related to FB5 were therefore obtained by screening 250,000 phage from a human fetal liver cDNA library in λgt11. Six clones were obtained, purified, subcloned, and compared as before by hybridization to genomic Southern blots. Once again, none of the clones detected exactly the pattern of fragments expected for the amyloid β protein gene, but one, FL5, revealed a number of fragments of the expected pattern. By virtue of this homology, we reasoned that FL5 might represent a

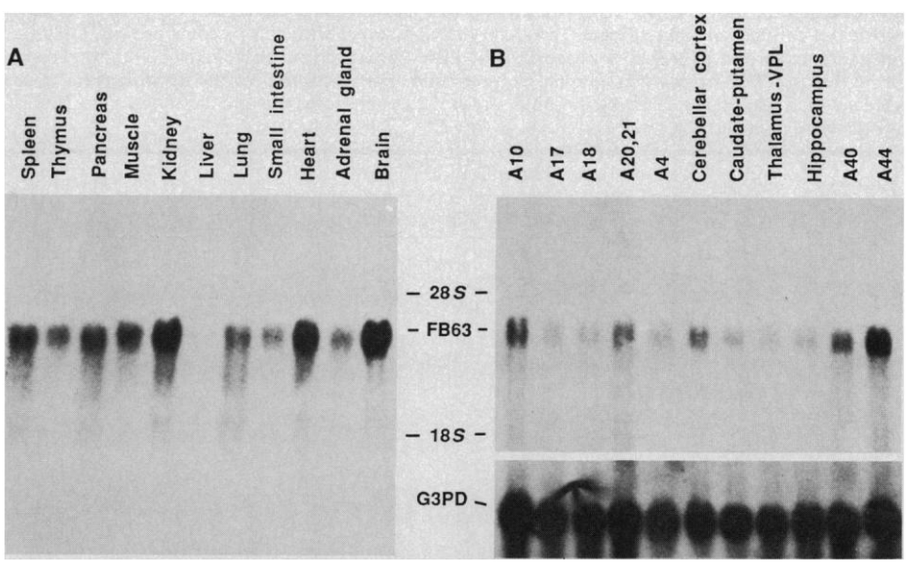

Fig. 2. Distribution of β protein gene transcripts. RNA was isolated by the guanidinium thiocyanate procedure (*26*). Total RNA was fractionated on agarose-formaldehyde gels, transferred to Biodyne A membrane, and hybridized with radiolabeled probe as described (*11*). Although the results shown were obtained with FB63, hybridization with the equivalent clone FB68L gave identical patterns. (**A**) Hybridization of FB63 to RNA (20 μg) from human 20- to 22-week fetal tissues. Fetal tissue was obtained from midtrimester elective abortuses under protocols approved by the institutional review board at Brigham and Women's Hospital. (**B**) Hybridization of FB63 to RNA (10 μg) from adult human brain subregions: A10, frontal pole of the cortex; A17, striate cortex; A18, extrastriate cortex; A20, 21, temporal association cortex; A4, motor cortex; thalamus-VPL, thalamus–ventral posterolateral nucleus; A40, posterior perisylvian cortex–supramarginal gyri; A44, anterior perisylvian cortex–opercular gyri. A control hybridization with a glyceraldehyde-3-phosphate dehydrogenase cDNA (G3PD) is shown below the FB63 hybridization.

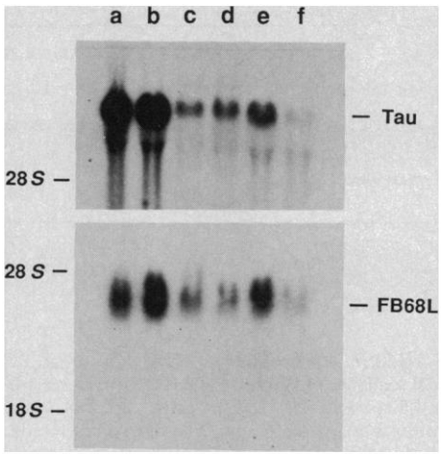

Fig. 3. Northern blot of FB68L hybridization to total RNA (25 μg) from 19-week normal (lane a) and trisomy 21 (lane b) brains, adult normal (lane c) and AD (lane d) cerebellum, and adult normal (lane e) and AD (lane f) frontal cortex. Fetal tissue was obtained from an abortus with a diagnosis of Down syndrome and from an age-matched normal abortus. Adult tissue was obtained from autopsy brains of a case of histologically confirmed Alzheimer's disease and from an individual without dementing illness. Control hybridization with a cDNA for the microtubule-associated protein tau (*11*) is shown above the results for FB68L. The two autoradiograms are from independent hybridizations with the same filter. 28*S* and 18*S* ribosomal RNA were used as size markers.

better probe for the amyloid β protein cDNA than did our short oligonucleotides.

Because we assumed that the β protein mRNA would most likely be found in brain, we rescreened the human fetal brain cDNA library with FL5. The probe unexpectedly hybridized to 0.2% of the clones in the library. Ten of these were "plaque-purified" and analyzed by excising the inserts with Eco RI. Nine contained a single 1.1-kb insert while one, FB68A, contained an additional fragment of 1.7 kb for a presumed total insert of 2.8 kb. Southern blot analysis revealed that FB68A hybridized to all of the genomic restriction fragments expected for the β protein gene together with a number of additional fragments. The 1.1-kb and 1.7-kb fragments of FB68A were subcloned and hybridized separately to genomic Southern blots. The entire array of genomic bands postulated to represent the β protein gene was detected by the 1.1-kb fragment. The 1.7-kb fragment hybridized to none of these. The 1.7-kb fragment was determined to be unrelated to the β protein cDNA, probably the result of cloning a double insert during construction of the cDNA library. The 1.1-kb fragment from FB68A was subcloned into pUC18 and designated FB68L for subsequent analyses. An identical 1.1-kb subclone was obtained from one of the nine phage with single inserts and designated FB63. FB68L and FB63 have identical restriction maps and hybridize to the same pattern of genomic fragments with all restriction enzymes tested.

Sequence analysis of the 1.1-kb fragment from FB68L was initiated, beginning at the Eco RI ends. At this juncture, we learned that a similar cDNA clone (λAm4) of approximately 1 kb had been isolated by Goldgaber *et al.* (*14*) and that a sequence corresponding to 26 of the 28 amino acids known for the β protein began at one Eco RI site. The initial 90 bp of sequence obtained from FB68L (Fig. 1) is that expected for amino acids 3 to 28 and confirms that our cDNA encodes the β protein. The portion of the sequence corresponding to the oligonucleotides that we constructed showed only a 75% match with the "best guess" sequence we chose. This explains our initial lack of success in retrieving amyloid β protein cDNAs with the oligonucleotides. Direct comparison of our cDNA clone with that of Goldgaber *et al.* (*14*) indicates that the two represent the same mRNA. The λAm4 clone from Goldgaber and colleagues was hybridized to genomic Southern blots and, like FB68L, detected all of the genomic fragments we had previously postulated derived from the amyloid β protein locus. We conclude, therefore, that like the λAm4 clone of Goldgaber *et al.* (*14*), the clone FB68L represents a cDNA encoding the amino acid sequence of the amyloid β polypeptide as part of a larger as yet unidentified protein. The fact that our clone was obtained by virtue of its homology with two other cDNAs, FB5 and FL5, suggests that there may be additional human proteins related to the amyloid β polypeptide.

The 1.1-kb cDNA was hybridized to a Northern blot consisting of RNA from 11 different human fetal tissues (20 to 22 weeks gestation) (Fig. 2A). A 3.7-kb mRNA species was detected in every tissue examined, but with variable intensity. The signal was strongest in brain, kidney, heart, and spleen. The weakest hybridization was observed in liver. This may explain why screening the fetal liver cDNA library with FB5 did not

Table 1. Segregation pattern of FB68L with DNAs from human-rodent somatic cell hybrids. The hybrids were derived from fusions of hypoxanthine phosphoribosyltransferase–deficient Chinese hamster E36 cells or mouse RAG cells with leukocytes from two female carriers of different reciprocal X/19 translocation chromosomes: the X/19W translocation t(X;19)(q24::q13) (*27*); and the X/19B translocation t(X;19)(q13::p13) (*28*). The human chromosome complements of the hybrids were determined by isozyme and cytogenetic techniques (*29*) and with cloned DNA probes for each autosome and the sex chromosomes. The column designations are: +/+, hybridization signal and chromosome both present; −/−, hybridization signal and chromosome absent; +/−, hybridization present but chromosome absent; and −/+, hybridization absent with chromosome present. Only the segregation pattern of the 2.9-kb band detected by FB68L was analyzed because the 8.7-kb band could not be distinguished from a comigrating hamster band. For calculation of the discordant fractions, hybrids with a rearranged chromosome or in which the chromosome was present in less than 15% of the cells were excluded.

Chromosome	Hybridization pattern				Discordant fraction
	+/+	−/−	+/−	−/+	
1	3	3	6	2	0.57
2	0	4	6	1	0.64
3	6	3	4	1	0.36
4	4	4	5	1	0.43
5	4	4	6	1	0.47
6	7	0	3	3	0.46
7	4	2	6	3	0.60
8	4	4	6	1	0.47
9	4	4	6	1	0.47
10	5	2	5	3	0.53
11	6	2	3	3	0.43
12	4	2	6	1	0.54
13	6	2	4	3	0.47
14	9	2	1	3	0.27
15	3	1	7	4	0.73
16	7	2	3	3	0.40
17	2	3	8	2	0.67
18	4	2	6	3	0.60
19 &19q+*	10	0	0	5	0.33
20	8	2	2	3	0.33
21	10	5	0	0	0.00
22	7	1	3	4	0.47
X &Xq−†	4	5	5	0	0.36

*Because the hybrids were derived from fusions with leukocytes from two different X/19 translocation carriers, this category represents the der19 translocation chromosomes. †This category includes hybrids with an intact X and those with derX translocation chromosomes.

yield the β protein cDNA. The cDNA also hybridized with RNAs derived from monocytes and peripheral blood leukocytes.

To determine the distribution of the β amyloid gene transcript in the adult human brain, the cDNA was hybridized to a Northern blot containing RNA from 11 different regions of the adult brain (Fig. 2B). Levels of mRNA were highest in the frontal pole of the cortex (A10) and the anterior perisylvian cortex–opercular gyri (A44). Moderate hybridization was observed in the cerebellar cortex, the posterior perisylvian cortex–supramarginal gyri (A40), and the temporal association cortex (A20, 21). A much weaker signal was detected in the striate, extrastriate, and motor cortices (A17, A18, and A4, respectively), the caudate-putamen, the hippocampus, and the thalamus. Thus, the highest level of β protein gene expression was detected in the association cortex, a region probably related to some of the clinical symptoms observed in AD. Although the distribution does not correlate with the occurrence of amyloid plaques, which are frequent in the hippocampus (*15*), it does coincide with the deposition of CA (*16*). Because the β protein gene is also expressed outside the central nervous system, however, it is not possible to conclude that the amyloid in either APC or CA derives solely from endogenous expression of the protein in the brain.

The hybridization of FB68L to RNAs from normal and fetal brains with DS, and from normal and adult brains with AD, is shown in Fig. 3. The intensity of FB68L hybridization to RNA from brains of 19-week abortuses with DS is clearly increased relative to its hybridization to RNA from normal 19-week brains. The FB68L transcript is expressed equally in AD and normal adult cerebellum, but is present at lower levels in AD cortex relative to normal. This probably reflects the nonspecific degradation of RNA in AD cortex during the terminal stages of the disease since a control hybridization of tau cDNA (*11*) to the same blot (Fig. 3, above the FB68L hybridization) also displays weaker signal in the AD cortex RNA.

To assign a chromosomal location to the amyloid β protein gene, a filter containing Eco RI–digested DNA from various human-rodent somatic cell hybrid lines was hybridized with FB68L. The probe detected two Eco RI fragments of 8.7 kb and 2.9 kb in human DNA. On this filter, only the 2.9-kb band was sufficiently resolved from cross-hybridizing hamster fragments to assign an unequivocal location, chromosome 21 (Table 1). The 8.7-kb human fragment was not distinguishable in all lanes from a 8.2-kb hamster band.

Table 2. Genetic linkage of β protein locus to D21S1/D21S11. Linkage of the two markers was assessed by calculation of a lod score with the computer program LIPED (*31*). The lod score, z, is a parameter representing the relative likelihood of linkage given the data set. It is calculated by taking the logarithm of the ratio of the likelihood of linkage at a recombination fraction θ to the likelihood of nonlinkage ($\theta = 0.50$). A lod score greater than +3 is generally accepted as proof of linkage. The maximum lod score, $\hat{z} = +4.70$, occurs at a recombination fraction, $\hat{\theta} = 0.04$, with a 1-lod unit confidence interval of 0.01 to 0.16.

	Recombination fraction (θ)						
	0.00	0.01	0.05	0.10	0.20	0.30	0.40
Lod score (z)	$-\infty$	+4.42	+4.68	+4.42	+3.51	+2.33	+0.97

We have extended the physical mapping of the β protein gene by determining its regional localization on chromosome 21 using somatic cell hybrids containing portions of the human chromosome 21 on a rodent background (*17*): WA17, intact 21; 72532x-6, intact 21; 153E7bx, 21cen→21qter; 2FU^{r}1, 21q11.2→21qter; 21-8Ab5-23a, 21pter→21q22.2; and R2-10, 21p11.2→21q22.3. These hybrids, which have been previously characterized with chromosome 21–specific DNA probes, divide the chromosome into four regions: 21pter-21q11.2, 21q11.2-21q22.2, proximal 21q22.3, and distal 21q22.3. The 8.7-kb and 2.9-kb Eco RI bands were detected by FB68L in all of these hybrids; this result places the amyloid β protein gene in the region 21q11.2-21q22.2. An additional cell line, the hybrid Acem2-9U, contains a complex rearrangement of chromosome 21–yielding duplications in certain regions and deficiencies on other areas of the chromosome (*18*). While the superoxide dismutase (*SOD1*) locus in band 21q22.1 is present in this hybrid, more proximal markers spanning the region 21q11.2→21q21 are absent. The failure to detect hybridization of FB68L to DNA from Acem2-9U indicates that the β amyloid gene is probably located in the region 21q11.2→21q21, above the obligate DS region (*19*).

We have previously used a large Venezuelan reference pedigree to construct a genetic linkage map of polymorphic DNA probes that spans the long arm of human chromosome 21 (*20*). To position the amyloid β protein gene on this linkage map, we first screened for restriction fragment length polymorphism (RFLP) at the locus. DNAs from five unrelated individuals were digested with a total of 32 restriction endonucleases, fractionated by agarose gel electrophoresis, transferred to nylon membrane and hybridized to FB68L (*21*). An RFLP apparently resulting from the presence or absence of a single restriction site was detected with Eco RI (Fig. 4). The allelic Eco RI fragments, 8.7 kb and 8.3 kb, displayed Mendelian transmission and had frequencies (118 chromosomes) of 0.94 and 0.06, respectively. Calculation of the polymorphism information content (*22*) of the locus to assess its value as a genetic marker gave a value of 0.11 for the Eco RI RFLP, indicating that the β amyloid protein locus is only weakly informative.

None of the parents of the large reference sibships used to construct the chromosome 21 linkage map was heterozygous at this locus. Thus, we were unable to determine the precise position of the β protein gene with respect to the other chromosome 21 markers. In view of the detection of genetic linkage on chromosome 21 between FAD and the highly informative marker D21S1/D21S11 (*3*), we decided to specifically test for linkage of the β amyloid protein gene to this DNA marker. We therefore typed parents of nuclear families from many of the disease pedigrees currently being investigated in our laboratory in an attempt to find sibships with a parent doubly heterozy-

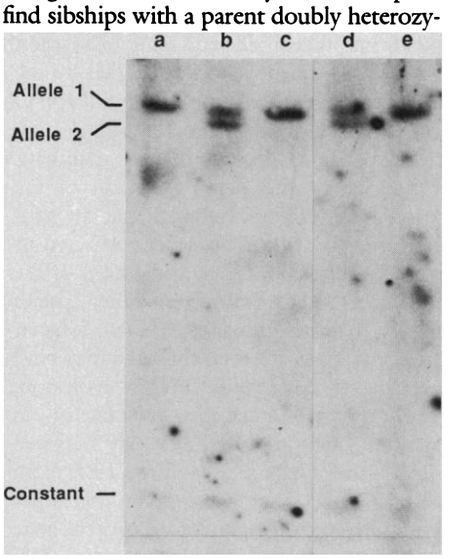

Fig. 4. Eco RI fragment length polymorphism detected by FB68L. FB68L was hybridized to Southern blots containing Eco RI–digested DNA from five related individuals. Conditions for Southern blot hybridizations have been described (*30*). The first two lanes (a and b) represent the mother and father, respectively, of the children in the subsequent three lanes (c, d, and e). Allelic fragments 1 and 2 are 8.7 kb and 8.3 kb, respectively; the constant band is 2.9 kb. The fragments were sized relative to Hind III fragments of λcI857 electrophoresed in a parallel lane.

gous for the FB68L Eco RI RFLP and D21S1/D21S11. Progeny of such parents were then typed for both markers and the data were analyzed for linkage. The results (Table 2) confirm that close linkage ($\hat{z} = 4.70$, $\hat{\theta} = 0.04$) is detectable between the β protein gene and D21S1/D21S11. Additional support for linkage is currently being sought to narrow the confidence interval (0.01 to 0.16) surrounding the most likely estimate, 4% of the frequency of recombination between the two loci. To precisely locate the amyloid β protein gene on the chromosome 21 linkage map may involve considerably more effort since it will require typing a large number of DNA markers on the specific families informative for linkage with this gene. The linkage of the amyloid β protein gene to D21S1/D21S11 places it within the same genetic region as the defect causing FAD (*3*). This raises the question of whether a defect in this particular gene might be the cause of FAD. Although analysis of linkage between the amyloid β protein gene and FAD is currently under way, the limited informativeness of the RFLPs detected thus far suggests that more polymorphisms will be needed before this issue can be resolved.

Although the exact relationship of amyloid formation to the fundamental cause of AD is not known, the deposition of this insoluble substance is clearly a major factor in the pathogenesis of the disorder. The 4.2-kD amyloid β polypeptide apparently derives from proteolytic processing of a larger, as yet unidentified, protein encoded by the 3.7-kb mRNA. Definition of the full amino acid sequence of this protein awaits cloning and sequencing of longer cDNAs than have been isolated thus far. Expression of the gene in many tissues suggests that the protein has a function that is not restricted to brain. In fact, positive hybridization of the FB68L cDNA to DNA from other species (mouse, hamster, bovine, *Xenopus*, lobster, *Drosophila*) indicates that the gene may have been highly conserved through evolution, implying a strong selective pressure on an essential function. The homology between FB68L and two other cDNAs, FB5 from brain and FL5 from liver, implies that other genes related to the amyloid β protein gene are present in the human genome. The potential relationship of these cDNAs to other known amyloidogenic proteins, such as the prion protein (*23*), is unknown. Genes related to the amyloid β protein gene might be involved in other human neurodegenerative disorders characterized by amyloid formation.

Assignment of the amyloid β protein gene to chromosome 21, together with the observation of increased β protein mRNA expression in DS brain, indicates that the deposition of amyloid in DS is likely to be the direct result of a dosage-related increase in concentration of the gene product. It is not yet known whether the amyloid plaques in AD are also due to such overexpression, or are due to some other alteration of the brain microenvironment that promotes precipitation of the β protein.

In view of the significant quantities of β protein mRNA present in all tissues tested, it is puzzling that amyloid plaque formation in AD and DS occurs exclusively in the brain. A number of explanations are possible. (i) Alteration of the protein to a component that can form plaque amyloid occurs only in the brain, perhaps as a result of specific proteolytic cleavage (*24*); (ii) the physicochemical environment in the brain differs (for example, the *p*H or specific ion concentration) from the other tissues in a way that promotes amyloid plaque formation; (iii) other tissues have mechanisms to eliminate amyloidogenic peptides; or (iv) part of the sequence of the mRNA produced in brain differs from that in other tissues.

Our results raise the prospect that a genetic defect in the amyloid β protein locus might be the basis for FAD, the inherited autosomal dominant form of AD. Our genetic linkage data mapping the β protein gene to the same vicinity of chromosome 21 as the locus causing FAD is consistent with, but does not prove, the possibility that the two loci are identical. The neuropathology observed in DS as a consequence of a 50% increase in gene dosage suggests that a similarly subtle change in amino acid sequence or expression level of the amyloid β protein could promote amyloid formation, thereby causing FAD. However, even if the amyloid β protein gene is not the site of the primary defect in FAD, genetic variation at the locus may play a role in the expression of apparently noninherited AD. It is conceivable that differences in genetic constitution at the β protein locus could alter the interaction of the protein product with the environmental influences triggering the disorder. Thus, particular genotypes at the β protein locus might predispose to AD without causing clearly detectable heritability of the disorder.

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