

Failure of familial Alzheimer's disease to segregate with the A4-amyloid gene in several European families

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The gene coding for the amyloid protein, a component of neuritic plaques found in brain tissue from patients with Alzheimer's disease, has been localized to chromosome 21, and neighbouring polymorphic DNA markers segregate with Alzheimer's disease in several large families. These data, and the association of Alzheimer's disease with Down's syndrome, suggest that overproduction of the amyloid protein, or production of an abnormal variant of the protein, may be the underlying pathological change causing Alzheimer's disease. We have identified a restriction fragment length polymorphism of the A4-amyloid gene, and find recombinants in two Alzheimer's disease families between Alzheimer's disease and the A4-amyloid locus. This demonstrates that the gene for plaque core A4-amyloid cannot be the locus of a defect causing Alzheimer's disease in these families. These data indicate that alterations in the plaque core amyloid gene cannot explain the molecular pathology for all cases of Alzheimer's disease.

Alzheimer's disease is the most common form of dementia, affecting as much as 5% of the population over 65 (ref. 1). It is characterized by the presence of large numbers of neuritic

plaques and neurofibrillary tangles, particularly in the hippocampus and the association cortex². The pathogenesis is not understood, although two groups are at high risk of developing Alzheimer's disease: those with a family history of the disease^{3,4} and persons with Down's syndrome⁵. In some pedigrees Alzheimer's disease segregates as an autosomal dominant disorder⁶⁻⁸. In four such pedigrees, molecular genetic linkage analyses have indicated a disease locus on chromosome 21 in the region of 21q11.2→21q22.2 (ref. 9), closer to the centromere than the region 21q22 associated with Down's syndrome¹⁰.

Recently the amyloid protein has been isolated from the Alzheimer's disease cerebral vessels (β -amyloid)¹¹ and plaque core (A4-amyloid)¹²⁻¹⁴ which have very similar amino-acid sequences and are antigenically related^{15,16}. Oligonucleotides corresponding to a part of the amino-acid sequence have been used to isolate complementary DNA recombinants that correspond to the coding sequence of A4-amyloid¹⁷ and β -amyloid¹⁸⁻²⁰. The amyloid protein gene has been localized to chromosome 21 (refs 17-20) in the region of q11-q22^{19,20}. It has been suggested that the amyloid locus is segregating close to the Alzheimer's disease locus in the 21q21 region^{9,19}. Furthermore a duplication of the chromosomal region around the amyloid protein gene has been reported in sporadic Alzheimer's cases²¹. These observations have been assimilated into a single theory that predisposition to Alzheimer's disease is due to genetically determined over-expression of the β -amyloid protein, or expression of an abnormal variant of this protein^{18,19,21}.

We have identified a common restriction fragment length polymorphism (RFLP) of the A4-amyloid gene, and observed its segregation in two families with early onset (at <35 years of age, Fig. 1), and five families with late onset (~65 years of age) Alzheimer's dementia. These families show autosomal dominant transmission of Alzheimer's disease (Figs 1 and 2; see Fig. 2 legend for details of the polymorphism). Our linkage analysis (Table 1) shows that the gene for amyloid protein is not closely linked to the gene for familial Alzheimer's disease and in two families crossovers between the amyloid locus and the Alzheimer's locus are observed (Figs 2 and 3). Furthermore, we did not detect amyloid gene duplication on Southern blots in any of our cases²¹. The recombination between the familial Alzheimer's disease gene and the amyloid gene demonstrates that these loci are genetically distinct unless the recombination events occurred within the amyloid locus. The linkage data suggest that the amyloid locus is further away from the Alzheimer's locus than the anonymous probes to which linkage

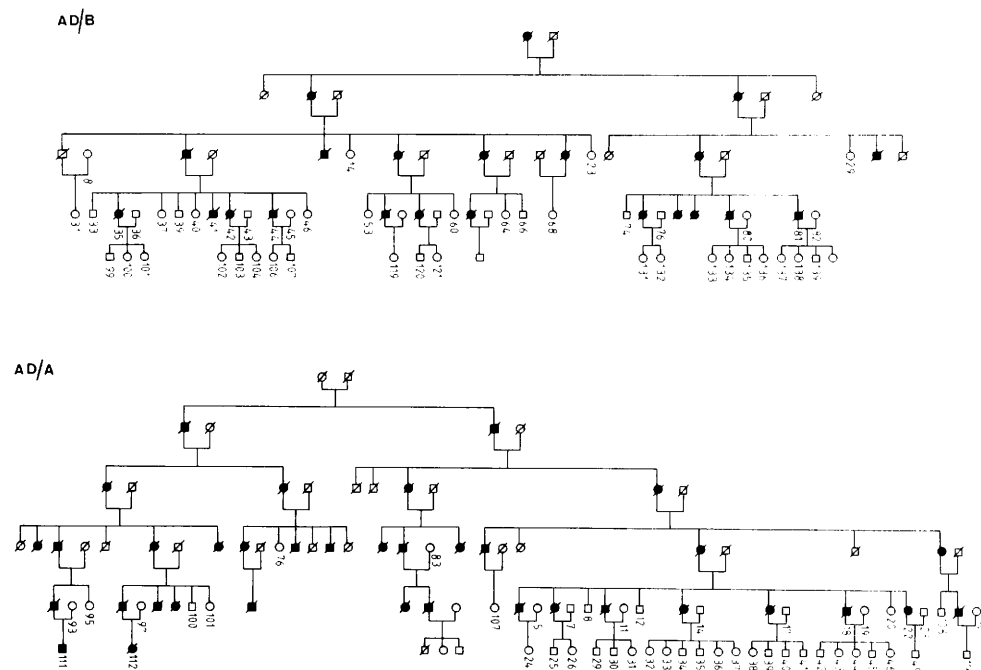


Fig. 1 Two large pedigrees with early onset Alzheimer's disease. Symbols: circle, female; square, male; diagonal line, deceased; filled symbol, affected individual. The numbers refer to the cases available for genetic linkage analysis. Family AD/A includes 36 Alzheimer cases in 6 generations of which 10 have been histopathologically confirmed. Mean age of onset was 33 ± 4 years. Family AD/B includes 22 Alzheimer cases in five generations of which four have been histopathologically confirmed. Mean age of onset was 34 ± 2 years.

Table 1 Lod scores for linkage

Histopathologically confirmed, early onset pedigrees	Recombinant fraction (θ)						
	0.00	0.01	0.05	0.10	0.20	0.30	0.40
Family							
AD/A	$-\infty$	-1.59	-0.73	-0.36	-0.08	-0.01	-0.02
AD/B	-0.63	-0.61	-0.55	-0.48	-0.34	-0.17	-0.03
Clinically diagnosed, late onset pedigrees*							
1	-1.15	-1.01	-0.69	-0.47	-0.22	-0.09	-0.02
2	-0.31	-0.31	-0.30	-0.28	-0.19	-0.09	-0.02
3	-0.22	-0.21	-0.19	-0.16	-0.09	-0.04	-0.01
4	+0.38	+0.37	+0.33	+0.28	+0.18	+0.09	+0.02
5	-0.33	-0.31	-0.24	-0.18	-0.09	-0.04	-0.01
Total (7 families)	$-\infty$	-3.67	-2.37	-1.65	-0.83	-0.35	-0.09

Values shown are lod scores for linkage between the A4-amyloid locus and Alzheimer's disease locus in seven informative families (two histologically confirmed). The exclusion limit is given by $z < -2$, $\theta = 0.07$.

The pedigrees used in this analysis were those of the two early-onset Alzheimer families illustrated in Fig. 1 (AD/A and AD/B) and five clinically diagnosed, late-onset pedigrees²⁴. In the early-onset pedigrees we used a gene frequency for the familial Alzheimer gene of 0.0001 (ref. 9) and assumed full penetrance of the disease. The late-onset pedigrees consisted of pedigree 1 (see Fig. 2, top) and four other informative sibships. These sibships contained 16 affected sibs with 21 unaffected sibs. The mean age of onset in all these clinically diagnosed families was between 55 and 70 years. An age of onset curve was constructed for each family to determine the risk for apparently unaffected family members. The gene frequency for an allele leading to familial Alzheimer's disease at an age of onset of ~65 was set at 0.01 (ref. 3). Calculation of the lod scores using these parameters for gene frequency and using an age-of-onset curve to determine the risk to all unaffected family members does not give an obligate recombinant in pedigree 1 because of the finite chances that the father of the sibship was an undiagnosed carrier of the familial Alzheimer trait or the mother was homozygous for the disease trait. All cases of Alzheimer's disease in the families were assumed to be of genetic rather than sporadic origin. These assumptions are justified because the clinical features of the disease within each family were homogeneous; however, recalculation of the data assuming less than full penetrance or assuming a low possibility (1%) that any individual case in a family is a sporadic case did not appreciably alter the exclusion limits (data not shown). Linkage was calculated using the MLINK program²⁵.

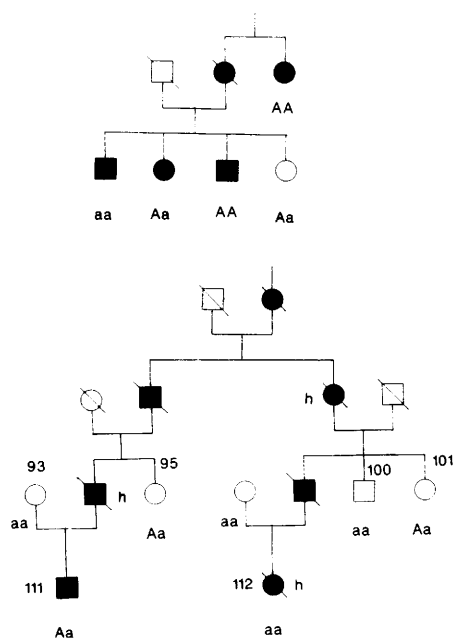


Fig. 2 Details of the haplotypes of pedigrees showing recombinations between Alzheimer's disease and the amyloid locus: h, histological confirmation at autopsy. The upper pedigree was diagnosed using the *American Psychiatric Association's Diagnostic and Statistics Manual*, 3rd edn criteria²⁴. The lower pedigree is a simplified section of the pedigree AD/A (Fig. 1). Symbols as in Fig. 1. Patient 111 is 33 years old and has been clinically diagnosed.

Methods. The *Bgl*II polymorphism used to trace the inheritance in pedigree analysis was detected using the 150-base pair (bp) *Sau*3AI fragment of the amyloid precursor cDNA spanning the coding region for the A4 polypeptide (base pairs 1,770-1,920, ref. 17). This fragment detects two allelic *Bgl*II fragments of 9.6 kb (A) and 6.9 kb (a). The allele frequencies are 0.36 and 0.64 as determined in 76 unrelated Caucasians (probe information content value 0.35); the alleles in this population are in Hardy-Weinberg equilibrium. Codominant inheritance has been demonstrated in three extended families. The *Bgl*II RFLP is obscured by co-migrating constant bands if larger fragments of the cDNA clone are used as a probe. No polymorphisms were found using this 150-bp probe with *Acc*I, *Asp*700, *Ava*I, *Ava*II, *Bam*HI, *Ban*I, *Ban*II, *Bcl*I, *Bgl*I, *Bst*NI, *Bst*XI, *Cl*aI, *Dra*I, *Eco*RV, *Hind*II, *Hind*III, *Hin*fI, *Msp*I, *Nci*I, *Pst*I, *Pvu*II, *Rsa*I, *Sac*I, *Sau*96T, *Stu*I, *Taq*I and *Xba*I using a panel of six unrelated Caucasians. In 38 chromosomes, the polymorphism reported here appears to be in linkage equilibrium with the polymorphism detected by *Eco*RI reported previously^{18,19}.

has been reported^{9,19}. Therefore, we studied the cosegregation of the amyloid polymorphism and a *Bgl*II polymorphism identified by the gene for superoxide dismutase (SOD); a marker localized at 21q22.1 (ref. 22). Preliminary data suggest that the amyloid protein gene may be linked to the SOD gene. (Maximum lod score, +1.92 at a recombination fraction of 0.07.) Such close proximity between the SOD gene and the amyloid gene has been recently reported in the homologous mouse chromosome using hybrid cell lines²³.

In conclusion our linkage data indicate that a mutation in the amyloid protein gene is not the primary defect causing familial Alzheimer's disease in all cases and suggest that the amyloid protein gene is closer to the 21q22 Down's syndrome phenotype region than has previously been reported¹⁹. Currently more Alzheimer's families are being investigated with both the

amyloid and SOD polymorphisms to confirm these preliminary findings.

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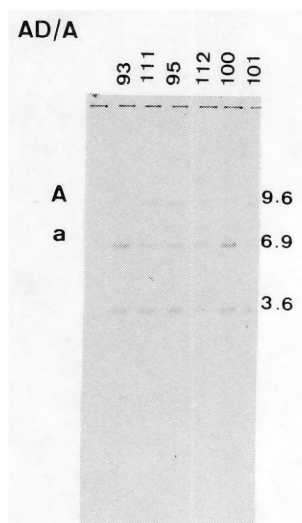


Fig. 3 Southern blot of members of pedigree AD/A (Fig. 2), showing the polymorphic bands at 9.6 kb (A) and 6.9 kb (a) and the constant band at 3.6 kb. Pedigree numbers are as in Fig. 2.

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