

Presenile dementia and cerebral haemorrhage linked to a mutation at codon 692 of the β -amyloid precursor protein gene

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Several families with an early-onset form of familial Alzheimer's disease have been found to harbour mutations at a specific codon (717) of the gene for the β -amyloid precursor protein (APP) on chromosome 21. We now report, a novel base mutation in the same exon of the APP gene which co-segregates in one family with presenile dementia and cerebral haemorrhage due to cerebral amyloid angiopathy. The mutation results in the substitution of alanine into glycine at codon 692. These results suggest that the clinically distinct entities, presenile dementia and cerebral amyloid angiopathy, can be caused by the same mutation in the APP gene.

A common pathological hallmark in Alzheimer's disease (AD), cerebral amyloid angiopathy (CAA) and in hereditary cerebral haemorrhage with amyloidosis Dutch type (HCHWA-D), an autosomal dominant form of CAA, is the progressive deposition of β -amyloid (β A)¹, a 4 kD proteolysis product of the β -amyloid protein precursor (APP)² in parenchymal senile plaques and in the blood vessel walls. In AD, the β A deposition is accompanied by a neurodegenerative response characterized by the appearance of dystrophic neurites (DN) and neurofibrillary tangles (NFT)³. The major component of the NFTs is abnormally phosphorylated tau protein⁴. Quantitative analysis using β -amyloid and tau antibodies reveals that the clinical symptoms and severity of dementia correlates with the density and the topography of the DNs and NFTs and not with that of the β -amyloid deposits⁵. In CAA, the common feature is cerebral haemorrhage due to extensive vascular amyloid deposition in the absence of DNs and NFTs suggesting that the neuronal cell population is not involved⁶.

Recently, single base changes have been reported in exon 17 of APP in familial AD and in HCHWA-D. In AD, three different amino acid substitutions were found at codon 717 while in HCHWA-D an amino acid at codon 693 was changed⁷⁻¹⁰. We have identified a family in which both patients with presenile dementia and patients with a cerebral haemorrhage due to CAA occurred. Direct sequencing of exon 17 in the affected individuals revealed a base change (C to G) at position 2075, substituting alanine into glycine (Ala to Gly) at codon 692 of APP. In this family the mutation may be responsible for the β -amyloid deposition in the blood vessel walls and in the brain parenchyma.

Family 1302

Family 1302 is a four generation Dutch family identified in an epidemiological study of genetic risk factors in AD¹¹ (Fig. 1). The six patients currently alive, one in generation III and 5 in generation IV, were diagnosed using neurological examination, neuropsychological testing and brain imaging techniques. Four patients, III-8, IV-2, IV-4 and IV-5, received a diagnosis of presenile dementia that fulfilled the NINCDS criteria¹² for probable AD. Their age at onset was respectively 61, 47, 49 and 45 years. The two

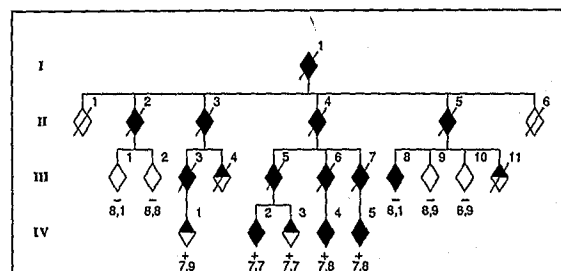


Fig. 1 Family 1302. The pedigree is disguised and shows only the non-affected and the patients for reasons of confidentiality. The Roman numbers left are the generations, the numbers above the symbols are the individuals. The filled symbols represent the patients with presenile dementia, the half filled symbols the patients who suffered a cerebral haemorrhage. Below the symbol are indicated the presence (+) or absence (-) of the mutation as determined by direct sequencing or SSCP analysis as well as the alleles of the 21-GT12 (D21S210) marker.

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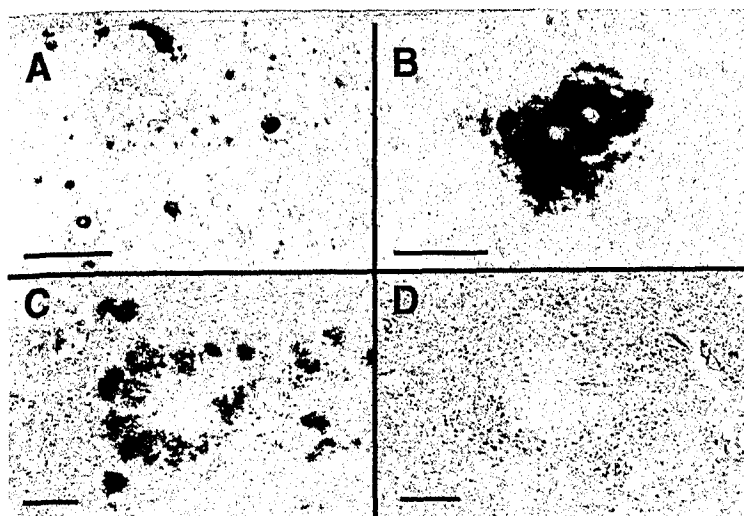


Fig. 2 Biopsy of patient IV-3. *a, b*, Senile plaques and congophilic angiopathy stained with the polyclonal antiserum β A 1-42³¹. *c*, Dystrophic senile plaque neurites were labelled with an ubiquitin polyclonal antiserum³² (Dako). *d*, In a semi-serial section, these neurites were negative with AT8, a monoclonal antibody directed to abnormally phosphorylated tau^{33,34}. Bar is 500 μ m in A, 50 μ m in B, and 20 μ m in C and D.

other patients, IV-1 and IV-3, survived a cerebral haemorrhage at the age of 41 and 42 years respectively. Prior to the cerebral haemorrhage these patients did not show signs of cognitive impairment. Medical records revealed that six patients died in a psychiatric hospital of presenile dementia, while three other patients died demented according to family informants. The age at death of these patients varied between 47 and 61 years. Further family information indicated that two patients did not survive a cerebral haemorrhage at the age of 40 and 35 years respectively. The available information, however, does not exclude the possibility that some of the demented patients may have suffered from a cerebral haemorrhage prior to their dementia. Also, since no autopsy was performed on any of the deceased patients we cannot determine whether they had a brain pathology consistent with AD or CAA. If all patients (with presenile dementia and cerebral haemorrhage), are taken together, an autosomal dominant segregation pattern is observed for the disease with a mean age at onset of 45.7 ± 7.3 years ($n = 11$, range 35 – 61 years) and a mean age at death of 53.3 ± 9.2 years ($n = 11$, range 35 – 61 years).

Immunohistochemistry

In one of the two patients, IV-3, who suffered from a cerebral haemorrhage, a large haematoma in the left parieto-occipital cortex was evacuated and a biopsy taken. Immunohistochemistry on two sections of the biopsy material with a β A polyclonal antiserum, showed extensive amyloid deposition in the blood vessel walls and in parenchymal senile plaques (Fig. 2*a, b*). The senile plaques, 11 mm^{-2} in the neocortex, were predominantly diffuse amyloid deposits but a few neuritic plaques with a dense amyloid core were also observed. DNs in the senile plaques and in the perivascular amyloid depositions were immunostained by a polyclonal antiserum to ubiquitin (Ub) (Fig. 2*c*). However, no NFTs were detected in the DNs using a monoclonal antibody to

pathological tau (Fig. 2*d*).

Mutation analysis

We examined whether a mutation was present in exon 17 of the APP gene in the cerebral haemorrhage patient IV-3. Leukocyte DNA was used for PCR amplification and direct sequencing (Fig. 3*a*). Neither the previously reported HCHWA-D mutation nor the known AD mutations were found. However, we detected a novel C to G transversion at position 2075 substituting alanine into glycine at codon 692 of the APP770 transcript¹³. Direct sequencing and single strand conformational polymorphism analysis (SSCP) were used to test for the mutation in all available patients. Theoretically the mutation can also be detected after restriction enzyme digestion since the C to G transversion destroys a *Cvi*RI recognition site. The mutation was present in patient IV-1 with cerebral haemorrhage and in patients IV-2, IV-4 and IV-5, but not in patient III-8, with dementia (Fig. 3*b*). We also tested the unaffected individuals for the mutation by SSCP analysis. In generation III (Fig. 1), the mutation is absent in III-1, III-2, III-9 and III-10 who, on the basis of their age ranging from 64–76 years, are judged to have escaped the disease. In generation IV, the age of the seven unaffected individuals analysed ranged from 38–59 years. The mutation was present in two individuals aged 41 years and recently reported to show suggestive symptoms (individuals not shown for reasons of confidentiality). Furthermore, SSCP analysis showed that the mutation did not occur in 100 normal unrelated Caucasians.

Linkage analysis

Segregation analysis in family 1302 with the highly polymorphic dinucleotide repeat marker 21-GT12 (D21S210) located near the APP gene, showed that the mutation segregated with allele 7 (Fig. 1). Linkage with the disease was calculated assuming that both the presenile dementia and the cerebral haemorrhage are phenotypes of the same autosomal dominant gene, and showed a peak lod score of 2.29 for the APP692 mutation and 1.13 for the 21-GT12 marker at a recombination fraction, θ , of 0.07 and 0.08 respectively (Table 1). If we excluded individual III-8 from the linkage analysis, we obtained peak lod scores respectively of 3.29 and 2.14 at zero recombination. These results indicate that the recombination fractions are the sole result of the absence of respectively the APP692 mutation and allele 7 of the 21-GT12 marker in patient III-8, confirming that the dementia in this patient is not linked to the APP gene. The recombinant was also observed with the *Bgl*II polymorphism detected by the β A fragment of the APP cDNA¹⁴ (data not shown).

Discussion

Our results indicate that in family 1302 both the presenile dementia and the cerebral haemorrhage are segregating with a mutation at codon 692 of the APP gene, with one exception — patient III-8. A likely explanation for this lack of segregation is that the dementia in individual III-8 has a different aetiology. In patient III-8, the onset at 61 years is considerably later than the mean age at onset (45.7 ± 7.3 years) of the other affected relatives, a finding that conflicts with our previous observation that in AD families the age at onset tends to cluster within a narrow onset range¹⁵. Furthermore, the current age of patient III-8 of 71 years is outside the range of the age at death (35–61 years)

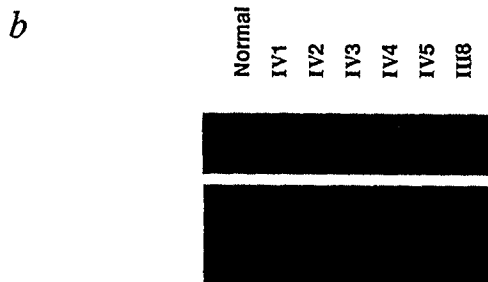
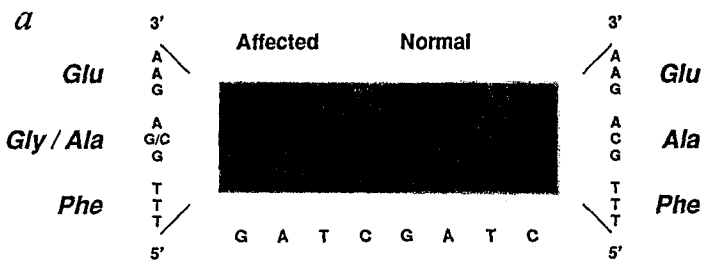


Fig. 3 a, Autoradiograph of the sequencing gel from part of APP exon 17 in individual IV-3 (Fig. 1) and in an unaffected family member showing a single base pair change at position 2075. The C to G transversion leads to an Ala to Gly amino acid substitution at codon 692 of the APP770 transcript¹³. The presence of the mutation was confirmed after sequencing of the opposite strand (data not shown). b, SSCP analysis of the mutation at codon 692 in one normal and 6 affected individuals from family 1302 showing the presence of the mutation in all affecteds with the exception of one, individual III-8. The numbers refer to the generation number of the individual in the pedigree (Fig. 1).

in family 1302. The finding within family 1302 of a patient with an unrelated dementia is not unusual since the prevalence of dementia is known to increase with age¹⁶. Another possible explanation is that the APP692 mutation is sufficient but not necessary for the disease in family 1302, and may have an epistatic effect on the onset, duration and severity of the disease symptoms. Although less likely, the possibility remains that the lack of segregation indicates that the APP692 mutation is not pathogenic; the predicted amino acid substitution (Ala to Gly) is not dramatic biophysically.

We have not yet analysed other exons or the promoter sequence of APP, therefore it remains possible that other sequence differences exist within the gene in the patients in family 1302. Nonetheless, mutations outside exon 17 have yet to be reported. The APP gene comprises 18 exons, of which exons 16 and 17 encode the sequence for the 42 amino acids of the β A¹³. The β A is partly inserted into the transmembrane domain with 28 amino acids protruding into the extracellular space². The mutation we have described causes a substitution (Ala to Gly) in the extracellular part of β A at amino acid 21. Interestingly, the mutation is located next to the HCHWA-D mutation which substitutes a glutamic acid for a glutamine (Glu to Gln) at amino acid 22 of β A¹⁰.

In the biopsy of patient IV-3, the distribution and quantity of β A reactive senile plaques is similar to that seen in HCHWA-D patients¹⁷. The senile plaques consisted mainly of diffuse amyloid deposits but immunohistochemistry with a Ub antiserum, showed

DNs in a few of the senile plaques and in the perivascular amyloid deposits. The DN, however, differed from the conventional DN seen in AD patients as they were negative with a monoclonal antibody against pathological tau and thus did not contain NFTs. Ub-immunoreactive but tau-negative DN are known to occur in senile plaques in normal aged individuals, early in Down's syndrome patients and in cerebellar plaques in AD, suggesting that APP and Ub immunoreactivity are more related to neurotic dystrophy than is abnormally phosphorylated tau^{18,19}. It is also known that in AD, tau immunoreactivity in DN occurs only when NFTs have formed in the perikarya^{20,21}.

In normal brains, extensive β A deposition is prevented since the APP is cleaved within the β A fragment at amino acid 16 by the APP secretase^{22,23}. The mutation in family 1302 and in HCHWA-D may alter the efficiency of the APP secretase allowing the processing of APP through alternative pathways that leave the β A fragment intact. In HCHWA-D families the common clinical manifestation is a cerebral haemorrhage²⁴. In most patients surviving one or more haemorrhages dementia was recorded and some patients showed progressive dementia in the absence of apparent cerebral haemorrhages²⁵. In family 1302, most patients demonstrate dementia in the absence of cerebral haemorrhages. How this can be explained by a mutation of one amino acid closer to the APP secretase cleavage site remains to be elucidated.

Methodology

Linkage analysis. The two-point likelihood of the odds (lod) scores were calculated using MLINK from the LINKAGE package²⁶ assuming a disease frequency of 0.001 and a phenocopy rate of 0.001. The penetrance for the unaffected individuals was estimated from an age at onset curve²⁷, using a mean age at onset of 45.7±7.3 years. The frequency for the APP692 mutation was set at 0.01. The allele frequencies of 21-GT12 (D21S210) are available from genome data base (GDB).

Immunohistochemistry. Unlabelled antibody bridge or avidin-biotin labelled immunoperoxidase techniques were used with diaminobenzidine as substrate. The sections were counterstained with haematoxylin.

Sequence analysis. The conditions and the primers for the polymerase chain reaction (PCR) amplification of exon 17 were as described previously²⁸. In the amplification we used a biotinylated 3' primer and the PCR product was bound to streptavidin coated magnetic beads (Dynal). After DNA denaturation the single-stranded DNA was sequenced²⁹ using the Sequenase kit version 2.0 (USB) and the 5' primer.

Single strand conformational polymorphism analysis (SSCP). The amplification of exon 17 was performed (ref. 28) in the presence of [α -³²P]dCTP. After denaturation the PCR product was loaded on a 5% non-denaturing polyacrylamide gel containing 10% glycerol³⁰.

Table 1 Lod scores for linkage

	Recombination fraction (θ)								
	0.0	0.01	0.05	0.10	0.20	0.30	0.40		z(θ)
APP692	1.20	1.89	2.25	2.18	1.75	1.16	0.51		2.29 (0.07)
21-GT12	0.03	0.73	1.12	1.12	0.87	0.49	0.12		1.13 (0.08)

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1. Castano, E.M. & Frangione, B. *Lab. Invest.* **58**, 122-132 (1988).
2. Kang, J. *et al. Nature* **325**, 733-736 (1987).
3. Selkoe, D.J. *Ann. Rev. Neurosci.* **12**, 463-490 (1989).
4. Lee, V.M.-Y., Ballin, B.J., Otvos, L. & Trojanowski, J.Q. *Science* **251**, 675-678 (1991).
5. McKee, A.C., Kosik, K.S. & Kowall, N.W. *Ann. Neurol.* **30**, 156-165 (1991).
6. Vinters, H.V. *Stroke* **18**, 311-324 (1987).
7. Goate, A. *et al. Nature* **349**, 704-706 (1991).
8. Murrell, J., Farlow, M., Ghetti, B., & Benson, M.D. *Science* **254**, 97-99 (1991).
9. Chartier-Harlin, M.-C. *et al. Nature* **353**, 844-846 (1991).
10. Levy, E. *et al. Science* **248**, 1124-1126 (1990).
11. Hofman, A. *et al. Neurology* **39**, 1589-1592 (1989).
12. McKhann, G. *et al. Neurology* **34**, 939-944 (1984).
13. Yoshikai, S. *et al. Gene* **87**, 257-263 (1990).
14. Van Broeckhoven, C. *et al. Nature* **329**, 153-155 (1987).
15. van Duyn, C.M. *et al. Brit. J. Psych.* **158**, 471-474 (1990).
16. Rocca, W.A. *et al. Ann. Neurol.* **30**, 381-390 (1991).
17. Timmers, W.F., Tagliavini, F., Haan, J. & Frangione, B. *Neurosci. Lett.* **118**, 223-226 (1990).
18. Craspedakis, M. *et al. Proc. natn. Acad. Sci. U.S.A.* **88**, 7552-7556 (1991).
19. Arai, H. *et al. Proc. natn. Acad. Sci. U.S.A.* **87**, 2249-2253 (1990).
20. Probst, A., Anderton, B.H., Brion, J.-P. & Ulrich, J. *Acta Neuropathol.* **77**, 430-436 (1989).
21. Barcikowska, M., Wisniewski, H.M., Baner, C. & Grundke-Iqbal, I. *Acta Neuropathol.* **78**, 225-231 (1989).
22. Sisodia, S.S., Koo, E.H., Beyreuther, K., Unterbeck, A. & Price, D.L. *Science* **248**, 492-495 (1990).
23. Esch, F.S. *et al. Science* **248**, 1122-1124 (1990).
24. Haan, J., Lanser, J.B.K., Zijderfeld, I., van der Does, I.G.F. & Roos, R.A.C. *Arch. Neurol.* **47**, 965-967 (1990).
25. Haan, J., Algra, P.R. & Roos, R.A.C. *Arch. Neurol.* **47**, 649-653 (1990).
26. Lathrop, G., Lalouel, J.M., Juller, C. & Ott, J. *Proc. natn. Acad. Sci. U.S.A.* **81**, 3443-3446 (1984).
27. Ott, J. in *Analysis of Human Genetic Linkage* (eds Boyer, S.H. *et al.*) 141-144 (The Johns Hopkins University Press, Baltimore, 1985).
28. Bakker, E. *et al. Am. J. hum. Genet.* **49**, 518-521 (1991).
29. Uhlen, M. *Nature* **340**, 733-734 (1989).
30. Orita, M., Suzuki, Y., Sekiya, T. & Hayashi, K. *Genomics* **5**, 874-879 (1989).
31. Masters, C.L. *et al. Proc. natn. Acad. Sci. U.S.A.* **82**, 4245-4249 (1985).
32. Perry, G., Friedman, R., Shaw, G. & Chau, V. *Proc. natn. Acad. Sci. U.S.A.* **84**, 3033-3036 (1987).
33. Blernat, J. *et al. EMBO J.* **11**, 1593-1597 (1992).
34. Mercken, M. *et al. Acta Neuropathol.* (in the press).