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Presentation of amyloidosis in carriers of the codon 692 mutation in the amyloid precursor protein gene (APP692)

Gerwin Roks,[1]* Frans Van Harskamp,[2]* Inge De Koning,[2] Marc Cruts,[6] Chris De Jonghe,[6] Samir Kumar-Singh,[6] Aad Tibben,[3,4] Herve Tanghe,[5] Martinus F. Niermeijer,[3] Albert Hofman,[1] John C. Van Swieten,[2] Christine Van Broeckhoven[6] and Cornelia M. Van Duijn[1]

Departments of [1]Epidemiology and Biostatistics, [2]Neurology, [3]Clinical Genetics, [4]Medical Psychology and Psychotherapy and [5]Radiology, Erasmus Medical Centre, Rotterdam The Netherlands and [6]Flanders Interuniversity Institute of Biotechnology (VIB), Born-Bunge Foundation (BBS), Department of Biochemistry, University of Antwerp (UIA), Belgium

Correspondence to: Cornelia M. van Duijn, Department of Epidemiology and Biostatistics, Erasmus Medical Centre Rotterdam, PO Box 1738, 3000DR Rotterdam, The Netherlands
E-mail: vanduijn@epib.fgg.eur.nl

**These authors contributed equally to the work*

Summary

Several mutations in the amyloid precursor protein (APP) gene may lead to either Alzheimer's disease or cerebral haemorrhage due to congophilic amyloid angiopathy (CAA). A single family is known in which both types of pathology are expressed because of a missense mutation at codon 692 of the APP gene (APP692). Here we describe the clinical and pathological expression of APP692 in eight patients with the mutation. Furthermore, 21 first-degree relatives with an *a priori* risk of 50% of being a carrier were tested for the APP692 mutation and studied for presymptomatic signs by neurological examination, neuropsychological testing and brain MRI. Patients with APP692 presented with haemorrhage, dementia or both. The dementia in patients with the APP692 mutation was compatible with Alzheimer's disease both clinically and neuropathologically. Of the 21 healthy relatives at 50% risk, five carried the APP692 mutation. The presymptomatic carriers showed a subtle, non-significant impairment of cognitive function compared with relatives without APP692. A significant increase in the number of periventricular and subcortical white matter lesions at young age was seen in presymptomatic carriers (mean age 26.4 years). The findings of this study suggest that a single (genetic) mechanism may underlie the pathology of Alzheimer's disease and CAA. These diseases are manifested subclinically by white matter pathology. Further insight into the relationship between CAA and Alzheimer's disease may provide clues about the aetiology of Alzheimer's disease.

Keywords: Alzheimer's disease; cerebral haemorrhage; congophilic amyloid angiopathy; amyloid precursor protein

Abbreviations: APOE = apolipoprotein E; APP = amyloid precursor protein; CAA = congophilic amyloid angiopathy; CVLT = California verbal learning test; GIT = Groningen intelligence test; HCHWA-D = hereditary cerebral haemorrhage with amyloidosis of the Dutch type; NINCDS–ADRDA = National Institute of Neurological and Communicative Diseases and Stroke–Alzheimer's Disease and Related Disorders Association; WAIS = Wechsler Adult Intelligence Scale

Introduction

A common pathological hallmark of Alzheimer's disease and cerebral amyloid angiopathy (CAA) is the deposition of amyloid β (Aβ) in parenchymal senile plaques and cerebral blood vessel walls. Several mutations in exons 16 and 17 of the amyloid precursor protein (APP) gene cause autosomal dominant forms of early-onset Alzheimer's disease (Goate *et al.*, 1991; Hendriks and Van Broeckhoven, 1996). However, APP mutations may alternatively cause CAA with cerebral haemorrhages without Alzheimer's disease pathology. A glutamic acid to glutamine mutation at codon 693 of APP (APP693) is associated with hereditary cerebral haemorrhage with amyloidosis of the Dutch type (HCHWA-D) (Levy

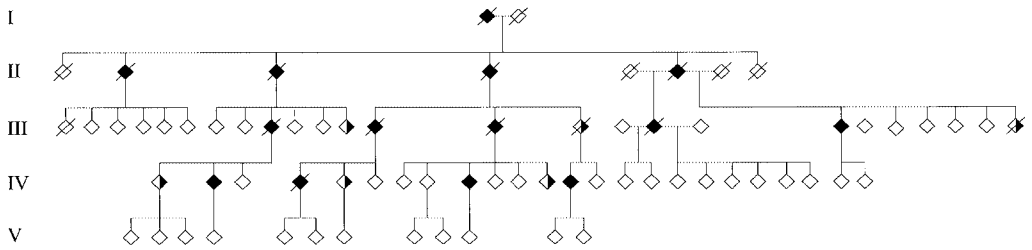

Fig. 1 Updated pedigree of the family in which the APP692 mutation was segregating. The pedigree is disguised for reasons of confidentiality. Filled symbols represent patients presenting with presenile Alzheimer's disease and half-filled symbols represent patients presenting with cerebral haemorrhage. Of the subjects alive in generations IV and V, only those who participated in this study after informed consent are indicated.

et al., 1990; Van Broeckhoven *et al.*, 1990). The pathology in carriers of APP693 shows extensive CAA but no lesions characteristic of Alzheimer's disease (Maat-Schieman *et al.*, 1996). Our group described a mutation at codon 692 of the APP gene (APP692) presenting with either presenile dementia or cerebral haemorrhage (Hendriks *et al.*, 1992). Little is known about the natural history of disease in carriers of APP692. We have conducted a follow-up study of the patients with Alzheimer's disease and CAA and their offspring. The disease progression and pathology was studied in eight patients carrying the APP692 mutation. Furthermore, 21 first-degree relatives at 50% risk were tested for the APP692 mutation and studied for presymptomatic signs by neurological examination, neuropsychological testing and brain MRI.

Table 1 *Characteristics of the asymptomatic carriers of the APP692 mutation*

	Carriers	Non-carriers	Age-matched unrelated controls
MRI studies			
Number of subjects	5	16	12
Male/female	4/1	8/8	5/7
Age (years) (range)	26.4 (21–30)	37.8 (24–60)	25.5 (19–31)
Neuropsychology			
Number of subjects	4	14	–
Male/female	3/1	7/7	
Age (years) (range)	26.8 (21–30)	38.2 (24–60)	

Methods

Family ascertainment

The family came to our attention when a 51-year-old patient presented with presenile dementia. A sibling was known with cerebral haemorrhage caused by CAA. The family history showed a genetic disorder segregating in this family, manifesting as either presenile dementia or cerebral haemorrhage (Fig. 1). In four cases, cerebral haemorrhage occurred in the offspring of a patient with dementia, whereas one dementia case was reported in the offspring of a haemorrhage patient. Both traits could be linked to the APP692 mutation (Hendriks *et al.*, 1992).

The disorder could be traced back to an ancestor born three generations back, in 1866, who was affected with a presenile dementia syndrome, according to heteroanamnestic data. All offspring of the siblings of this ancestor were traced using municipal genealogy records, and living descendants were contacted. None of the 81 descendants of these siblings were known to have had early-onset dementia or cerebral haemorrhage. Figure 1 shows the only branch of the family in which the APP692 mutation was segregating.

Subjects

Clinical data could be retrieved for nine patients with a cerebral haemorrhage, dementia or both. Seven patients were examined clinically. Descriptions of two patients were obtained from records from other hospitals. Twenty-one healthy individuals at 50% risk participated in our study of asymptomatic carriers, 10 from generation IV and 11 from generation V. All clinical, neuropsychological and MRI studies were conducted blind to APP692 status. The physical examination and MRI studies were performed in all respondents. The neuropsychological test battery was completed by 18 subjects.

Of the 21 subjects tested for the APP692 mutation, five carried the mutation. Characteristics of carriers and non-carriers participating in the study are given in Table 1. Carriers and non-carriers differed significantly in age ($P = 0.02$). Age is an important determinant of neurological performance and white matter lesions. For the MRI studies, an age-matched control group was used (Table 1). Subjects without known or suspected pathology involving the white matter were derived from the neurological out-patient clinic. To adjust for the difference in age distribution between carriers and non-carriers, we used age- and sex-standardized scores for neuropsychological tests.

Informed consent was obtained from all participants and the protocol of the study was approved by the Medical Ethics Committee of the Erasmus Medical Centre Rotterdam. According to the study protocol, the mutation tests were not disclosed to the participants. All subjects were offered

individual genetic counselling and presymptomatic testing irrespective of APP692 status. The results of the MRI studies and neuropsychological tests were reported to participants at the group level; no individual scores were given.

Clinical and neuropsychological examination

All subjects at risk, and seven out of nine patients diagnosed with cerebral haemorrhage or dementia underwent neurological examination. The examination included medical history, assessment of mental status, cranial nerves, muscle strength and tone, sensation, coordination, fine motor skills, tendon reflexes, primitive reflexes, posture and movement.

The neuropsychological evaluation of subjects at risk was performed by a neuropsychologist. The test battery included nine domains. Intelligence was tested with the condensed version of the Groningen Intelligence Test (GIT) (Luteijn, 1983). The expected premorbid IQ was calculated from the level and number of complete years of education for all persons, based on standardized scores for the Dutch population (Luteijn, 1971). The ratio of the current IQ to expected premorbid IQ was used to estimate any deterioration in IQ. Tests for measuring attention and concentration included Part A of the Trail Making Test (Reitan, 1958) and the substitution test of the Wechsler Adult Intelligence Scale (WAIS) (Stinissen *et al.*, 1970). We used the digit span forward and backward versions of the WAIS (Stinissen *et al.*, 1970) to assess the span of immediate verbal recall and as a measure of attention capacity. Auditory verbal memory was examined by the Dutch-validated and -standardized version of the California Verbal Learning Test (CVLT) (Delis *et al.*, 1987; Mulder *et al.*, 1996). The total score is the number of words recalled correctly in five trials. Recognition score is related to the score after five presentations, represented as 'hits' and 'false positives'. Recall of the complex figure of Rey was used for measuring non-verbal memory (Osterrieth, 1944). The Similarities subtest of the WAIS provided a measure for abstraction and verbal concept formation (Stinissen *et al.*, 1970). Executive (control) functions were examined with Part B of the Trail-Making test (Reitan, 1958), and by testing verbal fluency (Luteijn, 1983). Visuoconstructive performance was assessed with the Rey–Osterrieth complex figure test (Osterrieth, 1944) and visuospatial abilities were examined with the Line Orientation test (Benton *et al.*, 1978).

Neuroimaging

In the subjects at 50% risk, MRI scanning was performed with a Gyroscan T5-II, using T_1- and T_2-weighted series in the axial plane. A neurologist and a research physician, who were blind to the clinical and genetic data, scored the MRI scans with a semiquantitative scale separately for periventricular and subcortical white matter lesions (Scheltens *et al.*, 1993). This scale produces a score related to the size and number of white matter lesions (Table 2). Lesions were defined as areas of higher signal intensity compared with the surrounding brain tissue on T_2-weighted scans.

Table 2 *Semiquantitative MRI scores for white matter lesions*

	Range	Score
Periventricular		
Caps: occipital	0–2	0 = absent
Caps: frontal	0–2	1 = <4 mm
Lines: lateral ventricles	0–2	2 = >4 mm
Total periventricular	0–6	
Subcortical		0 = no abnormalities
Frontal lobe	0–6	1 = <4 mm, $n < 6$
Parieto-occipital lobe	0–6	2 = >4 mm, $n > 6$
Total white matter	0–12	3 = 4–10 mm, $n < 6$
		4 = 4–10 mm, $n > 6$
		5 = >10 mm
		6 = confluent
Total score		0–18

Caps = white matter lesions adjacent to anterior or frontal ventricles; lines = white matter lersions adjacent to lateral ventricles.

DNA testing

Genomic DNA was extracted from peripheral blood leucocytes. Screening for the APP mutation was performed either by PCR–RFLP (polymerase chain reaction–restriction fragment length polymorphism) analysis (Nishiwaki *et al.*, 1996) or by SSCP (single-strand conformation polymorphism) analysis, as follows. Exon 17 was amplified (Bakker *et al.*, 1991) and the PCR product was loaded on a 1× HydroLink MDE gel (J. T. Baker, Philipsburg, New York, NY, USA) with 10% glycerol and the gel was stained with silver (Nelis *et al.*, 1997). Apolipoprotein E (*APOE*) genotypes were analysed as described elsewhere (Wenham *et al.*, 1991; van Duijn *et al.*, 1994).

Statistical analysis

Differences between mutation carriers, non-carriers and unrelated controls were analysed with the non-parametric Mann–Whitney test. Exact one-sided P values were used because our hypothesis was that mutation carriers were more likely to score worse on psychometric tests and MRI.

Results

Clinical course and pathology of affected subjects

The clinical characteristics of the nine patients are summarized in Table 3; a detailed report of the clinical course for each patient is given in Appendix I. Three patients presented with cerebral haemorrhage (Patients 2, 3 and 5). One of these patients was diagnosed with probable

Table 3 *Clinical characteristics of affected relatives*

Patient	Age at onset of dementia (years)	Age at first haemorrhage (years)	Age at death (years)	First symptoms	Disease course	Behavioural problems	Neuroimaging	APP692 mutation	*APOE* genotype	Pathology
1	48	–	57	Memory loss	Gradual decline in cognitive function	+	Cortical + central atrophy, WML	+	34	SP, NFT, CAA*
2	–	43	–	R hemiplegia	L hemiplegia, vegetative state	–	Haematoma, WML	+	34	–
3	48	42	–	L hemiplegia	Gradual decline in vegetative state	–	Haematoma, WML	+	34	CAA†
4	49	–	–	Memory loss	Gradual decline in cognitive function	+	Cortical + central atrophy, WML	+	34	–
5	?	44	–	R hemiplegia	?	–	Haematoma	+	34	–
6	46	–	55	Memory loss	Gradual decline in cognitive function	+	Normal	+	33	SP, NFT, CAA*
7	46	–	59	Memory loss	Gradual decline in cognitive function	?	?	+	?	–
8	61	–	68	Memory loss, depression	Gradual decline in cognitive function	–	Cortical + central atrophy	–	34	–
9	41	46	–	Memory loss	Gradual decline in cognitive function L hemiplegia	–	Cortical + central atrophy, WML Haematoma	+	34	–

+/–/? = present/not present/unknown; R/L = right/left; WML = white matter lesions; SP = senile plaques; NFT = neurofibrillary tangles. *Autopsy; †biopsy.

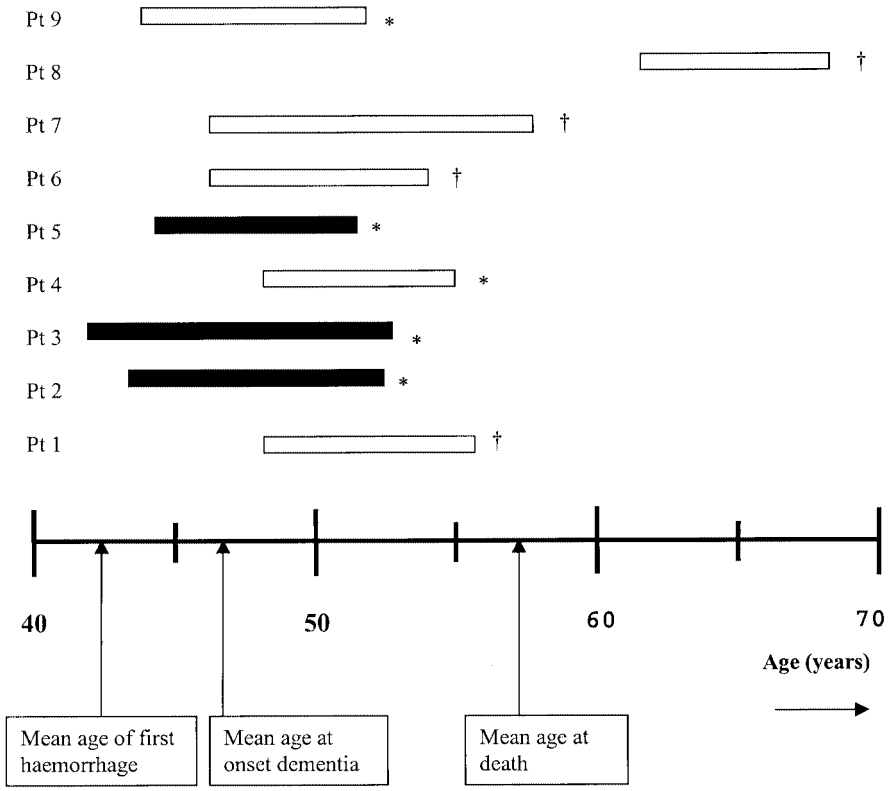

Fig. 2 Duration of follow-up of patients. Mean ages at first symptoms and death were calculated for APP692 carriers. Open bars represent patients presenting initially with dementia; filled bars represent patients presenting initially with haemorrhage. *Age at end of follow-up; †age at death.

Alzheimer's disease 6 years after the haemorrhage (Appendix I, Patient 3). Cognitive function could not be assessed in the other two patients presenting with cerebral haemorrhage because one patient reached a vegetative state within 1 year after the first stroke and the other refused further participation. All patients presenting with cerebral haemorrhage carried the APP692 mutation and had the *APOE 34* genotype. A brain biopsy was taken from Patient 3 during surgery at the initial stroke. The biopsy showed extensive amyloid deposition in the blood-vessel walls and in parenchymal senile plaques (Hendriks *et al.*, 1992). The senile plaques were predominantly of the diffuse type, with a few neuritic plaques but without neurofibrillary tangles.

In total, six patients presented with dementia. In these patients, the clinical course of disease was compatible with probable Alzheimer's disease (McKhann *et al.*, 1984). One patient developed a haemorrhage after the diagnosis of Alzheimer's disease (Patient 9). Each patient was screened for the APP692 mutation, and, except for Patient 8, all were carriers (Hendriks *et al.*, 1992). As shown in Fig. 2, the clinical course of Patient 8 differed in that the ages at onset and death were outside the range for patients carrying the mutation. The diagnosis of Alzheimer's disease was confirmed pathologically for Patients 1 and 6 (van Harskamp *et al.*, 1997; Cras *et al.*, 1998). Both patients showed cortical and subcortical neuronal loss, which was accompanied by the presence of numerous senile plaques and neurofibrillary tangles, and congophilic angiopathy (van Harskamp *et al.*, 1997; Cras *et al.*, 1998). The senile plaques were larger than reported for classical Alzheimer's disease (van Harskamp *et al.*, 1997; Cras *et al.*, 1998). Of the five patients diagnosed with dementia with a known *APOE* genotype, four had the *APOE 34* genotype.

When comparing the APP692 carriers presenting with haemorrhage and dementia, the vascular pathology occurred at an earlier age (mean 43 years) than the dementia syndrome (mean 52 years) (see also Fig. 2). However, Patients 3 and 9 both show that the vascular pathology and Alzheimer's disease may occur in a single patient presenting with either haemorrhage or dementia. White matter lesions were found on neuroimaging in two out of the three patients presenting with cerebral haemorrhage, whereas three out of the five patients presenting with dementia showed white matter lesions (no scan was available for one patient). There was no evidence that *APOE* influences the age at onset of dementia or cerebral haemorrhage.

Preclinical signs in subjects at risk

None of the 21 subjects at risk showed abnormalities at the neurological examination. Scores of the neuropsychological tests were within the normal range. The four APP692 carriers

Table 4 *Scores for neuropsychological testing*

	Mutation (n = 4)		No mutation (n = 14)		P
	Median	Range	Median	Range	
Intelligence					
GIT-IQ	86	79–107	103	79–125	0.06
Expected IQ	108	86–108	108	87–127	0.30
Ratio GIT/expected IQ (%)	80	73–99	99	88–116	0.06
Attention and concentration					
Trail-Making A*	50–75	50–100	50–75	10–100	0.29
Substitution WAIS*	54	36–93	83	30–99	0.16
Auditory verbal memory					
Total score CVLT†‡	−2	−5 to 0	−1	−4 to +2	0.19
Recognition CVLT†§	+2	+1 to +3	+1	−2 to 4	0.20
Short-term memory					
Digit span WAIS*	38	14–66	52	8–84	0.16
Non-verbal memory					
Rey recall score*	90–100	10–100	80–90	10–100	0.22
Abstraction					
Similarities (WAIS)*	50	14–95	78	18–99	0.14
Executive control functions					
Trail Making B*	50–75	25–75	50–75	10–100	0.44
Word fluency test†	28	26–30	31	18–34	0.22
Visuoconstructional					
Rey complex figure*	80–90	20–100	90–100	10–100	0.36
Visuospatial					
Line orientation test*	48	22–100	74	3–100	0.36

*Percentile score; †standardized score; ‡number of words learned in five trials of 16 words; §difference between recognition and long-term free recall.

tended to have lower scores on tests of intelligence, short-term memory, abstraction and visuospatial abilities than the 14 non-carriers (Table 4). However, none of the differences between carriers and non-carriers was statistically significant. The most pronounced difference in test scores between mutation carriers and non-carriers was found for the overall IQ test (P = 0.06) and the IQ test adjusted for expected IQ (P = 0.06). For the tests measuring attention and concentration, only the substitution test of the WAIS showed lower scores for carriers. All other tests showed similar scores for carriers and non-carriers.

MRI did not show evidence for cerebral haemorrhage in any of the asymptomatic family members. The results of the semiquantitative score of the MRI scans were computed for the periventricular and subcortical region (Table 2). The five mutation carriers had a statistically significant higher score of total periventricular white matter lesions (median = 1) than the 16 non-carriers (median = 0, P = 0.001) and the clinic-based control group (median = 0, P = 0.001). The median scores for the subcortical region did not differ between carriers (median = 1) and non-carriers (median = 1), but differed with borderline significance from the age-matched controls (median = 0, P = 0.04). The total white matter lesion score for the periventricular and subcortical regions in mutation carriers (median = 2) was not different from scores in related non-carriers (median = 1), but differed significantly from those in the age-matched hospital controls (median = 0, P = 0.001). *APOE E4* was not related to the load of WMH. However, as in the affected relatives, a high proportion of subjects at risk carried the *APOE E4* allele (15 out of 21).

Discussion

Our study of eight patients with the APP692 mutation showed variable onset and progress of disease. Patients with APP692 presented with haemorrhage, dementia or both. The dementia in patients with the APP692 mutation was compatible with Alzheimer's disease both clinically and neuropathologically. The presymptomatic carriers showed a subtle, non-significant impairment of cognitive function compared with relatives without APP692. A significant increase in the number of periventricular and subcortical white matter lesions at early age was seen in presymptomatic carriers (mean age 26.4 years).

Although the APP692 and APP693 mutations both involve missense mutations at neighbouring codons, the clinical expression is very different. Patients with the APP693 mutation diagnosed with HCHWA-D suffer from recurrent strokes with a high mortality rate (Bornebroek *et al.*, 1996). Mortality after haemorrhage in carriers of the APP692 mutation is low; all patients described here are still alive after multiple haemorrhages. Few patients with the APP693 mutation diagnosed with HCHWA-D develop dementia (Bornebroek *et al.*, 1996). If dementia occurs, the clinical course is compatible with vascular dementia rather than

with Alzheimer's disease (Bornebroek *et al.*, 1996). No neurofibrillary tangles have been observed on pathological examination in patients with the APP693 mutation (Maat-Schieman *et al.*, 1996). Although it is difficult to disentangle the cause of dementia in the presence of CAA, the clinical course of dementia was compatible with Alzheimer's disease in the patients with APP692. For two patients with the APP692 mutation presenting with dementia, the clinical diagnosis of Alzheimer's disease was confirmed pathologically (Cras *et al.*, 1998). At the level of pathology, senile plaques and neurofibrillary tangles were present. Also, in the biopsy sample from Patient 3, which was taken during vascular surgery, senile plaques were found but no neurofibrillary tangles (Hendriks *et al.*, 1992). These pathological lesions did not fulfil the criteria for Alzheimer's disease because of the absence of neurofibrillary tangles (Braak and Braak, 1991). This patient was diagnosed with Alzheimer's disease 6 years after the haemorrhage. As there was no evidence for subsequent vascular events and the cognitive deficits gradually progressed, we hypothesize that the patient's cerebral pathology has developed further towards Alzheimer's disease. An implication of our hypothesis is that the vascular pathology and Alzheimer's disease are processes that occur simultaneously in carriers of APP692.

The APP692 mutation leads to mutated forms of the secreted Aβ peptides Aβ 40 and Aβ 42. The expression of APP692 as Alzheimer's disease or cerebral haemorrhage may be explained by the effect of the mutation on the levels of Aβ 40 and Aβ 42 (Hardy, 1997). The Aβ 42 peptide is deposited predominantly in senile plaques and is selectively increased in Alzheimer's disease patients carrying APP mutations (Hardy, 1997), whereas an increased Aβ 40 level may lead to vascular amyloid deposition (Gravina *et al.*, 1995). In contrast to other APP mutations, *in vitro* cDNA transfection studies show that the APP692 mutation leads to increased secretion of both Aβ 42 and Aβ 40 (De Jonghe *et al.*, 1998), which is compatible with an increased risk of Alzheimer's disease pathology and vascular amyloid deposition.

Although the findings of neuropsychological tests in presymptomatic carriers were not conclusive statistically, in general carriers appeared to perform worse on the intelligence, short-term memory, visuospatial and abstraction tests at early age (mean age 26.4 years). However, the differences between carriers and non-carriers were small, and as the findings are based on only four mutation carriers who completed the neuropsychological testing no conclusions can be drawn from this sample.

White matter pathology was found more frequently in asymptomatic mutation carriers in the periventricular regions than in the related and the age-matched controls. This finding suggests that white matter lesions are early manifestations of APP692-related pathology. Also, in the general population the strongest relation between white matter lesions and cognitive function is observed in the periventricular region (de Groot *et al.*, 2000). However, compared with age-matched hospital controls, white matter lesions in the subcortical region were more frequently present in presymptomatic carriers of the APP692 mutation. White matter lesions were also found in 60% of the patients described in this paper. The pathophysiology of white matter lesions and their role in the development of dementia are unclear. None of the subjects studied suffered from hypertension, the most important risk factor for white matter lesions in the general population (de Leeuw *et al.*, 1999). Vascular amyloid deposits leading to stenosis of arterioles may cause chronic hypoperfusion leading to white matter lesions as well as Alzheimer's disease pathology (Gray *et al.*, 1985). Patient 9 showed white matter lesions adjacent to previous haemorrhages (Fig. A2), which is in line with the observation that white matter lesions are predominantly the result of secondary damage after cortical haemorrhages and/or infarcts (Hendricks *et al.*, 1990; Silbert *et al.*, 1995).

Recently, the *E4* and *E2* alleles of the *APOE* gene have been found to modify the clinical presentation of CAA (Nicoll *et al.*, 1997). We did not find any association of the *E4* allele with age at onset and the presence of dementia in symptomatic carriers of APP692 or with the presence of white matter lesions or cognitive (dys)function in the asymptomatic APP692 carriers. Also, for the APP693 mutation no association with the *APOE* gene was found (Bornebroek *et al.*, 1997). However, 75% of the subjects in the present study carried the *APOE E4* allele and none carried the *E2* allele. The statistical power of our analysis of the effect of *APOE* on the clinical expression of the APP692 mutation was therefore low.

The findings in our family in which the APP692 mutation was segregating suggest that a single mechanism may underlie the pathology of Alzheimer's disease and CAA. Furthermore, the findings in asymptomatic carriers suggest that these diseases are subclinically manifested as white matter pathology at an early age. These observations suggest that preventive strategies in these high-risk subjects should start at early age. It remains to be determined whether our findings in carriers of APP692 mutations can be extrapolated to the vast majority of patients, who have sporadic Alzheimer's disease. Given the presence of CAA in patients with sporadic Alzheimer's disease, further studies of the pathophysiology of the APP692 mutation may be relevant to our understanding of the common origin of the CAA and Alzheimer's disease lesions.

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Appendix I
Patient descriptions

Patient 1 was seen at age 51 years because of headaches and behavioural problems. The spouse reported memory loss, aggressive behaviour, loss of initiative and gradual cognitive decline for 2–3 years. No epileptic seizures or myoclonic jerks were reported. Neurological examination was normal. Neuropsychological testing showed cognitive dysfunction (Cognitive Screening Test 6/20), with disorientation in time, memory problems (immediate and delayed recall 0/15 words), impaired visuospatial skills (inability to draw or copy a clock) and language disturbances (resembling transcortical sensory aphasia on the Achener Aphasia Test). Blood and CSF tests were normal. EEG showed a low dominant rhythm with episodes of delta activity bilaterally in the frontal regions. CT showed marked cortical and central atrophy with multiple white matter lesions. Single photon emission computed tomography (SPECT) showed symmetrical temporoparietal hypoperfusion. The clinical diagnosis was probable Alzheimer's disease according to the NINCDS–ADRDA criteria (National Institute of Neurological and Communicative Diseases and Stroke–Alzheimer's Disease and Related Disorders Association) (McKhann *et al.*, 1984). Cognitive function and capabilities declined gradually during the disease course, leading to admission to a nursing home and death at age 57 years following pneumonia.

At autopsy, moderate cortical and central atrophy was found (van Harskamp *et al.*, 1997; Cras *et al.*, 1998). The meninges were thickened and discoloured brown at the frontal and temporal lobes. Small cavities were found in the cortical and subcortical white matter, often surrounded by a brownish hue. Numerous leptomeningeal vessels with amyloid deposits were present. Throughout the whole cortex, senile plaques of different types were found (Fig. A1). Several of these plaques had a larger core than in classical Alzheimer's disease. Lumen-reducing amyloid deposits were found in the vessels. Neurofibrillary changes were numerous, especially in the superior temporal gyrus. In areas CA1 and CA2 there was extensive neuronal loss, whereas areas CA3 and CA4 were relatively spared. Immunohistochemistry showed numerous amyloid deposits, dystrophic neurites and neurofibrillary tangles (Fig. A1). Few abnormalities were found in the subcortical areas.

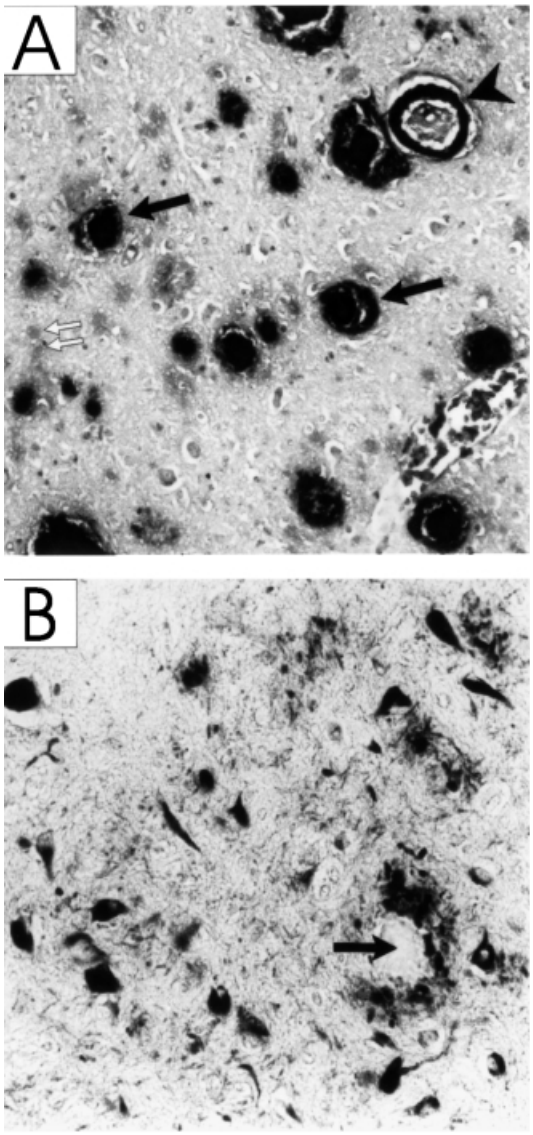

Fig. A1 (**A**) Numerous dense-core (filled arrows) and diffuse amyloid plaques (white arrows) in the neocortex stained by monoclonal antibody 4G8 against Aβ (Senetek, Maryland Heights, Mo., USA). Note also the presence of congophilic angiopathy (arrowhead). (**B**) Numerous neurofibrillary tangles and dystrophic neurites in the neocortex, immunostained with monoclonal antibody AT8 (against hyperphosphorylated tau) (Innogenetics, Belgium). Arrow indicates the core of a cored plaque.

Patient 2 was treated successfully with sodium valproate for generalized epileptic seizures at age 30 years. Neither neurological examination nor EEG showed evidence of focal pathology. Seizures recurred at age 41 years, with left-sided symptoms. Neurological

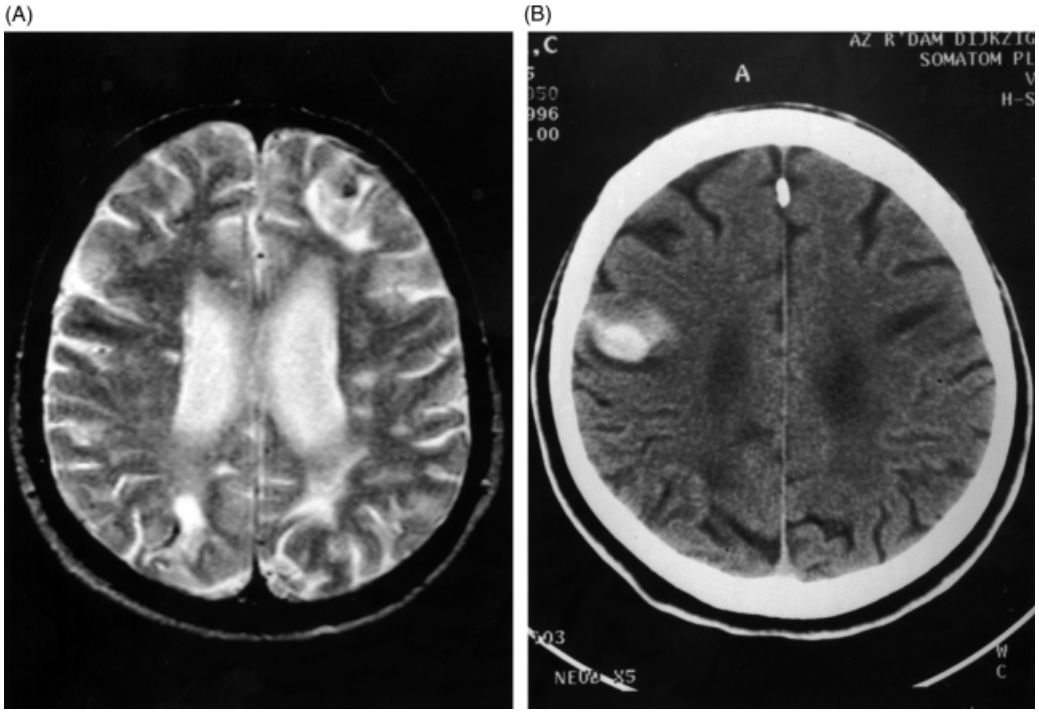

Fig. A2 (**A**) MRI scan typical of patients with dementia carrying the APP692 mutation. There are cortical and central atrophy and numerous white matter lesions, which are most prominent adjacent to old haemorrhages in the frontal and occipital cortexes. (**B**) Circumscribed haemorrhage in the right parietal cortex and global cortical atrophy.

examination was normal. Paroxysms of theta and delta activity in the central temporal region were seen on EEG. CT revealed a hypodensity in the right basal ganglia and in the left parietal region. Calcifications were present in the white matter of the centrum semiovale on the right side. At age 43 years the patient was admitted with right-sided hemiparesis, aphasia and right-sided homonymous hemianopia. Tendon reflexes in the right extremities were increased, with an ankle clonus and a right-sided extensor plantar response. CT revealed a large parietotemporal occipital haematoma with extension into the lateral ventricle. A second stroke 1 year later resulted in left-sided paresis and complete loss of consciousness. The patient remained in a vegetative state until the last follow-up (December, 1999).

Patient 3 was admitted to the neurosurgery department because of headache, nausea, vomiting and loss of consciousness at age 42 years. Rating on the Glasgow Coma Scale was E3M5V1. The patient was hemiplegic with a dilated pupil (left). CT showed a large left-sided parieto-occipital haematoma with midline shift. The haematoma was successfully removed by surgery. A brain biopsy showed extensive amyloid deposition in the blood vessel walls and in parenchymal senile plaques (Hendriks *et al.*, 1992). The senile plaques were predominantly of the diffuse type, with a few neuritic plaques but without neurofibrillary tangles. The patient was discharged with dysphasia, hemianopia and Gerstmann syndrome, without cognitive dysfunction.

The cognitive function stayed stable over the next 3 years but declined afterwards, resulting in dementia 6 years after the haemorrhage. Neurological evaluation showed an insidious onset of cognitive decline >3 years after the haemorrhage without evidence of stepwise progression. At MRI, no evidence was found for a second haemorrhage. Neurological evaluation and neuropsychological screening tests resulted in the diagnosis of probable Alzheimer's disease (NINCDS–ADRDA criteria).

Patient 4 had had symptoms since age 49 years. Neurological examination showed bradyphrenia, dysphasia and severe memory disturbances. Neuropsychological testing revealed a decline in intellectual performance (GIT IQ 57), memory loss (Rivermead Behavioural Memory Test 2/12), reduced verbal (immediate and delayed recall 0/15 words) and visual recall (recall Rey figure, 10th percentile), time orientation problems, moderate aphasia, severe disturbances of executive functions (semantic word fluency standardized score 17, mean population standardized score 25) and severe visuospatial problems (copy of Rey figure, <10th percentile). Routine blood and CSF tests were normal. CT showed white matter lesions and both cortical and central atrophy. Reduced perfusion in all cortical regions, white matter and the left thalamus was revealed with SPECT, while EEG showed diffuse slowing of activity. The diagnosis of probable Alzheimer's disease was made on the basis of the NINCDS–ADRDA criteria. Cognitive function declined gradually and the patient was admitted to a psychogeriatric nursing home and was still alive at the last follow-up (December 1999).

Patient 5 was referred to the emergency unit at age 44 years with a severe headache, nausea, vomiting and right-sided hemiplegia. Memory and orientation were normal. The patient had reported visual complaints 2 years earlier. Neurological examination showed paralysis of the right extremities with right-sided homonymous hemianopia, increased tendon reflexes and extensor responses bilaterally. CT showed a subcortical haemorrhage in the left frontoparietal region with rupture into the ventricles. The patient was lost to follow-up after admission to a rehabilitation centre.

Patient 6 had had increasing memory problems since age 46 years and was examined at age 49 years. Neurological examination was normal. Time orientation and memory were slightly disturbed and were masked by confabulations. Blood tests and CT were normal; EEG showed diffuse cortical disturbances. The diagnosis of probable Alzheimer's disease was made on the basis of NINCDS–ADRDA criteria. Within 1 year, the patient was admitted to a nursing home, where mood disturbances were treated with antidepressives. During the stay in the nursing home, cognitive function gradually deteriorated. The patient developed hypokinesia and severe rigidity. Parkinsonism was ascribed to the use of neuroleptics. Behavioural problems were marked, with screaming, aggression, agitation and obsession. At 55 years the patient died from pneumonia and bowel obstruction. Macroscopic examination of the brain showed temporal, frontal cortical and subcortical atrophy, and a pale substantia nigra (van Harskamp *et al.*, 1997; Cras *et al.*, 1998). Microscopy findings fulfilled the criteria for definite Alzheimer's disease, with amyloid plaques, numerous neurofibrillary tangles and congophilic amyloid angiopathy. A few Lewy bodies were present in the substantia nigra.

Patient 7 developed cognitive dysfunction insidiously at age 46 years. In about 1920 the patient was diagnosed with presenile dementia at age 51 years. EEG showed no alpha rhythm with bilateral synchronous, often intermittent theta activity, and diffuse delta activity. Pneumo-encephalography revealed dilated lateral ventricles, predominantly in the right frontal and occipital lobes. At age 59 years the patient died of bronchopneumonia. Autopsy was not performed.

Patient 8 first presented with memory problems, initiative loss, roaming and depressive mood at age 59 years. At age 63 years a presenile dementia syndrome was diagnosed. The patient was known to have glaucoma and diabetes mellitus type II. Besides loss of ankle reflexes, the neurological examination was normal, as were laboratory studies. EEG showed a diffuse slow rhythm with more abnormalities in the left frontotemporal region. CT scanning showed cortical and central atrophy. A diagnosis of Alzheimer's disease was made (NINCDS–ADRDA criteria). The patient was admitted to a psychogeriatric institution at age 64 years. Cognitive functions declined rapidly. The patient developed severe rigidity and died at the age of 68 years. No autopsy was performed.

Patient 9 presented at the age of 41 years with memory complaints. Cognitive screening showed slight disturbances in immediate and delayed recall (Rivermead Behavioural Memory Test 9/12). Neurological examination and laboratory studies were all normal. CT showed white matter lesions and a small hypodense lesion in the frontal cortex. EEG showed diffuse slow activity. Six months later, a marked decline in non-verbal memory (recall Rey figure, $<$10th percentile) was found. At that time the patient was diagnosed with probable Alzheimer's disease (NINCDS–ADRDA criteria). By age 42 years the patient could perform only simple tasks at work and showed a marked decline in cognitive function when tested. At follow-up, MRI showed extensive white matter lesions, two haemorrhages (both old, one not noticed earlier on CT), and cortical and central atrophy (Fig. A2A). The hippocampus was symmetrically reduced in volume. At age 46 years the patient developed a left-sided hemiplegia. CT revealed an intracerebral haemorrhage of the right parietal lobe (Fig. A2B). During recovery, partial epileptic insults occurred that were treated successfully with anti-epileptic drugs. The patient was still alive at the last follow-up (December 1999).