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ORIGINAL INVESTIGATION

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A 50-year perspective of a family with chromosome-14-linked Alzheimer's disease

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Abstract A Swedish family with two generations suffering from presenile dementia with an unusually severe Alzheimer encephalopathy was first reported in 1946. The hypothesis that the disease was inherited through a dominant gene is strongly supported by the follow-up 50 years later of three additional generations and molecular genetic findings of a novel presenilin-1 gene mutation in the family. The pedigree contains six cases with well-documented dementia in four consecutive generations. The Alzheimer encephalopathy was unusually severe in the three cases studied post-mortem, with a pronounced involvement of the central grey structures, such as the claustrum, the nuclei around the third ventricle, the central thalamic nuclei and the brain stem. There were no vascular lesions and little amyloid angiopathy. All six affected cases showed the typical temporoparietal symptom pattern and other core symptoms of Alzheimer's disease, such as logoclonia, myoclonic twitchings and major motor seizures. Other predominant features were psychomotor slowness, increased muscular tension, a stiff stooped gait and a rapid loss of weight. The symptom pattern is convincingly explained by the consistent and severe involvement of cortical and central grey structures and is probably linked to the presenilin-1 gene mutation.

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Introduction

In 1946, Essen-Möller in Lund, Sweden described a family in which the father, the daughter and one of two sons had died from a slowly progressive dementia with presenile onset (Essen-Möller 1946). The neuropathological examination of the son's brain showed an unusually severe Alzheimer encephalopathy but no damage of the vascular type. Essen-Möller gave a detailed account of the clinical histories of the three individuals involved. His conclusion 50 years ago was that "perhaps there will be cause to return to this family in a few years. A case of Alzheimer's disease (AD) in the third generation would be evidence in favour of the hypothesis that the disease is inherited through a dominant gene". We have followed up this specific family for three additional generations and conclude from molecular genetic findings that a novel presenilin-1 gene mutation is responsible for the family's AD.

Subjects and methods

Subjects

The pedigree of the first five generations studied are presented in Fig. 1. The sixth generation is omitted to make identification of the family more difficult. The first of the new generations contains two post-mortem verified cases of AD, one of which (IV:9) was the daughter who had earlier asked for genetic counselling. The fifth generation has so far presented one male patient (V:4), who is a son of case IV:9, with the clinical picture of AD.

Neuropathology

The brains were fixed in formalin at autopsy and sectioned in coronal whole-brain slices, out of which about every other slice was embedded in paraffin and sectioned at 5 μm. Sections were stained by routine staining procedures to reveal plaques, tangles and dystrophic neurites. Immunomethods to show the presence of amyloid were applied (Brun and Gustafson 1993).

Table 1 Clinical findings in a family with early onset Alzheimer disease (*0* clinical information not available, ? questionable, + marked, (+) moderate presence of symptom or symptoms)

Case	II:1	III:2	III:3	IV:2	IV:9	V:4	
Sex	M	M	F	M	F	M	
Age at onset	35	43	35	48?	49	46	
Age at death	53	58	44	51	56		
Duration	18	15	9	3?	7	(9)	
Insidious onset	+	+	+	+	+	+	100%
Early amnesia	+	+	+	+	+	+	100
spatial disorientation	0	+	–	+	+	–	50
preserved insight	+	+	+	+	+	+	100
Dyspraxia, dysgnosia	+	+	+	+	+	+	100
Expr and rec dysphasia	+	+	+	+	+	+	100
Logoclonia	+	+	–	+	–	+	67
Vocally disruptive	+	+	–	–	+	–	50
Late mutism	+	+	–	(+)	(+)	(+)	83
Dyslexia	0	–	+	+	+	+	67
Restlessness - agitation	–	+	+	–	+	+	67
Suspiciousness - delusions	–	+	–	+	+	+	50
Psychomotor slowness	+	+	+	+	+	+	100
Increased muscular tension	+	(+)	+	+	+	+	100
Gait disturbance	+	–	+	+	+	(+)	83
Grand mal and myoclonia	–	–	–	+	+	–	33
Late incontinence	+	–	–	+	+	+	67
Rapid loss of weight	+	+	+	+	+	+	100

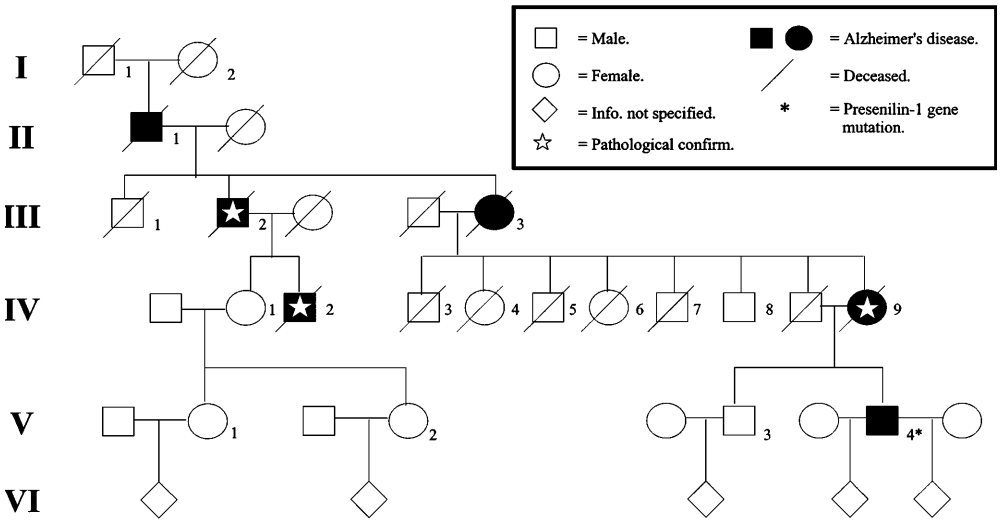

Fig. 1 Pedigree of the E-M family

DNA analyses

Blood samples from the subjects were collected in tubes containing EDTA as anticoagulant and genomic DNA was isolated from the leucocyte fraction (Miller et al. 1988). Apolipoprotein E (Apo E) genotyping was accomplished as previously described (Kontula et al. 1990). The presenilin-1 gene was screened for mutations in the five fragments containing the major fraction of known AD-related mutations by using the polymerase chain reaction (PCR) primer pairs described by Sherrington et al. (1995). For all fragments, amplification was performed by 1 min denaturation at 94°C, 1 min annealing at 54°C and 1 min extension at 72°C, repeated over 35 cycles. The cycles were preceded by 5 min denaturation at 95°C and followed by 10 min extension at 72°C.

Single-strand conformation polymorphism (SSCP) analysis of the PCR fragments was accomplished as described earlier (Ponjavic et al. 1996). Direct dideoxy sequencing of both strands of PCR products, indicated as containing sequence alterations by SSCP, was carried out on heat-denatured samples following enzymatic removal of excess primers and nucleotides, by using 35S-dATP for labelling and the modified T7 DNA polymerase, buffer and nucleotides from the Sequence PCR Product Sequencing Kit (United States Biochemical, Cleveland, Ohio, USA). The sequencing primers for the exon 5 fragment of the presenilin-1 gene were MA415, 5-GAG ACT GTC GGC CAG AGA-3 and MA417, 5-GAA AGC AAA GAT CTG TGT CT-3. Both strands of the PCR fragments were sequenced in order to cover the entire sequence and subsequently to be able to verify the mutation.

Loss of a recognition site for the restriction enzyme, *Bsph*I, resulting from the Met-146–Ile causing mutation, was analyzed exactly as described for the Met-146–Leu mutation (Sherrington et al. 1995), by using enzymes from Amersham. This assay was used for routine mutation analysis in the family under study.

Results

Clinical findings

A typical and mainly similar clinical picture of AD was described in all six affected cases in four generations (Table 1). There was an insidious onset and slow progression of the cognitive decline starting between 35 and 49 years of age. Memory failure was recognised early and commented upon by the patients themselves. Case II:2 said that he could not remember "as far as from his mouth to his nose". Case III:2 who was a farmer "would plough the same field twice over". Insight and habitual personality traits were not greatly affected when the first symptoms of cognitive deterioration appeared. Spatial disorientation was an early sign in 50% of the cases and the patients became increasingly dyspractic. Severe visual dysgnosia was described in four cases of three generations, in case V:4 in combination with a disturbing "mirror sign". This male patient would stand in front of a mirror for an hour or more, indulging in fantasies about the unknown person at whom he was looking. Word-finding difficulties and reduced spontaneity of speech were noted in all cases, later with the addition of severe receptive dysphasia and almost incomprehensible speech. Four cases from three generations showed the stuttering-like phenomenon of logoclonia, whereas slight echolalia was present in two cases. Three cases developed vocally disruptive behaviour (Hallberg et al. 1990) and finally all cases became mute or semimutistic.

Other predominant characteristics in this family were a general psychomotor slowness, increased muscular tension of the extrapyramidal type and a peculiar disturbance of gait. All cases except III:2 developed increased muscular tension, with slight tremor and cog-wheel phenomena being observed in two cases. The gait was described as slow and stiff in four cases, three of these having a stooped habit. Case II:2 walked, bent forward with long slow steps, "curtseying" at the knees, stiffly and heavily. Myoclonic twitchings and generalised epileptic seizures were seen in the two cases of the fourth generation. The electroencephalogram (EEG) recordings in these cases and in V:4 were severely pathological. Regional cerebral blood flow measured by the xenon inhalation technique in case V:4 showed the typical bilateral temporoparietal flow decrease of AD (Risberg et al. 1993). The neurological and physiological examination showed no other somatic cause of the dementia process. All six cases were surprisingly free from vascular risk factors and none of the cases suffered from diabetes. Blood pressure was recorded in three cases and showed fairly low values, viz. 110/175, 100/60 and 110/70 mm/Hg, respectively. The medical records of all six cases described periods of six months or more with rapid weight loss in spite of fairly normal food intake.

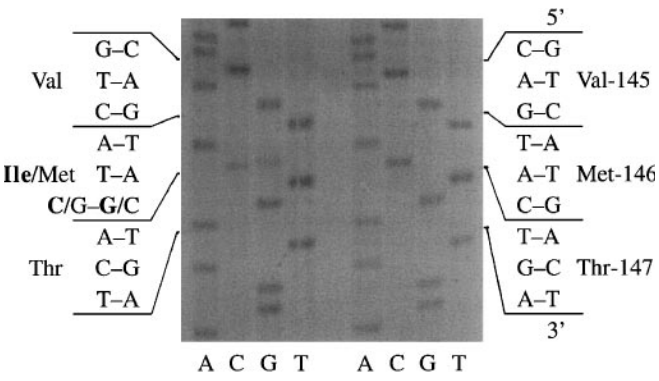

Fig. 2 Presenilin-1 gene mutation in the E-M family. PCR fragments corresponding to exon 5 of the presenilin-1 gene show aberrant SSCP patterns for AD-affected individuals from the family. Part of the sequence for the non-coding strand is shown for case V:4 (*left*) and for a non-affected individual (*right)*

DNA findings

Patient V:4 had previously been screened for mutations in the amyloid precursor protein (APP) gene. Complete sequencing of exons 16 and 17 of the gene demonstrated an unusual silent mutation in exon 17 but no potential disease-causing mutations (Balbín et al. 1992). ApoE typing showed that the patient was homozygous E3/3. Following

Table 2 Neuropathological findings in a family with Alzheimer's disease (++ severe, + moderate, (+) mild degeneration)

Case	III:2	IV:2	IV:9
Sex	M	M	F
Age on onset	43	48?	49
Age at death	58	51	56
Duration	15	3?	7
Brain weight		1175	913
Pattern of atrophy			
temporoparietal		++	++
frontal		+	+
anterior cingulate		(+)	(+)
posterior cingulate		++	++
sensory motor cortex		(+)	(+)
Hypothalamus		+	+
Corpora mamillaria		+	(+)
Claustrum		+	+
Central thalamic nuclei		+	+
Brain stem		+	+
Substantia nigra		+	(+)
Pontine nuclei		(+)	(+)
Amyloid angiopathy		(+)	(+)

[a] Neuropathological findings published by Essen-Möller (1946) showed severe degeneration similar to that in cases IV:2 and IV:9

the report of Sherrington et al. (1995), the presenilin-1 gene on chromosome 14 was screened for mutations by SSCP analysis of the five exons harbouring the majority of known AD3 mutations. A deviating SSCP gel pattern for the exon 5 fragment (Clark et al. 1995) from the patient was found. Sequencing revealed a single base substitution in codon 146 (ATG→ATC), resulting in the amino acid substitution Met-146-Ile (Fig. 2). The base change destroys a normal recognition site for *Bsph*I, meaning that the mutation could be followed in the family by direct digestion of the exon 5 PCR fragment (not shown). As far as we know, this mutation has not been detected before but it is interesting to note that two other mutations in the same codon resulting in Met-146–Val (in three unrelated families) or Met-146–Leu have been described recently (Clark et al. 1995). The indication that residue 146 is important for normal presenilin-1 function prompted us to summarise all available clinical and neuropathological data, as elucidation of the genotype–phenotype correlations for AD3 should provide important clues regarding the molecular basis for the disease.

Neuropathological findings

Post-mortem neuropathological investigations were performed in three cases. The findings in case III:2 were presented in 1946 by Essen-Möller and the present communication describes the findings in the fourth generation (cases IV:2 and IV:9), as summarised in Table 2.

Case IV:2 died at the age of 51 because of pulmonary embolism. The brain weighed 1175 g and was diffusely and moderately atrophic with a slightly widened ventricular system. Microscopy showed the classical Alzheimer changes deviating from the average sporadic Alzheimer case in terms of intensity and distribution of changes. Thus, all cortical areas were unusually severely involved, including the frontal areas with large numbers of plaques, tangles and dystrophic neurites in all laminae and fewer (but still many) plaques and tangles in the hypothalamus and mamillary bodies, the central nuclei of the thalamus and the claustrum, fewer still in the putamen and few to none in the globus pallidus. In the brain stem, these same changes were also seen in the substantia nigra and the midline nuclei beneath the aqueduct, with occasional tangles in the pontine nuclei. The sensory-motor cortex was mildly affected, although the changes here were more advanced than is usually the case. Amyloid angiopathy was seen only in a few places in meningeal vessels and only occasionally in intracortical vessels.

Case IV:9 was a 56-year-old woman who died of bronchopneumonia. The brain was diffusely and severely atrophic, weighing only 912 g. The ventricular system was moderately widened with thinning of the callosal body. The microscopical changes were the same as those reported above and were again extremely intense; in addition to being found in the cortex, they occurred in the central grey structures, as in case 1V2, being somewhat more pronounced in the mesencephalon, especially in its tegmental portions. Again, the motor cortex was not as badly affected as other areas but was still more involved than is usually the case. Amyloid angiopathy was observed only in occasional vessels.

These cases thus had in common an unusual severity of Alzheimer changes, including a more pronounced involvement of the central grey structures than is usually seen in Alzheimer's disease, e.g. in the claustrum, the nuclei around the third ventricle, the central thalamic nuclei and the brain stem. In addition to these quantitative and topographic similarities, amyloid angiopathy was very mild in both cases. In all these respects, these cases differ from the majority of sporadic Alzheimer cases, probably reflecting a special genetic background. These findings are similar to those previously reported in case III:2. He was described as an extremely severe case of AD, with a profusion of senile plaques and fibrillary degeneration, these changes being more generalised than ever previously noticed by the experienced examiner. The cortical layers were disordered and showed intense hyperplasia of the macroglia and distinct marginal gliosis (Essen-Möller 1946).

Discussion

The presenilin-1 gene mutation found in the family under study results in a substitution in codon 146 leading to the amino acid substitution Met-146-Ile. The clinical picture of this pedigree of a multi-generational Alzheimer family is highly consistent with very small phenotypic variation, as are the neuropathological findings in the three cases studied post-mortem. They all had in common an unusual severity and widespread distribution of changes and the two cases studied by us both had a mild form of amyloid angiopathy. The symptom pattern is convincingly explained by the progressive degeneration of the hippocampus, the posterior cingulate gyrus and the temporoparietal association cortex (Brun and Gustafson 1976). Several patients passed through a stage with vocally disruptive behaviour and the late stage was characterised by inertia and almost total loss of verbal communication. These language disturbances may be related to the marked frontal lobe involvement in addition to the post central cortical degeneration. Logoclonia has so far not been linked to any specific localisation or type of brain damage. The high prevalence of myoclonus and the combination of myoclonus and generalised epileptic seizures frequently reported in early onset and familial AD have never been fully understood. An association with temporal limbic, subcortical and cerebellar lesions suggested by Sourander and Sjögren (1970) and others is in agreement with the neuropathological and EEG findings in cases IV:4 and IV:9.

The predominant features of psychomotor slowness, increased muscular tension and a stiff stooped gait were described by independent observers in almost all our cases. It is tempting to relate these symptoms to the extrapyramidal pathology, although the cerebellar involvement may also contribute. The consistent degeneration of the claustrum is interesting with respect to the extensive connections be-

tween the claustrum and the neocortex and that these connections are topographically organised. This involvement of the claustrum in AD could be primary or the result of anterograde degeneration secondary to pathological changes in the cortex (Morys et al 1993).

A rapid loss of weight with no somatic explanation was found in all six cases of our family. It has recently been pointed out that this feature, first described by Alzheimer, is a neglected and misunderstood manifestation of AD (Aronson et al. 1993). The findings in our material suggest a relationship to the consistent hypothalamic involvement or to the reduction of neuropeptide Y in AD (Minton et al. 1996) and its relationship to hypothalamic regulation of metabolism and body weight (Frankish et al. 1995, Davies and Marks 1994).

The striking pathological similarities between the two cases and the previously reported family member tallies with a common etiology and might point to a picture typical for the chromosome-14 mutation found in this family. A general conclusion might be that the present family shows important similarities to other families with chromosome-14-encoded AD (Haltia et al. 1994; Van Broeckhoven et al. 1992; Martin et al. 1991). Our cases showed a marked variation in the age of onset (35–48 years) and in the the duration of the disease (3–18 years). This indicates the importance of other etiological and modulating genetic and environmental factors and the difficulties of recognising the first symptoms of dementia in some cases.

The extended time perspective on our pedigree adds support to the hypothesis of a dominant inheritance of AD as suggested for this family 50 years ago; the cause is now identified as a mutation in the presenilin-1 gene of chromosome 14. The present design makes it possible to analyse clinicopathological correlates and their relationships to genetic factors in additional family members now exhibiting signs of the genetic curse of this devastating disorder.

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