CASE REPORT

A Second Pedigree with Amyloid-less Familial Alzheimer’s Disease Harboring an Identical Mutation in the Amyloid Precursor Protein Gene (E693delta)

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Abstract

A 59-year-old woman developed early-onset, slowly progressive dementia and spastic paraplegia. positron emission tomography (PET) imaging revealed a large reduction in the level of glucose uptake without amyloid deposition in the cerebral cortex. We identified a homozygous microdeletion within the amyloid β (Aβ) coding sequence in the amyloid precursor protein (APP) gene (c.2080_2082delGAA, p.E693del) in three affected siblings and a heterozygous microdeletion in an unaffected sibling. The identical mutation was previously reported in the first Alzheimer’s pedigree without amyloid deposits. Furthermore, an increase in high-molecular-weight Aβ-reactive bands was detected in the patient’s CSF. Our findings suggest that soluble Aβ-oligomers induce neuronal toxicity, independent of insoluble Aβ fibrils.

Key words: Alzheimer’s disease, familial Alzheimer’s disease, APP gene, Aβ oligomers, PET

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Introduction

Alzheimer’s disease (AD) is distinguished pathologically from other forms of dementia by amyloid deposition in the brain (1). Amyloid deposits are comprised of insoluble fibrils of 40 and 42-residue amyloid β (Aβ) peptides, derived from the amyloid precursor protein (APP). To date, approximately 40 missense mutations in the APP gene have been identified in over 80 AD families, most of which are located near processing sites or within the Aβ coding sequence (2). Almost all mutations in the APP gene cause the disease in a dominant manner, suggesting that these mutations confer a gain-of-function that results in the enhanced formation and deposition of insoluble Aβ fibrils (3, 4). However, one AD pedigree was reported to have a single amino acid deletion within the Aβ coding sequence (E693delta), inherited as a recessive trait, with a lack of Aβ deposition (5). Recently, soluble Aβ oligomers, the precursors of insoluble Aβ fibrils, have been suggested to play a pivotal role in the pathogenesis of AD (6, 7). In this study, we report a second recessive AD pedigree negative for amyloid plaque, harboring the identical E693 deletion. Our findings suggest a link between this recessive mutation and the enhanced formation of soluble Aβ oligomers.

Case Report

We examined three patients from a single generation in a pedigree from an isolated island in the Seto Inland Sea, Japan (Fig. 1A). The subjects’ parents were first cousins and had no history of apparent episodes of memory or motor impairment.

The proband (II-8) was a 59-year-old woman admitted to our hospital for treatment of aspiration pneumonia. She had been well until 35 years of age, when her family members...
noticed short-term memory disturbances, particularly as she took her dog for a walk numerous times each day. She was diagnosed with AD at 42 years of age based on progressive cognitive impairment and prominent spatial disorientation. At 48 years of age, she first complained of difficulty walking in a straight line and consequently required a wheelchair for mobility. She became bedridden with urinary incontinence by 50 years of age. She was mute and unable to obey simple commands. She was admitted to our hospital at 56 years of age, at which time she had spastic paraparesis and mild dysphagia. The Aβ level in the serum and the total and phosphorylated tau levels in the cerebrospinal fluid (CSF) were normal. Fluid-attenuated inversion-recovery (FLAIR) magnetic resonance imaging (MRI) imaging showed remarkable brain shrinkage at 56 years of age (Fig. 1B). [18F]-fluorodeoxyglucose (FDG) positron emission tomography (PET) revealed a greater reduction in the level of glucose uptake in the cerebral cortex compared to that observed in normal control subjects (Fig. 1C, right).

Patients II-2 and II-5, the 76-year-old brother and 65-year-old sister of the proband, respectively, had milder clinical signs and symptoms than the proband. The onset of memory impairment in the brother and sister at 59 and 44 years of age, respectively, was succeeded by difficulty in walking due to spasticity of the lower limbs at 66 and 58 years of age, respectively. Both patients exhibited spastic paraparesis and mutism and were being treated at other hospitals.

The local ethics committee approved the present genetic study (No. 552-1), which was performed with informed consent from an unaffected sibling (II-6) and the spouses of the affected siblings (II-8, II-2, II-5). By sequencing exons 16 and 17 of the APP gene, we identified a homozygous microdeletion (c.2080_2082delGAA, p.E693del) in the affected siblings, whereas the unaffected sibling had a heterozygous deletion. Codon 693 in the APP gene codes for amino acid protein 22 (E22) of the Aβ peptide (Fig. 2A). The proband displayed normal sequences for all exons in two presenilin genes (PSEN1 and PSEN2), with an APOE genotype of ε3/ε3.
Denaturing electrophoresis demonstrated the levels of high-molecular-weight bands recognized by the anti-Aβ antibody to be markedly elevated in the proband (Fig. 2B, lower panel), thus suggesting enhanced formation of soluble Aβ oligomers. Compared to the control levels, the total Aβ level was decreased under the denaturing conditions (Fig. 2B, upper panel). Interestingly, however, non-denaturing electrophoresis demonstrated the levels of high-molecular-weight bands recognized by the anti-Aβ antibody to be markedly elevated in the proband (Fig. 2B, lower panel), thus suggesting enhanced formation of soluble Aβ oligomers.

**Figure 2.** DNA and protein analyses. (A) Schematic representation of the APP protein structure (upper). The amino acid E693 in the APP protein corresponds to amino acid E22 of the Aβ peptide (Aβ22, middle). DNA sequences of the proband (P, II-8; lower) and an unaffected sibling (Unaffected, II-6; lower) are shown. The microdeletion of the APP gene and the deduced amino acid sequence of the APP protein are shown. (B) Increased formation of high-molecular-weight bands reactive against the anti-Aβ antibody in the CSF obtained from the proband. Aliquots of CSF obtained from the proband (lane 1) or normal control subjects (lanes 2-5) were size fractionated under denaturing (upper) or non-denaturing (lower) conditions and subsequently immunoblotted with a mouse monoclonal anti-Aβ antibody (82E1). This analysis showed the enhanced formation of high-molecular-weight Aβ products (visible as diffuse banding) under non-denaturing conditions in the CSF obtained from the proband (arrowhead, lower), despite a reduction in the level of total Aβ under denaturing conditions (upper). Synthetic Aβ peptides were run on the same gel for comparison purposes (lane 6).

We then examined the level of Aβ in the CSF sample obtained from the proband. An immunoblot analysis using an anti-Aβ antibody was performed under both denaturing and non-denaturing conditions. Compared to the control levels, the total Aβ level was decreased under the denaturing conditions (Fig. 2B, upper panel). Interestingly, however, non-denaturing electrophoresis demonstrated the levels of high-molecular-weight bands recognized by the anti-Aβ antibody to be markedly elevated in the proband (Fig. 2B, lower panel), thus suggesting enhanced formation of soluble Aβ oligomers.

**Discussion**

In this report, we described a recessive familial AD pedigree harboring a single amino acid deletion mutation (E693 delta) within the APP gene, identical to one previously reported (5). The most remarkable phenotypic features of this mutation are the lack of amyloid deposition and increased soluble Aβ oligomers in the CSF. It may be inappropriate to categorize this form of dementia without amyloid deposition as AD; however, recent findings indicate that Aβ oligomers...
play a critical role in synaptic dysfunction, at least in the early stage of AD (9-11). This case report further indicates that Aβ oligomers induce neuronal degeneration without amyloid deposition. Because abnormal metabolism of APP or Aβ is a molecular pathogenetic feature in the current pedigree, our subjects can be diagnosed to be within the range of the AD spectrum.

It remains unclear whether Aβ oligomers accumulate in synapses or somata or how they impair synaptic transmission and induce neuronal dysfunction (12). A synthetic E693 delta Aβ peptide was recently shown to facilitate Aβ oligomerization, although this did not lead to Aβ fibrillation (5). Additionally, APP-E693delta transgenic mice exhibit a brain pathology partially resembling that of AD, including the presence of intracellular Aβ oligomers, although without extracellular Aβ deposition (12). Indeed, the CSF obtained from the current proband showed an increased level of high-molecular-weight Aβ-reactive bands, presumably corresponding to toxic Aβ40/42 oligomers. In accordance with that observed in the first report of this condition, the homozygous E693 deletion of the APP gene in this pedigree may cause dementia solely via the formation of toxic Aβ oligomers, not the deposition of insoluble Aβ fibrils.

Kinship with the previously reported pedigree (5) is not clear in our investigation, which was limited to the identification of second- and third-degree relatives of the proband. Compared with the patient in the first report (5), our proband exhibited an earlier onset of dementia (33 vs. 55 years), more profound motor impairment (paraplegia vs. mild pyramidal tract signs) and more severe brain shrinkage (whole brain atrophy vs. parietal lobe atrophy). Other differences in genetic background may modify the severity of these phenotypes. Further studies are therefore required to clarify the pathogenetic mechanisms underlying the phenotypic differences caused by identical amino acid deletions.

The authors state that they have no Conflict of Interest (COI).

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