SHORT REPORT

Novel amyloid precursor protein gene missense mutation (D678N) in probable familial Alzheimer's disease

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Objective: To describe a novel missense mutation, Asp678Asn (D678N), in the amyloid precursor protein (APP) gene in a Japanese pedigree of probable familial Alzheimer's disease (FAD).
Subject: The proband was a women of 72. Symptoms of dementia that fulfilled the criteria for probable Alzheimer's disease appeared at about 60 years of age, and slowly worsened over more than 10 years without evident cerebrovascular complications, either clinically or neuroradiologically.
Methods: Polymerase chain reaction single strand conformational polymorphism (PCR-SSCP) analysis followed by sequence analysis was used to examine genomic DNA of the proband for mutations in the APP gene exons 16 and 17.
Results: Analysis of the APP exon 16 in the proband showed a GAC to AAC nucleotide substitution in codon 678 of the APP gene, causing an amino acid substitution of Asp to Asn (D678N). Heterozygosity of the APP D678N mutation was found in the proband and in the demented elder sister.
Conclusions: The production and accumulation of mutated Abeta (Asn7-Abeta) or the misfunction of D678N mutant APP may have pathogenic properties for the development of Alzheimer's disease in this pedigree.

Mutations in three causative genes—amyloid precursor protein (APP),[1] presenilin 1 (PS1), and presenilin 2 (PS2),[2 3]—have been implicated in the pathogenesis of familial Alzheimer's disease (FAD). Mutations in the APP gene located close to the β- and γ-secretase cleavage sites lead to early onset FAD, whereas the clinicopathological representations of genes within the sequences coding for amyloid β peptide (Abeta) sequence are variable—that is, hereditary amyloid angiopathy with cerebral haemorrhage (HCHWA-D, Dutch mutation; E693Q, Italian mutation; E693K),[4] Alzheimer's disease with repeated stroke (Flemish mutation; A692G),[5] dementia with severe amyloid angiopathy (Iowa mutation; D694N), or Alzheimer's disease without stroke (Arctic mutation; E693G).[6 7] Here, we describe a novel APP mutation (D678N) in a Japanese-Tottori FAD (Jp-T) pedigree. The D678 mutation replaces the aspartate (Asp; D)7 of Abeta with asparagine (Asn; N) and was clinically linked to clinically diagnosed FAD without signs of vascular involvement.

METHODS

Patients

The Jp-T pedigree consisted of three demented patients and nine siblings (fig 1A). The proband (II-12) of this pedigree (fig 1A) was admitted to our hospital at 65 years of age, at which time he had been experiencing amnesia, disorientation, loss of interest in previous household activities, and occasional aggressive behaviour for over six years, without stroke-like episodes. Initial examination revealed moderate intellectual deterioration, severe memory impairment without focal neurological signs or parkinsonism. The mini-mental state examination (MMSE) score at this time was 9/30. Magnetic resonance imaging (MRI) showed mild cortical atrophy of the bilateral medial temporal lobes and parietal lobes. On ^{132}I-IMP SPECT there was marked hypoperfusion in the temporal and parietal cortices and normal blood flow in the cerebellum, motor cortex, and basal ganglia. The total tau protein and Abeta42 levels in the spinal fluid were 738 pg/ml (normal <250 pg/ml) and 295 pg/ml (normal >700), respectively, consistent with the characteristic findings of Alzheimer's disease.[8 9] The dementia syndrome gradually progressed over the next seven years.

Serial MRI at age 72 showed marked cortical atrophy including the hippocampal region bilaterally, without focal cerebral infarction or haemorrhagic lesions (fig 1B).

At the time of writing, her dementia symptoms had progressed to such a degree that she required total assistance in dressing, hygiene, and food intake. Her communication skills had severely deteriorated and dyskinetic movements in face and head without myoclonus were observed.

The proband's elder sister (II-10) had been experiencing amnesic symptoms that appeared when she was 60. Five years after the onset of her symptoms, she was unable to recognise her family. Over the next 15 years, she showed slowly progressive cognitive decline without stroke-like episodes. At age 75, she was severely demented, bed ridden, and fully dependent for personal care, and oral dyskinesia was observed. MRI at the age of 77 showed severe cerebral atrophy with mild periventricular hyperintensity lesions in the T2 weighted images but without focal cerebral infarction or haemorrhagic lesions (fig 1, C). The mother had no symptoms of dementia before she died at age 85. The father died at age 64 from an accident and it is unclear whether he was demented. It was reported that the eldest brother (II.1), who died at age 69, had an almost identical medical history of dementia beginning in his late 50s. The fourth to ninth and the 11th siblings all died aged less than 50 years.

Genetic analysis

After obtaining informed consent (from relatives where appropriate), genomic DNA was isolated from peripheral white blood cells of three family members: patient 1, II-12; patient 2, II-10; and a non-demented 68 year old member of the family, III.1, who is a son of the eldest affected brother (II.1). Mutation screening in APP exons 16 and 17, which code the Abeta peptide sequence and the surrounding region,

Abbreviations: Abeta, amyloid β peptide; APP, amyloid precursor protein; FAD, familial Alzheimer's disease; PCR-SSCP, polymerase chain reaction single strand conformational polymorphism

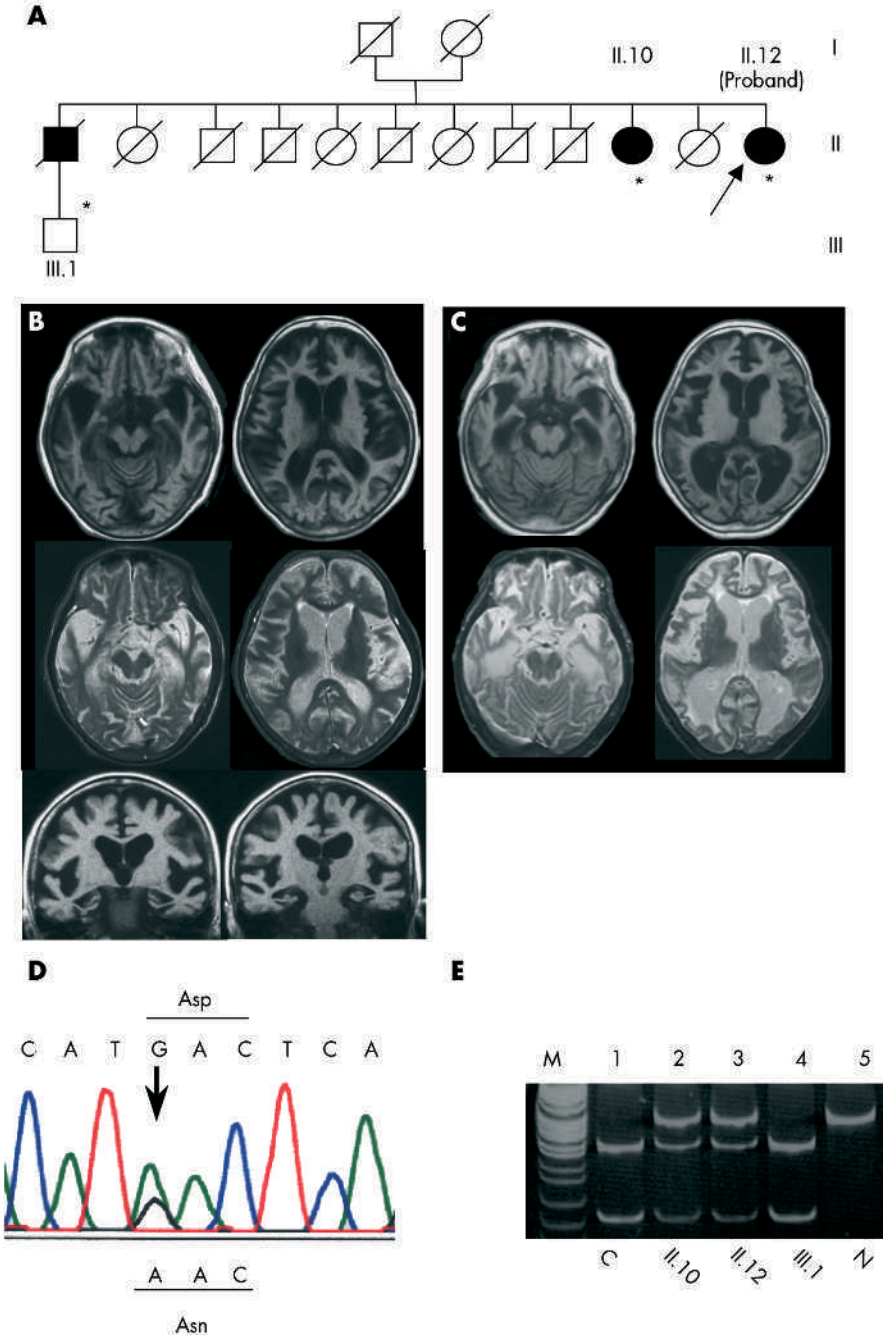

Figure 1 Family tree, neuroimaging findings, and molecular analysis of the Japanese-Tottori pedigree. (A) The pedigree: the proband is indicated by an arrow. Samples (*) were taken from three individuals for DNA analysis. (B) Axial and coronal magnetic resonance images (MRI) of the proband (II.12) at age 71 showing diffuse and severe cortical atrophy predominantly in the internal portion of the temporal lobes, without focal cerebral infarction or haemorrhage. (C) Axial MRI of the elder sister at age 71 showing marked cortical atrophy with mild periventricular hyperintensity lesions in T2 weighted images. (D) Direct sequencing of APP exon 16 PCR product derived from patient 1 (II.12) demonstrates heterozygosity for GAC-to-AAC nucleotide substitution in codon 678 of APP gene (APP770 numbering), that corresponds to an aspartic acid (Asp) to an asparagine residue (Asn) (D678N). (E) Characterisation of the D678N missense mutation in the APP gene. *Hinf*I RLFP analysis shows that only the affected members (II.10, lane 2; II.12, lane 3) are heterozygous for the D678N mutation. C = control (lane 1). III.1 = a 68 year old non-demented family member (lane 4). N = undigested PCR product (lane 5).

were carried out by polymerase chain reaction single strand conformational polymorphism (PCR-SSCP) analysis. The primer sequences are as follows: APP exon16, APP Ex16Fw; 5′-TAGAAAGAAGTTTTGGGTAGGCTTT-3′ and APP Ex16-Rv; 5′-AGAGTTAATAGGTCATTTGGCAAGACA-3′, APP exon17, APP Ex17-Fw; 5′-AATGAAATTCTTCTAATTGCGTTT-3′ and APP Ex17-Rv; 5′-TTCTCTCATAGTCTTAATTCCCACTT-3′. PCR was done using a Hot Start PCR kit (TaKaRa, Tokyo, Japan), following the manufacturer's instructions. For SSCP analysis, the PCR products were denatured in formamide containing buffer, and electrophoresed on 12% acrylamide gels with 10% glycerol at 4℃ for 20 hours at 200 V, followed by visualisation using silver staining. The PCR product showing abnormal bands on SSCP analysis, predicting the presence of a mutant allele, was purified and sequenced. Sequence analyses of PS1 and PS2 coding regions using the RT-PCR product from

cultured skin fibroblasts of patients 1 and 2 were also undertaken. The *APOE* genotype was determined by a standard PCR *Hha*I restriction enzyme digestion assay.[10]

RESULTS

PCR-SSCP analysis of APP exon 16 showed abnormal bands in the proband sample (data not shown). Sequence analysis of the APP exon 16 revealed a GAC to AAC nucleotide substitution in codon 678 of the APP gene that causes an amino acid substitution of Asp to Asn (D678N) (fig 1D). No pathogenic mutations in APP exon 17 were detected in the proband sample (data not shown). Because D678N mutation abolishes the *Hinf* I restriction site, we looked for the presence of this mutation by *Hinf* I restriction fragment length polymorphism (RFLP) analysis of genomic amplicons. Analysis of the samples from the two affected members (patients 1 and 2) and a 68 year old unaffected member showed that only the affected members were heterozygous for the D678N mutation (fig 1E). The *Hinf* I RFLP analysis also showed the absence of this mutation in samples from 215 patients with sporadic Alzheimer's disease, five members of another five independent early onset FAD pedigrees, and 102 age matched control subjects. These data indicate that the D678N mutation is specific to the Jp-T pedigree. In addition, all members of this pedigree tested had the epsilon3/epsilon3 genotype of the *APOE* gene. Sequence analyses of PS 1 and PS2 coding regions using the reverse transcriptase polymerase chain reaction products from cultured skin fibroblasts of patients 1 and 2 failed to identify a pathogenic mutation (data not shown).

DISCUSSION

We report a novel D678N mutation in the APP gene identified in a Japanese FAD pedigree. This mutation causes an amino acid substitution of Asp at position 7 of Abeta (Asp7-Abeta) with Asn (Asn7-Abeta). The clinical manifestations of the mutations located close to the β- and γ-secretase cleavage sites—which directly affect the proteolytic processing of APP—are those of typical early onset FAD.[1 11–20] In contrast, the mutations causing amino acid substitutions within internal sequence of Abeta represent variable clinical and pathological features, presenting with different degrees of amyloid angiopathy or Alzheimer's disease pathology—for example, hereditary amyloid angiopathy with cerebral haemorrhage (E693Q and E693K),[4] Alzheimer's disease with repeated stroke (A692G),[5] dementia with severe amyloid angiopathy (D694N), or Alzheimer's disease without stroke (E693G).[6 7] Although the pathological features of our D678N mutant patients are still unknown, their clinical manifestations were those of typical Alzheimer's disease, characterised by progressive dementia without cerebrovascular complications in the clinical history and neuroradiological examination, fulfilling the NINCDS-ADRDA criteria for Alzheimer's disease.[21]

A recent report described the identification of novel APP H677R mutation adjacent to the D678N mutation, which causes an amino acid substitution of His at position 6 of Abeta (His6-Abeta) with Arg (Arg6-Abeta).[22] Because the H677R mutation was present in one of two siblings with pathologically proven Alzheimer's disease in this report (mean age at onset 55 years), the pathogenicity of this intra-Abeta sequence variant needs to be clarified. However, it is assumed that the APP D678N and H677R mutations have a substantial impact on the pathomechanism of the development of FAD.

Currently we are studying the APP metabolism in cells transfected with D678N APP, as well as carrying out in vitro fibrillisation assays using synthetic Asn7-Abeta, but we have not seen increased production of Abeta (including Abeta42), or acceleration of Abeta aggregation (Hashimoto T, Saido T C, Iwatsubo T, unpublished observations). Alternatively, it has been reported that Asp residues of Abeta peptide (Asp1, Asp7, and Asp23) play an important role not only in fibril production but also in protofibril formation and initial α-helix formation. Artificial replacement from Asp residue to Asn residue at position 7 of Abeta has been reported to show altered fibrillogenesis kinetics (delayed helix formation) relative to wild type Abeta peptide.[23] Another study revealed that synthetic Asn7-Abeta peptide has the reduced ability to activate the classical complement pathway activation compared with wild type Abeta, the implications of which may also be relevant to the pathogenesis of Alzheimer's disease caused by this mutation.[24]

From these results, we postulate that D678N may be a novel type of APP mutation linked to FAD, which may cause Alzheimer's disease by an as yet uncharacterised mechanism—for example, alteration in fibrillogenic or catabolic properties of mutated Abeta or misfunction of APP, eventually leading to Alzheimer's disease. We await further structural modelling and Abeta metabolism studies, or in vitro models such as animals that are transgenic for this novel mutation.

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