

## **Mutation in Gelsolin Gene in Finnish Hereditary Amyloidosis**

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### **Summary**

Familial amyloidosis, Finnish type (FAF), is an autosomal dominant form of familial amyloid polyneuropathy. The novel amyloid fibril protein found in these patients is a degradation fragment of gelsolin, an actin-binding protein. We found a mutation (adenine for guanine) at nucleotide 654 of the gelsolin gene in genomic DNA isolated from five FAF patients. This site is polymorphic since the normal allele was also present in all the patients tested. This mutation was not found in two unaffected family members and 11 normal controls. The A for G transition causes an amino acid substitution (asparagine for aspartic acid) that was found at position 15 of the amyloid protein. The mutation and consequent amino acid substitution may lead to the development of FAF.

**F**amilial amyloidosis, Finnish type (FAF) (1), also known as familial amyloid polyneuropathy type IV (2), is an age-associated systemic amyloidosis with lattice corneal dystrophy and progressive cranial neuropathy as the principal clinical manifestations (1, 3). It is found primarily in families residing in Southeastern Finland (4), but cases have also been reported from the Netherlands (5), Denmark (6), and the United States (7–9). The amyloid fibril protein found in these patients is a low molecular weight degradation fragment of gelsolin (10–12), an actin-binding and modulating protein with cytoplasmic and plasma forms (13, 14). The amyloid protein starts at a position corresponding to residue 173 of the mature plasma gelsolin (10, 13) and has an amino acid substitution (asparagine for aspartic acid) at codon 187 (position 15 of the amyloid protein) (11, 15). We show here that the variant protein is the result of an abnormality in the gelsolin gene located on chromosome 9 (16).

### **Materials and Methods**

High molecular weight genomic DNA was isolated from autopsied tissues or lymphocytes of five patients with FAF and 13 unaffected controls.

Specific fragments were amplified using the *thermus aquaticus* (Taq) heat-stable DNA polymerase (17). Amplification reactions in a volume of 100  $\mu$ l contained 1  $\mu$ g of DNA, 0.125  $\mu$ M of each primer, and 2.5 u of Taq polymerase in reaction buffer (Perkin-Elmer-Cetus, Norwalk, CT). The samples were subjected to 25 cycles set at 94°C for 1 min to denature the DNA, 56°C for 30 s to anneal the primers, and 72°C for 1 min to extend the annealed primers.

The amplified fragments were subcloned into an M13 bacteriophage vector and sequenced by the dideoxy chain termination method (18).

Slot blots were performed by applying 25  $\mu$ l of the PCR-amplified fragment to nitrocellulose, in duplicate. An oligonucleotide that contains the mutation was synthesized, 5' labeled with  $\gamma$ -[<sup>32</sup>P]ATP and T4 polynucleotide kinase, and hybridized to the blots. High stringency washes of the blots demonstrated the existence of the mutated allele in the DNA samples tested.

### **Results and Discussion**

The amyloid protein isolated from patients with FAF has an amino acid substitution, asparagine for aspartic acid at position 15, corresponding to position 187 of the mature plasma gelsolin (11, 13).

Aspartic acid is encoded by GAC; thus, only a guanine to adenine transition is necessary to cause the change to asparagine (AAC). To test the possibility that the mutation exists at nucleotide 654 (numbering as for the human plasma gelsolin cDNA [13]), high molecular weight genomic DNA was isolated from tissues of five unrelated FAF patients. We amplified a fragment that contains this nucleotide (nucleotides 565–680) in the PCR (17) using oligonucleotides that were synthesized based on the cDNA sequences of gelsolin (13) (Fig. 1). The resulting sequences demonstrated that all five patients had one allele containing a point mutation, at nucleotide 654, as well as one normal allele (Fig. 1).

In an attempt to facilitate the identification of the mutation in multiple DNA samples, a different approach was taken. An oligonucleotide containing the mutation (5' TGA AGC AGT TGC CAT TGT 3') was hybridized to amplified DNA

565  
 |  
 TG GTG GTG CAG AGA GTG TTC CAG GTC AAA GGG CGG CGT GTG GTC CCT GCC ACC  
 Val Val Val Gln Arg Leu Phe Gln Val Lys Gly Arg Arg Val Val Arg Ala Thr  
 157

854  
 |  
 A  
 GAG GTA CCT GTG TCC TGG GAG AGC TTC AAC AAT GGC GAC TGC TTC ATC GTG GAG  
 Glu Val Pro Val Ser Trp Glu Ser Phe Asn Asn Gly Asp Cys Phe Ile Leu Asp  
 187

680  
 |  
 GTC GGC AAC AAC ATC CAC CAG TGG TGT GGT TCC AAC AGC AAT CGG TAT GAA AG  
 Leu Gly Asn Asn Ile His Gln Trp Cys Gly Ser Asn Ser Asn Arg Tyr Glu Arg  
 724  
 210

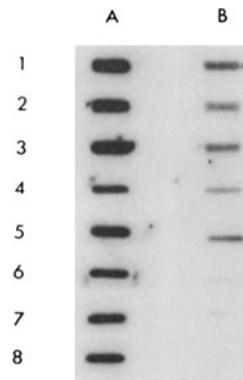
**Figure 1.** The mutation and amino acid substitution found in gelsolin in FAF patients. The nucleotides and predicted amino acids are numbered as for plasma gelsolin (13). Sequences used to synthesize the oligonucleotides are underlined; the NH<sub>2</sub> terminus of amyloid protein is indicated by an arrow; the intron is indicated by an arrowhead.

fragments. At low stringency (48°C), the oligonucleotide probe hybridized to all DNA tested (five FAF patients and 11 controls). However, at high stringency (65°C), only DNA isolated from FAF patients hybridized to the probe (Fig. 2).

This technique was used to show the absence of the mutation in DNA isolated from lymphocytes of two unaffected family members (a sister, no. 6; and a son, no. 7) of two of the FAF patients examined (no. 5 and no. 4, respectively) (Fig. 2). Our results suggest that the mutation (G to A) segregates with the disease, and that the slot blot analysis can be used as a diagnostic assay for prenatal evaluation of FAF for high risk populations.

Use of another pair of oligonucleotides designed to amplify sequences 565–724 resulted in a fragment >1,300 nucleotides long. Sequence analysis revealed the existence of an intron between nucleotides 680 and 681 (Fig. 1). The gelsolin amyloid protein deposited in FAF patients is encoded by at least two exons, similar to the Alzheimer's disease amyloid β protein (19).

The guanine to adenine transition causes an amino acid substitution in a repetitive motif of gelsolin, conserved among species (20). The asparagine for aspartic acid substitution was found at position 15 of the amyloid protein extracted from kidney tissue of a second patient (J.A.A.) (15), while the sequence of our original patient (V.U.O.) showed both amino



**Figure 2.** Slot blot analysis demonstrating the existence of a point mutation in DNA isolated from FAF patients (nos. 1–5) and its absence in DNA isolated from normal control (no. 8) and two unaffected family members of FAF patients (nos. 6 and 7). Genomic DNA sequences 565–680 (Fig. 1) were amplified with the PCR, blotted in duplicate and washed in low stringency (lane A) and in high stringency (lane B).

acids at this position (11). Although the aspartic acid found at this position in amyloid fibrils isolated from one patient may be due to deamination of asparagine, it is likely that the normal allele is also expressed in this patient, as is the case in other forms of hereditary amyloidoses (21, 22). The amino acid substitution appears not to exert a significant effect on the secondary structure of gelsolin; nevertheless, its biological consequence can be profound, as shown in some hemoglobinopathies (23).

Mutations have been found in genes encoding various amyloid proteins in familial amyloidoses with an autosomal dominant mode of inheritance, such as hereditary cerebral hemorrhage with amyloidosis, Icelandic type (24) and Dutch type (25), several types of familial amyloid polyneuropathy (FAP) (26), Gerstmann-Sträussler-Scheinker syndrome (27), and FAF, described here. Point mutation may affect the regulatory mechanisms of expression of the mRNAs or the post-translational processing of the precursor protein, thus enhancing amyloid fibril formation and deposition. Abnormalities in genes encoding amyloid proteins may indicate that the precursor molecules themselves play an effective role in amyloidogenesis, rather than the amyloid being the consequence of other pathological conditions involving cell damage. Thus, understanding the mechanisms of amyloid fibril formation and deposition in this familial disease may shed light on the causation of amyloidoses in general.

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