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.

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 µl contained 1 µg of DNA, 0.125 µM 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 µl of the PCR-amplified fragment to nitrocellulose, in duplicate. An oligonucleotide that contained the mutation was synthesized, 5' labeled with γ-[32P]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.

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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 (15). Sequences used to synthesize the oligonucleotides are underlined; the NH2 terminus of amyloid protein is indicated by an arrow; the intron is indicated by an arrowhead.

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).
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