Familial 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, 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 μl 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.
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 NH₂ 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 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 amyloidosis (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 amyloidosis 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.

We thank Fran Hitchcock for assistance with manuscript preparation.

This research was supported by National Institutes of Health grants AG-05891 and AR-02594. E. Levy is the recipient of a grant from the Alzheimer’s and Related Disorders Association. M. Haltia is the recipient of a fellowship from the Paulo Foundation, Helsinki, Finland.

Address correspondence to Blas Frangione, Department of Pathology, New York University Medical Center, 550 First Avenue, New York, NY 10016.

Received for publication 18 July 1990.
References


