Brief Definitive Report

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 contains 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 TGCCAT TGT 3') was hybridized to amplified DNA.
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 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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