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Finnish hereditary amyloidosis is caused by a single nucleotide substitution in the gelsolin gene

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The amyloid protein in Finnish hereditary amyloidosis is a fragment of the actin-filament binding region of a variant gelsolin molecule. Here we demonstrate, using polymerase chain reaction and allele-specific oligonucleotide hybridization analyses of genomic DNA, a single base mutation ($G^{654} \rightarrow A^{654}$) in the gelsolin gene segment encoding the amyloid protein. The mutation is responsible for the expression of the variant (Asn^{187}) gelsolin molecule in Finnish hereditary amyloidosis. The nucleotide substitution was found in all five unrelated patients with Finnish amyloidosis studied, but not in 45 unrelated control subjects. The mutation co-segregated with the disease phenotype in a family with Finnish amyloidosis. The results show that a single substitution in the gelsolin gene causes Finnish hereditary amyloidosis. The allele-specific oligonucleotide hybridization method provides a simple and accurate means of detecting this mutation.

Finnish hereditary amyloidosis; Familial amyloid polyneuropathy; Gelsolin gene; Point mutation; Oligonucleotide hybridization; DNA-test

1. INTRODUCTION

Finnish hereditary amyloidosis (McKusick No. 105120; familial amyloidosis, Finnish type; Meretoja amyloidosis) constitutes a clinically and biochemically distinct entity among the autosomal dominant amyloid polyneuropathy syndromes [1]. It is characterized by lattice corneal dystrophy, progressive cranial neuropathy and systemic amyloid deposits [2,3]. We have demonstrated that the amyloid protein in Finnish hereditary amyloidosis represents a novel type that shows amino acid homology with the actin-modulating protein gelsolin [4]. The gelsolin-nature of the amyloid was confirmed by others [5]. Our recent studies show that the amyloid protein is derived from an Asp^{187} Asn mutant of gelsolin [6,7]. Based on this substitution and the gelsolin cDNA sequence [8,9] we predicted that a single base mutation ($G^{654} \rightarrow A^{654}$) in genomic DNA would be responsible for the protein variant. To demonstrate this mutation we amplified the target DNA with polymerase chain reaction for oligonucleotide hybridization analyses. The $G^{654} \rightarrow A^{654}$ nucleotide substitution in the gelsolin gene was found in all studied patients with Finnish hereditary amyloidosis, but in none of the control subjects. The co-segregation of the mutation and the disease phenotype in a family with Finnish hereditary amyloidosis is also shown.

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2. METHODS

2.1. *Samples and DNA extraction*

Blood samples were collected from 5 unrelated patients with Finnish hereditary amyloidosis, 9 family members of one of the patients, and 45 healthy unrelated individuals. DNA was extracted from white blood cells using a standard detergent lysis and proteinase K digestion protocol, and purified by phenol extraction and ethanol precipitation [10]. 200–400 ng genomic DNA was used for each polymerase chain reaction.

2.2. *Polymerase chain reaction (PCR) for gelsolin*

Primers flanking the predicted mutated region were designed according to a published plasma gelsolin sequence [8]. The following primers were used (5′ to 3′): TCC TGG GAG AGC TTC AAC AAT GGC (forward) and GTT GCC CAG GTC CAG GAT GAA GCA (reverse). The PCR [11] program was 94°C for 1.2 min, 55°C for 1.0 min, and 72°C for 1.0 min for 30 cycles. The reaction was in 50 mM KCl, 10 mM Tris pH 9.0, 1 mM $MgCl_2$, and 0.01% gelatin with 2 units of Cetus Taq polymerase in a final volume of 100 μl. The PCR products were analyzed by electrophoresis in 3% agarose gels containing 0.5 μg/ml ethidium bromide.

2.3. *Oligonucleotide hybridization*

Ten μl of each polymerase chain reaction was heated to 95°C to denature DNA and dotted on nylon filters (Zeta-Probe, Bio-Rad, Richmond, California). The filters were ultraviolet irradiated to crosslink DNA. Prehybridization and hybridization were in 1 mM EDTA, 0.5 M NaH_2PO_4 pH 7.2, 7% SDS at 62°C. Oligonucleotides specific for the normal plasma gelsolin sequence (AAC AAT GGC GAC TGC TTC ATC) or the predicted mutation sequence (AAC AAT GGC AAC TGC TTC ATC) were end-labelled with ^{32}P using the kinase reaction [12] and hybridized to the filters. Filters were washed at high stringency at 62°C and autoradiographed overnight with an intensifying screen.

3. RESULTS

3.1. *Polymerase chain reaction and oligonucleotide hybridization*

The PCR products were analyzed by electrophoresis.

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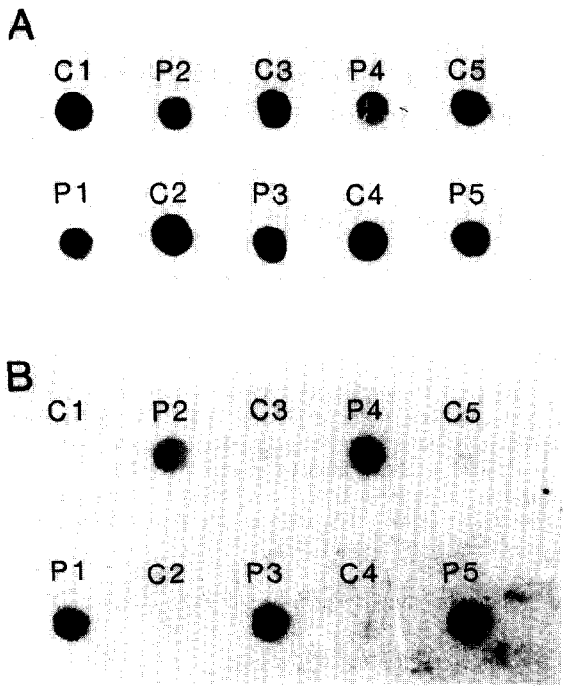

Fig. 1. Oligonucleotide hybridization to amplified DNA samples. (A) a 21 bp oligonucleotide corresponding to the normal plasma gelsolin sequence hybridizes to both patient (P1-P5) and control (C1-C5) samples. Note the weaker signal with patient DNA (heterozygous) compared with control DNA (homozygous, normal). (B) a 21 bp oligonucleotide with the single base G→A change hybridizes to patient samples (P1-P5) but not to control samples (C1-C5). The hybridization and washes were under the same conditions at 62°C for both oligonucleotides.

A single fluorescent band was visible under ultraviolet light, corresponding in size to the expected 51 bp reaction product [8]. Filters containing 10 μl amplified DNA from 5 unrelated patients and 5 healthy individuals were hybridized to end-labelled oligonucleotides specific for the normal sequence or the predicted mutation. The oligonucleotide with the normal sequence hybridized to all samples, but the signal was less intense in the 5 patient samples (Fig. 1A). The oligonucleotide containing the predicted G to A change hybridized under the same conditions to the 5 patient samples but not to any of the normal controls (Fig. 1B). In further experiments, hybridization to the normal oligonucleotide but not to the mutation sequence was observed in altogether 45 unrelated healthy individuals.

3.2. *Family study*

Samples from a family with Finnish hereditary amyloidosis were analyzed for the mutation. The pedigree and the results for oligonucleotide hybridization are shown in Fig. 2. The mutant gene was found in all members with clinical symptoms of the disease. One family member (no 13), a 26-year-old woman, was found to have the mutation without having disease manifestations. She apparently represents a presymptomatic gene carrier.

4. DISCUSSION

This study shows that in Finnish hereditary amyloidosis the gelsolin gene, located on chromosome 9 [9], contains a single base mutation $G^{654} \rightarrow A^{654}$ in the DNA segment encoding the amyloid protein. This mutation is responsible for the expression of the variant Asp^{187} Asn gelsolin molecule which is the precursor of the amyloid subunit protein in Finnish hereditary amyloidosis [6,7]. The mutation was demonstrated in all patients, but in none of the 45 unrelated Finnish control subjects. In the family study, the mutant gene was demonstrated in all members with clinical symptoms of the disease, as well as in one asymptomatic family member apparently representing a presymptomatic gene carrier. This is the first demonstration of a human disease linked to a single base mutation in the gelsolin gene. Recently, the torsion dystonia gene has been shown to be linked to the gelsolin gene [13], but the pathogenetic significance of this association is unclear.

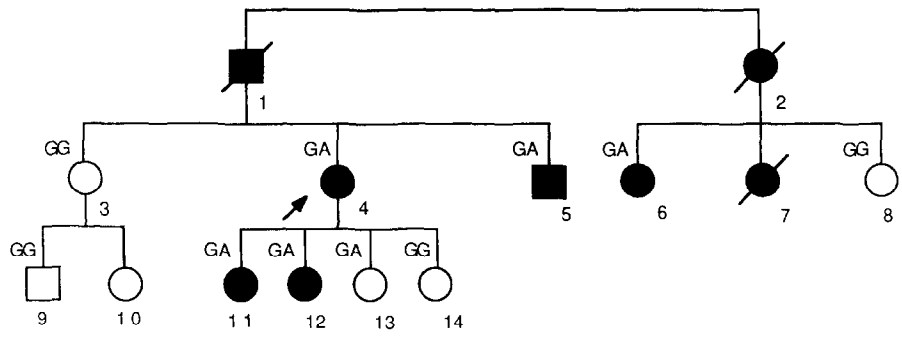

Fig. 2. Pedigree of a family with Finnish hereditary amyloidosis in 3 generations. The results of oligonucleotide hybridizations are given for each individual studied. G, normal allele; A, mutated allele. Clinically affected individuals are indicated by solid symbols. The arrow indicates the proband. Cases 1, 2, 7 (deceased) and 10 were not examined. Circles, females; squares, males.

By the end of the 1970's more than 300 cases of hereditary amyloidosis characterized by lattice corneal dystrophy and cranial neuropathy had been recorded in Finland [14]. The disease shows strong geographic isolation; individuals in the neighbouring districts in Southern Finland, South Häme and Kymenlaakso, have been known to be affected at least since the 17th century. It has been suggested that the mutation occurred or was introduced in this region at least 500 years ago [3]. The disease is, however, not limited to Finland. Cases have also been described from the United States [15–17], Denmark [18] and the Netherlands [19]. It remains to be determined what mutations cause the disease in other populations than the Finns.

The disease is inherited in an autosomal dominant fashion. In heterozygous patients, the disease has a late onset and is slowly progressive. By the age of 20 years, corneal dystrophy is usually manifested and by the age of 40 years most patients have developed cranial neuropathy. Skin, renal and cardiac manifestations also occur. In the rare presumptively homozygous subjects, the disease has a more severe clinical course characterized by renal insufficiency and a life-expectancy of about 30 years [3]. The demonstration of a base mutation in the gelsolin gene and its association with the amyloid disease now opens a new approach for the accurate diagnosis of Finnish hereditary amyloidosis. By use of the polymerase chain reaction the DNA segment can be amplified and the mutation easily demonstrated by allele-specific oligonucleotide hybridization. It is thus possible to detect presymptomatic gene carriers and perform prenatal diagnosis of the disease.

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REFERENCES

[1] Andrade, C., Araki, S., Block, W.D., Cohen, A.S., Jackson, C.E., Kuroiwa, Y., McKusick, V.A., Nissim, J., Sohar, E. and VanAllen, M.V. (1970) Arthritis Rheum. 13, 902–915.
[2] Meretoja, J. (1969) Ann. Clin. Res. 1, 314–324.
[3] Meretoja, J. (1973) Clin. Genet. 4, 173–185.
[4] Maury, C.P.J., Alli, K. and Baumann, M. (1990) FEBS Lett. 260, 85–87.
[5] Haltia, M., Prelli, F., Ghiso, J., Kiuru, S., Somer, H., Palo, J. and Frangione, B. (1990) Biochem. Biophys. Res. Commun. 167, 927–932.
[6] Maury, C.P.J. (1990) Biochim. Biophys. Acta, in press.
[7] Maury, C.P.J. (1990) Lab. Invest., in press.
[8] Kwiatkowski, D.J., Stossel, T.P., Orkin, S.H., Mole, J.E., Colten, H.R. and Yin, H.L. (1986) Nature (Lond.) 323, 455–458.
[9] Kwiatkowski, D.J., Westbrook, C.A., Burns, G.A.P. and Morton, C. (1988) Am. J. Hum. Genet. 42, 565–572.
[10] Kunkel, L.M., Smith, K.D., Boyer, S.H., Borgaonkar, D.S., Wachtel, S.S., Miller, O.J., Breg, W.R., Jones, H.R. and Rary, J.M. (1977) Proc. Natl. Acad. Sci. USA 74, 1245–1249.
[11] Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B. and Erlich, H.A. (1988) Science 239, 487–491.
[12] Maniatis, T., Hardison, R.C., Lacy, E., Lauer, J., O'Connell, C., Quon, D., Sim, G.K. and Efstratiadis, A. (1978) Cell 15, 687–701.
[13] Ozelius, L., Kramer, P.L., Moskowitz, C.B., Kwiatkowski, D.J., Brin, M.F., Bressman, S.B., Schuback, D.E., Falk, C.T., Risch, N., de Leon, D., Burke, R.E., Haines, J., Gusella, J.F., Fahn, S. and Breakefield, O. (1989) Neuron 2, 1427–1434.
[14] Meretoja, J., Hollmen, T., Meretoja, T. and Penttinen, R. (1978) Med. Biol. 56, 17–22.
[15] Darras, B.T., Adelman, L.S., Mora, J.S., Rodzinger, R.A. and Munsat, T.L. (1986) Neurology 36, 432–435.
[16] Purcell Jr, J.J., Rodrigues, M., Chishti, M.I., Riner, R.N. and Dooley, J.M. (1983) Ophthalmology 90, 1512–1517.
[17] Sack, G.H., Dumars, K.W., Gummerson, K.S., Law, A. and McKusick, V.A. (1981) Johns Hopkins Med. J. 149, 239–247.
[18] Boysen, G., Galassi, G., Kamieniecka, Z., Schlaeger, J. and Trojaborg, W. (1979) J. Neurol. Neurosurg. Psychiatry 42, 1020–1030.
[19] Winkelman, J.E., Delleman, J.W. and Ansink, B.J.J. (1971) Klin. Monatsbl. Augenheilkd. 159, 618–623.