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Human Genetics

Homozygosity for the met30 transthyretin gene in a Turkish kindred with familial amyloidotic polyneuropathy

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Summary. A Turkish family is described with two members suffering from familial amyloidotic polyneuropathy. Their transthyretin genes were examined using the polymerase chain reaction, and both patients possessed the met30 mutation in both of their transthyretin genes. In this family, only individuals who are homozygous for the met30 mutation have developed symptoms.

Introduction

Familial amyloidotic polyneuropathy (FAP; MIM 10480) is a dominant genetic disease caused, in nearly all cases, by fibrillar deposits of abnormal transthyretin (TTR). That is, symptoms occur in individuals with one normal and one mutant TTR gene. The most common mutation leads to substitution of methionine for valine at position 30 of the 127 amino acid transthyretin monomer. In addition to these met30 substitutions, ile33, his58, ala60, tyr77, ser84, and asn90 substitutions have been found associated with FAP. Met111 and ile122 substitutions have been found associated with an amyloid cardiomyopathy that occurs late in life.

It was recently reported (Holmgren et al. 1988a) that individuals exist in Sweden who have the met30 mutation in both of their transthyretin genes. We report individuals from Turkey who are also met30 homozygotes.

Materials and methods

DNA extraction and blot hybridization were performed as in Skare et al. (1989). The polymerase chain reaction (PCR) was as described by Skare et al. (1990), but the annealing temperature was 60C. Primers were cacgtgtcttctctacaccc and gtaccaagtgaggggcaaac.

Individual III-3 in Fig. 1 is now 61 years old. At the age of 51 he acquired numbness and weakness in his legs as well as difficulty in speech. These problems have become more severe, with tingling and weakness extending to his hands. He cannot walk without assistance, and deep tendon reflexes are absent. He also has urinary incontinence and cardiomyopathy. An electromyogram (EMG) showed demyelinating polyneuropathy, and a rectal biopsy had amyloid deposition.

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Individual III-7 is 55 years old and acquired vitreous opacities at the age of 51. He has developed sensory neuropathy. An echocardiogram indicated cardiomyopathy with hypertrophy of his interventricular septum. Vitreous material and rectal biopsy tissue were found to contain amyloid.

No other members of the family have been examined. Individuals in generation I were related only by marriage.

Results

The transthyretin (TTR) gene has been sequenced (Sasaki et al. 1985), so it was possible to select 20 base primers on each side of exon 2 that could be used to amplify the DNA between them. Table 1 shows the sequence of the PCR product containing exon 2 from a TTR gene with methionine substituted for valine at position 30. This met30 exon has a cleavage site for *Nsi*I as indicated in Table 1. *Nsi*I will not cleave the exon 2 PCR product from normal TTR genes. The met30 PCR product is 219 base pairs (bp), and cleavage with *Nsi*I results in a fragment of 101 bp with a 4-base tail and a fragment of 114 bp with a 4-base tail.

Figure 1 shows the pedigree of the Turkish FAP kindred, and Fig. 2 shows *Nsi*I digests of PCR products made using their DNA. Note that *Nsi*I does not cut the product from III-1, but completely cuts the products from III-3 and III-7. Both FAP patients (III-3 and III-7)

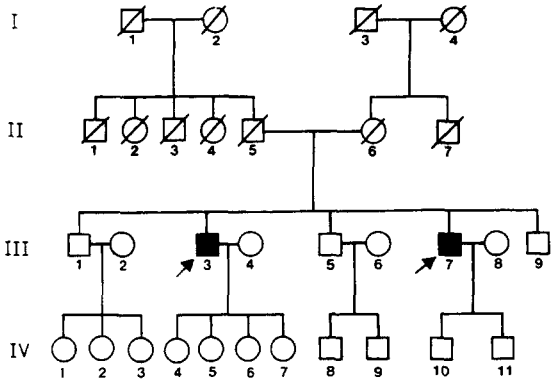

Fig. 1. Pedigree of the Turkish familial amyloidotic polyneuropathy family. *Solid symbols* represent the only individuals with symptoms. □ Male, ○ female, ⊠ deceased, ■ affected

Table 1. Polymerase chain reaction product from a met30 transthyretin (TTR) gene. Exon 2 is in upper case, and the *Nsi*I recognition sequence is underlined. Normal TTR has a G at position 101

cacgtgtctt ctctacaccc agGGCACCGG TGAATCCAAG TGTCCTCTGA	50
TGGTCAAAGT TCTAGATGCT GTCCGAGGCA GTCCTGCCAT CAATGTGGCC	100
ATGCATGTGT TCAGAAAGGC TGCTGATGAC ACCTGGGAGC CATTTGCCTC	150
TGGgtaagtt gccaaagaac cctcccacag gacttggttt tatcttcccg	200
tttgcccctc acttggtac	

Fig. 2. DNA from members of a Turkish family was amplified and cut with *Nsi*I. The products were subjected to electrophoresis on 6% polyacrylamide and stained with ethidium bromide. Polymerase chain reaction products are from *lane a* III-1, *lane b* III-3, *lane c* III-7, *lane e* IV-10, and *lane f* IV-11. *Lane d* is pBR322 cut with *Msp*I, and *lane g* is product from III-7 before *Nsi*I digestion

are therefore homozygous for the met30 mutation. That is, neither of their two TTR genes are normal. As expected, both sons of III-7 have one normal TTR gene and one met30 TTR gene.

The same conclusions were obtained when the family was examined using blot hybridization (data not shown). Equivalent amounts of DNA from the individuals resulted in equivalent hybridization signals, so the PCR data cannot be the result of deletion of the TTR gene from one of the patient's chromosomes 18. Two copies of the met30 TTR gene are present in each cell.

Discussion

This is the first report of Turkish individuals with FAP, and the second report of individuals who are homozygous for met30 transthyretin. Holmgren et al. (1988a) reported a Swedish brother and sister homozygous for the met30 mutation. The male developed peripheral neuropathy at about age 51, vitreous opacities at age 54, and subsequently gastrointestinal problems. His sister was still asymptomatic at age 62. They had two aunts who were asymptomatic and met30 heterozygotes.

Holmgren et al. (1988a, b) and Sandgren et al. (1988) concluded that genetic factors in addition to the met30 mutation are necessary for the deposition of amyloid fibrils. Some Swedish individuals with met30 TTR lived to be 80 years old without developing symptoms. Late age of onset explains why 15 of 35 Swedish FAP patients had no family history of FAP.

This situation might be even more pronounced in Turkey, and it may explain why the first Turkish FAP patients are also homozygotes. Individuals II-5 and II-6 are deceased, but in order to have a child with two normal TTR genes as well as children with two met30 TTR genes, they must both have been met30 heterozygotes. According to their children, they showed no FAP symptoms even though II-5 lived to be 75 years old and II-6 lived to be 84. Furthermore, II-5 should have inherited his mutation from one of his parents, and they lived to be 80 and 104 years old without evidence of symptoms. (The parents of II-6 lived to the age of 75 without symptoms).

Since none of the heterozygotes in generations I or II had symptoms and two of them lived to at least 80 years of age, we predict that heterozygotes in generation IV will live 80 years without developing symptoms. Even the homozygotes had a late onset of symptoms, as did their Swedish counterparts.

It has been estimated that about 3% of the population in one region of Sweden are met30 heterozygotes. Given the fact that two heterozygotes married in this family, many nonpathogenic met30 mutations might also exist in regions of Turkey.

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