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Transthyretin Pro 36 associated with familial amyloidotic polyneuropathy in an Ashkenazic Jewish kindred

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Summary. Mutations in the serum protein transthyretin (TTR) cause amyloidosis involving the peripheral nerves, heart, and other organs. In Ashkenazic Jews, the only TTR variant described to date has been TTR Ile 33. We have studied DNA from another Ashkenazic Jewish kindred with familial amyloidotic polyneuropathy. Single-strand conformation polymorphism analysis, DNA sequencing, and restriction analysis indicated that this kindred has the TTR Pro 36 variant, previously described only in a Greek kindred.

Introduction

Several point mutations in the serum transport protein transthyretin (TTR) lead to its deposition as amyloid material, primarily along the peripheral nerves (familial amyloidotic polyneuropathy, FAP), in the heart (familial amyloid cardiomyopathy), or both (Jacobson and Buxbaum 1991). In Ashkenazic Jews, TTR Ile 33 is the only previously described TTR variant (Nakazato et al. 1984; Jacobson et al. 1988a). We now report genetic studies of another Ashkenazic Jewish kindred with FAP.

Material and methods

Proband and family

The proband, born in New York City of Polish Ashkenazic Jewish descent, died at age 36 after a 7-year illness with FAP (Rosenthal et al. 1986). Autopsy revealed amyloidosis of the peripheral nerves, heart, gastrointestinal tract, liver, kidney, thyroid, and spleen. His father died at age 62 with TTR-positive FAP, his paternal grandfather died at age 65 with generalized amyloidosis not studied immunohistochemically, and his two sisters are clinically unaffected in their sixth decades.

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DNA analysis

DNA isolation, polymerase chain reaction (PCR) analysis of the four TTR exons, single-strand conformation polymorphism (SSCP) analysis, and DNA sequencing were performed as previously described (Jacobson et al. 1992). Exon 2 PCR products of DNA from the proband, his unaffected mother and two sisters, and controls, were digested with *Fnu*4HI and subjected to electrophoresis on agarose gels containing ethidium bromide.

Results

SSCP analysis revealed a shifted exon 2 band in the proband DNA (Fig. 1, lane 5). For exons 1, 3, and 4, no

Fig. 1. Single-strand conformation polymorphism (SSCP) analysis of exon 2 polymerase chain reaction (PCR) product. *Lane 5* DNA from the proband, containing the variant band *(arrow); lanes 1–3* control DNA samples. *Lane 4* is derived from DNA from a patient with another exon 2 variant, and reveals a different shifted band. Similar experiments on exons 1, 3, and 4 revealed identical patterns for the study sample and controls (not shown)

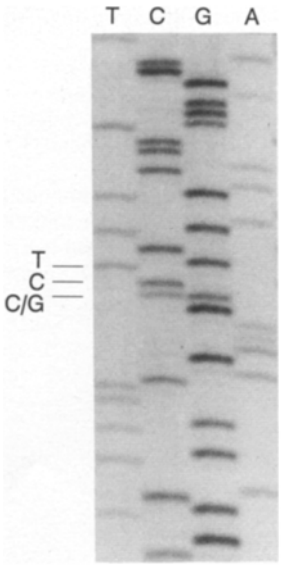

Fig. 2. Autoradiograms of the DNA sequence gel of transthretin (TTR) exon 2, demonstrating a heterozygous G→C transversion at codon 36, position 1. The remainder of the sequence was normal

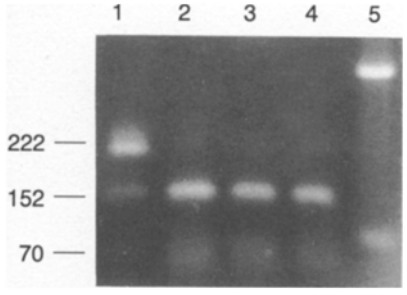

Fig. 3. *Fnu*4HI restriction digest of PCR-amplified DNA spanning TTR exon 2. *Lane 1.* DNA from the proband; *lanes 2–4* DNA from the proband's mother and sisters; *lane 5* marker DNA (121 and 383 bp). Other controls all gave the pattern seen in lanes 2–4

shifted bands were seen for the study sample, while known exon 3 and 4 mutations were demonstrated, indicating a mutation in exon 2, but none elsewhere. Sequencing of the exon 2 PCR product revealed two bands at codon 36 position 1, one corresponding to the normal sequence and the other to a G→C change; the latter encodes an Ala→Pro change (Fig. 2). The G→C transversion should abolish an *Fnu*4HI restriction site; restriction analysis of exon 2 PCR products confirmed the loss of this site from one allele in the proband. DNA from his mother, unaffected sisters, and normal controls demonstrated the normal pattern (Fig. 3). Loss of this site has been shown also in Southern blotting experiments on genomic DNA (Jacobson et al. 1988b).

Discussion

Most TTR variants associated with FAP have been found in single kindreds or ethnic groups, and probably arose from single founders. The notable exception, TTR Met 30, derives from a point mutation at a CG dinucleotide "hot spot" (Cooper and Krawczak 1990) and evidently arose separately in the Portuguese and Japanese populations (Yoshioka et al. 1989). We now demonstrate another FAP-associated TTR variant present in two ethnic groups: TTR Pro 36, formerly found only in a Greek patient from Samos with FAP (Jones et al. 1991), has been associated with FAP in an Ashkenazic Jewish kindred.

Whether the Greek and Jewish kindreds descended from one founder is unknown. Unlike TTR Met 30, TTR Pro 36 did not arise from a mutation at a CG dinucleotide, perhaps decreasing the likelihood of multiple founders. If the Greek and Jewish patients descended from a single founder many generations ago, then this variant will likely be found in additional patients of Greek, Jewish, and perhaps other ethnic backgrounds. In the present case, the variant gene was shown only in the proband; however, his father had TTR-positive FAP, and his paternal grandfather also had amyloidosis. The absence of the variant in the proband's mother and unaffected sisters confirms the association of the variant with disease.

How TTR mutations cause amyloidosis is not understood. The TTR molecule contains abundant β pleated sheet structure, thought to contribute to amyloid fibril formation. Changes in primary structure may affect higher-order structure such that amyloid formation is promoted. Amino acid 36 is near a transition point from β pleat to α helix; proline disrupts α helices. Another amyloidogenic variant, TTR Pro 55, also substitutes a proline near a β pleat − α helix boundary (Jacobson et al. 1992). Other mutations, however, are located throughout the molecule. Thus, the mechanisms by which mutations lead to amyloid fibril formation remain obscure.

This report illustrates the value of the recommendation of the nomenclature committee of the VIth International Conference on Amyloidosis, that whenever possible the amyloid syndromes should be designated chemically rather than with names based on the ethnicity or nationality of an affected patient (Husby et al. 1991). As specific amyloidogenic proteins are found in varied ethnic groups, and as multiple mutations are found in single ethnic groups, designations such as Jewish-type FAP become meaningless, and should be abandoned in favor of their chemical counterparts.

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