Oculoleptomeningeal Amyloidosis Associated With a New Transthyretin Variant Ser64

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Background: A Canadian family with oculoleptomeningeal amyloidosis with both central and peripheral nervous system disorders was described in 1988. Death of affected family members resulted from recurrent cerebral hemorrhage.

Objective: To determine if oculoleptomeningeal amyloidosis is caused by a mutation in transthyretin (prealbumin).

Methods: DNA isolated from peripheral blood and archival tissues of affected members of the kindred was studied by direct DNA sequencing and restriction fragment length polymorphism analysis.

Results: Direct DNA sequencing identified a thymine-to-cytosine transition at the second base of codon 64, which resulted in a replacement of serine for phenylalanine. This mutation, which creates an additional Hinfl site was detected by restriction fragment length polymorphism analysis in each affected individual.

Conclusion: In this kindred, oculoleptomeningeal amyloidosis is related to a mutation in transthyretin (Phe64Ser).

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Familial amyloidosis is a group of autosomal dominant diseases caused by homologous protein deposition, amyloid, to extracellular space of various target organs. It is biochemically classified into several subtypes by the precursor proteins, which include transthyretin (prealbumin), apolipoprotein A-I, gelsolin, fibrinogen Aa chain, lysozyme, β-amyloid precursor protein, cystatin C, and prion protein. Among the disease subtypes, transthyretin amyloidosis is presumably the most common form. Transthyretin (TTR) is a plasma protein of 127 amino acid residues. Structurally, it has 4 pairs of β-strands and exists as a tetramer in plasma. Transthyretin plays an important role in transportation of thyroxine and retinol binding protein in serum. Transthyretin is coded by a single gene on chromosome 18 and synthesized in liver, choroid plexus, and retina.

Since the first TTR variant was identified in a patient with familial amyloidotic polyneuropathy in 1983 by Dwulet and Benson, more than 60 mutations in the TTR gene, most of which are associated with systemic amyloidosis of autosomal dominant inheritance, have been described. Clinical manifestations of TTR amyloidosis include peripheral neuropathy, autonomic dysfunction, cardiomyopathy, gastrointestinal tract disorders, renal dysfunction, carpal tunnel syndrome, and vitreous opacity.

In 1988, Uitti et al described a family with amyloidosis showing various symptoms of central and peripheral nervous system disorders. This type of amyloidosis, called oculoleptomeningeal amyloidosis, had been reported only in a few kindreds, and its genetic background was unknown until 1996 when Vidal et al reported a mutation in the TTR gene in a kindred with this type of amyloidosis.

In this study, we examined the TTR gene of the family with oculoleptomeningeal amyloidosis reported by Uitti et al to reveal their predisposing genetic variation.

RESULTS

Direct DNA sequencing of the exon 3 PCR products of the affected individuals in this family showed both thymine and cytosine at position 3293 of the TTR gene (Figure 2). Thus, they were heterozygous with normal TTT (Phe) and variant TCT (Ser) codons at amino acid position 64.

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PATIENTS AND METHODS

CASES

Three members in 2 generations were affected with amyloidosis presenting various neurological symptoms (Figure 1). The grandfather of the propositus (I-1) died in an accident at the age of 39 years and the 85-year-old grandmother (I-2) is healthy with no signs of amyloidosis. The kindred is of Italian descent presently living in Canada. Their clinical picture and pathological findings have been reported previously and their unique clinical manifestations are summarized in the Table. Case 1, case 2, and case 3 of the 1988 report are kindred members II-1, II-3, III-1, respectively.

DNA ISOLATION

Total genomic DNA was extracted from peripheral blood cells of member II-3 by a standard method and stored at 4°C until used. DNA from member III-1 was isolated from paraffin-embedded postmortem brain tissue as previously described.8

DIRECT DNA SEQUENCING ANALYSIS

Exons 2, 3, and 4 of the TTR gene of the affected individuals were enzymatically amplified by polymerase chain reaction (PCR) using Taq DNA polymerase (Boehringer Mannheim Biochemicals, Indianapolis, Ind) and primers complementary to flanking intron sequences as previously described.9 They were then separated by electrophoresis through an agarose gel. DNA bands of the expected sizes were excised from the gel and used as templates for asymmetric PCR. Asymmetric PCR was performed using the same sets of primers (primer ratio 1:30) and the PCR products were purified by a microconcentrator (Centricon-30; Amicon, Beverly, Mass). DNA sequencing was performed using a dideoxy chain termination method (Sequenase Version 2.0; US Biochemicals, Cleveland, Ohio) according to the manufacturer’s protocol. Sulfur 35–radiolabeled DNA samples were then electrophoresed through 6% polyacrylamide gel at 1800 V for 3 hours. The gel was dried and exposed to x-ray film.

RESTRITION FRAGMENT LENGTH POLYMORPHISM ANALYSIS

For the detection of the Ser64 mutant gene, exon 3 of the TTR gene was amplified by PCR as described previously. Ten units of restriction enzyme HinI (New England Biolabs Inc, Beverly, Mass) was directly added to 10 µL of PCR products, and incubated at 37°C for 2 hours. These samples were electrophoresed through 3% agarose gel, stained with ethidium bromide, and photographed over UV light. Analysis. The normal PCR product containing exon 3 has a single HinI site, which should create 206–base pair (bp) and 62-bp bands. On the other hand, the Phe64Ser allele is expected to have an additional HinI site, therefore resulting in 170-bp, 62-bp, and 36-bp bands.

The individuals with the Phe64Ser mutation had a digestion band of 170 bp in addition to the normal band of 206 bp, which indicates that they are heterozygous for the mutation (Figure 3). Undigested 268-bp bands were invisible on the gel, indicating that the HinI digestion was performed properly.

COMMENT

Three kindreds where patients developed symptoms of central nervous system disorder due to mutations in the TTR gene have been reported. Affected members of a Hungarian kindred were reported to have central nervous system symptoms including dementia, spasticity, ataxia, and hearing loss. Amyloid deposits immunohistologically reactive to anti-TTR antibody were found in the meningeal vessels and subpial matter in the brain as well as in systemic organs. A mutant TTR gene coding glycine instead of aspartic acid at codon 18 was demonstrated in this kindred. A German family living in Ohio who had seizures, strokes, coma, and visual deterioration have been described. Amyloid deposits were found in systemic organs, including subarachnoid vessels and leptomeninges. A point mutation in the TTR gene resulting in Gly-for-Val amino acid substitution at codon 30 was associated with the condition. In another report, a patient with intracranial hemorrhage due to extensive meningeal amyloidosis has been described as having a mutant TTR gene coding proline in place of leucine at position 12.

Among more than 60 TTR variants reported to give autosomal dominant amyloidosis, Phe64Ser in this study and Leu12Pro, Asp18Gly, and Val30Gly described above, are the only mutations that cause predominantly oculo-leptomeningeal amyloidosis. The factor common among
these TTR variants is not clear. The position 64 is located in the D-E loop of the TTR molecule while positions 12, 18, and 30 are at the near-end of the A or C β-strand. Pro is a hydrophobic nonpolar residue while Gly and Ser are polar uncharged hydrophilic residues. Although Gly, Ser, and Pro residues are relatively small, there are many other TTR variants with Gly, Ser, or Pro replacing larger amino acids and not manifesting as cerebral hemorrhage.2

Another mutation at position 64, which led to amino acid substitution of Leu for Phe, was reported originally in an Italian-American family and subsequently in 2 Italian families.16,17 Affected members in these families had autonomic and sensorimotor neuropathy and cardiomyopathy but not central nervous system disorder. It has been observed that different amino acid substitutions at the same position of the TTR molecule lead to different clinical manifestations. At position 18, an Asp-to-Gly amino acid substitution is responsible for meningeal amyloidosis while a Glu-for-Asp change leads to amyloidotic polynuropathy.14 This is also true for mutations at codon 30, where only Val-to-Gly substitution causes oculoleptomeningeal amyloidosis, and the other 3 known variants, Met, Ala, and Leu, do not.4,18,19

Besides TTR, β-amyloid precursor protein (β-APP) and cystatin C are known to be related with hereditary cerebral hemorrhage due to amyloid angiopathy. β-Amyloid precursor protein with an amino acid substitution at either 692 or 693 is associated with cerebral hemorrhage in Dutch families.20,21 It is notable that cerebral amyloid angiopathy of β-APP is common in brains of elderly persons even without mutations.22 β-Amyloid precursor protein amyloid deposition is restricted to the brain. A variant form of cystatin C, Leu68Glu, is associated with cerebral hemorrhage in Icelandic families.23 Amyloid deposits have been demonstrated in cerebral blood vessels. Although amyloid is demonstrated also in systemic organs, there have been no reports of systemic organ damage due to amyloid deposition. Although oculoleptomeningeal amyloidosis of the TTR form manifests as cerebral hemorrhage similar to β-APP and cystatin C amyloidoses, it is characteristic of TTR amyloidosis to have noncerebral symptoms such as vitreous opacity and carpal tunnel syndrome.

Amyloid in systemic organs of patients with TTR amyloidosis is believed to be derived from plasma TTR that is synthesized in liver. The vitreous amyloid seems to be of retinal origin, since liver transplantation for patients with familial amyloidotic polyneuropathy failed to halt the progression of vitreous opacities. The source of TTR deposited in the cerebral blood vessels has not been determined. Since TTR is synthesized by epithelial cells of the choroid plexus24 and exists in cerebrospinal fluid at a level of 0.017 g/L,25 the amyloid in leptomeninges might be from TTR in cerebrospinal fluid rather than from plasma TTR. In affected individuals with cystatin C amyloidosis, the concentration of cystatin C in cerebrospinal fluid is very low, which may be related to pathogen-
esis of amyloid angiopathy.28 In cerebral amyloid angiopathy of β-APP type, studies have shown that vascular smooth muscle cells express β-APP and the blood vessel wall is speculated to be the site of precursor protein production.27,28 While this may explain why the cerebral blood vessels are preferentially affected in β-APP amyloid angiopathy, synthesis of TTR in blood vessel walls has never been demonstrated.

In the kindred presented herein, affected individuals showed various neurological symptoms. Although hemorrhage due to amyloid angiopathy is the cause of cerebral and cerebellar symptoms, compression of the spinal cord by leptomeningeal amyloid may be responsible for myelopathy. As described in the previous report by Uitti et al.,29 leptomeninges of patients in this kindred were markedly thickened with amyloid deposits and their spinal cords were severely atrophic with gliosis, which was presumed to be the cause of paraplegia. Myelopathy in patients with TTR amyloidosis has been reported by Har-arts et al.29 In their report, 3 patients with familial amyloidotic polyneuropathy due to TTR Ile84Ser mutation developed spinal claudication. In contrast to our cases, ligamentum flavum with marked amyloid deposition was responsible for compression of the spinal cord. Although amyloid deposits could be found in leptomeninges of patients with TTR amyloidosis of polynu-ropathy type,29 severe leptomeningeal amyloid deposition causing myelopathy is characteristic for this kindred.

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