Letters to the Editor

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A Kerato-Epithelin (βig-h3) Mutation in Lattice Corneal Dystrophy Type IIIA

To the Editor:

The lattice corneal dystrophies (LCDs) are a class of inherited stromal amyloidoses characterized by pathognomonic, branching "pipestem" lattice figures in the cornea (Klintworth 1967). Four different LCD subtypes have been described. Type I (MIM 122200), the autosomal dominant form not associated with systemic amyloidosis (Gorevic et al. 1984), has its onset early in childhood and possesses a delicate network of interdigitating filaments in the cornea (fig. 1*A*). Type II (MIM 105120), the Finnish type (Meretoja 1972), on the other hand, is a condition associated with systemic amyloidosis. LCD type III has a presumed recessive inheritance pattern, is characterized by thicker lattice lines, and is not associated with systemic amyloidosis (Hida et al. 1987). Type IIIA resembles type III clinically but differs in that type IIIA has an onset age of 70–90 years and an autosomal dominant inheritance pattern (Stock et al. 1991). To the best of our knowledge, only two families with LCDIIIA have been reported (Stock et al. 1991), and, unlike LCD1, which along with three other 5q31-linked (Stone et al. 1994) autosomal dominant corneal dystrophies is the result of a mutation in the βig-h3 gene (Munier et al. 1997), the molecular defect in LCDIIIA has not been identified.

We encountered three Japanese families with LCDIIIA, and, in a molecular analysis of nine affected patients from these families, we detected a novel missense mutation in the βig-h3 gene. The same mutation was also detected in four additional sporadic LCDIIIA patients from whom no family history was available. βig-h3 encodes an extracellular adhesion protein inducible by transforming growth factor-β (TGF-β), first isolated by Skonier et al. (1992) and recently termed "kerato-epithelin" (Munier et al. 1997).

All affected individuals had late-developing thick, ropy lattice lines in the corneal stroma typical of LCDIIIA (fig. 1*B*). In each family, the disease showed an autosomal dominant inheritance pattern (fig. 2). No corresponding systemic abnormalities were seen in any of the patients. Histopathological examination (two cases) revealed characteristic accumulations of amyloid deposits in the stroma (fig. 1*C, D*). Furthermore, of 13 LCDIIIA patients (9 members of three families and 4 sporadic cases), 8 had a history of recurrent corneal erosions like those described by Stock et al. (1991).

After obtaining informed consent, we analyzed genomic DNA isolated from leukocytes of the LCDIIIA patients and their family members, using standard methods. The 13 exons of the βig-h3 gene (Munier et al. 1997) were amplified using the PCR with oligonucleotide primers. The PCR products were then subjected to SSCP analysis (Orita et al. 1989). In LCDIIIA patients, we identified an abnormal conformer of exon 11. Sequencing analysis demonstrated that one of the alleles of every patient had a C→A transition (CCA→ACA) at position 1501 (fig. 3) that caused a proline-to-threonine substitution (Pro501Thr). To specifically rule out mutations in codons 124 and 555, where mutations have been found in other corneal dystrophies including LCDI (Munier et al. 1997), we sequenced exons 4 and 12 of the βig-h3 gene. No mutations were found, and codons 124 and 555 were intact.

The LCDIIIA families were analyzed using a mutation-specific primer we synthesized that generates a *Dra*III site. Under standard PCR conditions (94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s, with a final extension step at 72°C for 10 min), the following forward and reverse PCR primers were used; BIGexon11F (Munier et al. 1997) (5′-CTC GTG GGA GTA TAA CCA GT-3′) and BIG11RLCDIII (5′-GAC ATC CAT GAC AGT CCA CAT-3′). We observed the mutation-specific *Dra*III digestion pattern in all LCDIIIA-affected individuals but not in unaffected family members (fig. 4), indicating that the missense change Pro501Thr perfectly cosegregated with the disease. In addition, this mutation was not found among 41 patients with granular corneal dystrophy type I (MIM 121900), Reis-Bücklers corneal dystrophy, or Avellino corneal dystrophy, nor was it found in 106 normal individuals (data not shown). On the basis of this evidence, we conclude that the Pro501Thr mutation is the cause of LCDIIIA.

The βig-h3 gene encodes an adhesion molecule char-

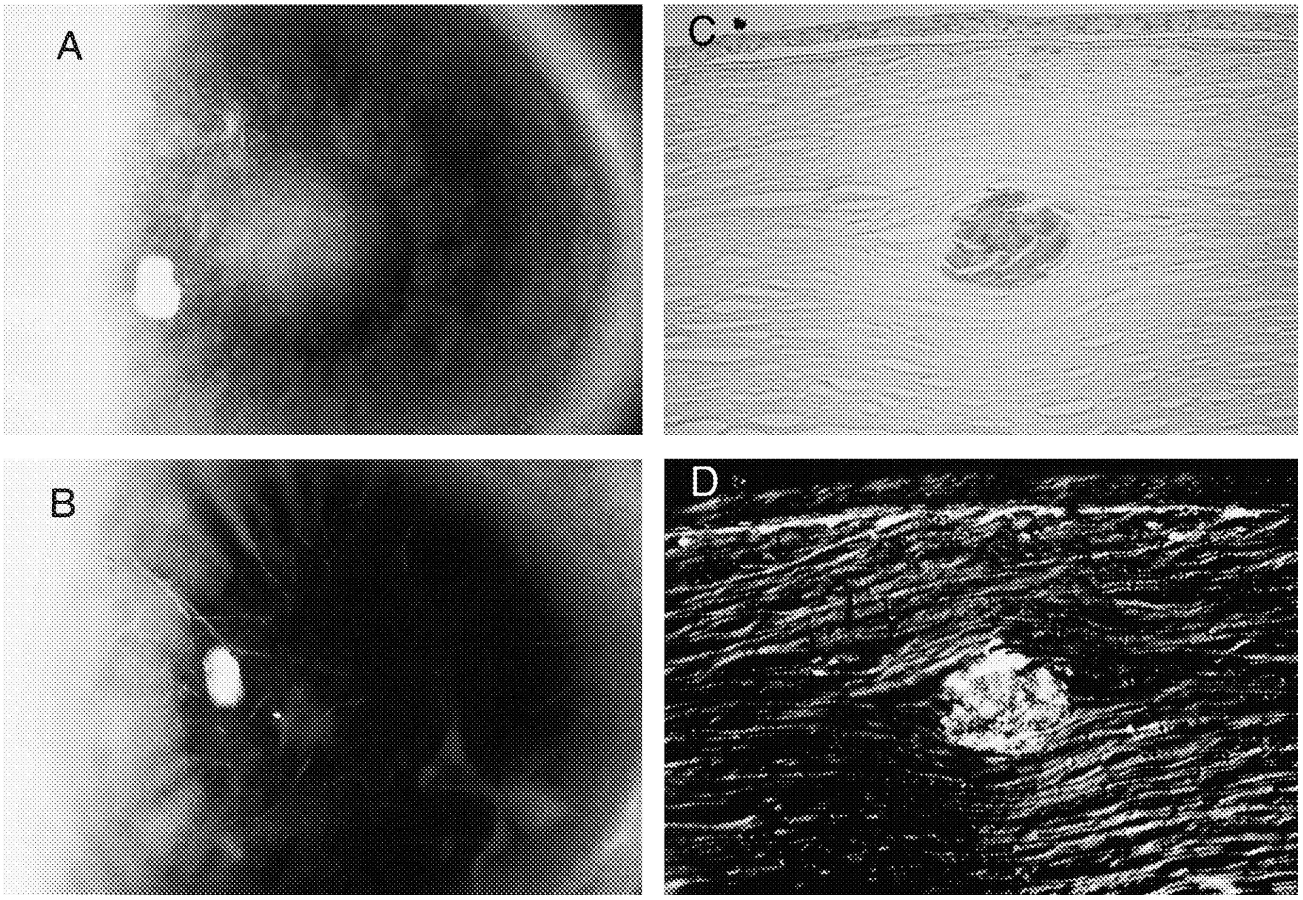

Figure 1 Clinical and histological appearance of LCD corneas. *A,* LCD type I, showing central opacities. Fine lattice lines are present in this patient, but they are difficult to reproduce photographically. In this case, we identified a heterozygous missense mutation, R124C, that was identical to that of a previous report (Munier et al. 1997) (original magnification × 5). *B,* LCD type IIIA, showing thick, ropy branching lattice lines throughout the cornea on sclerotic scatter (patient A-3, in family A) (original magnification × 5). *C* and *D,* Histology of the stroma from one of our four sporadic LCD type IIIA cases stained with congo red (*D* is viewed under polarized light), showing an amyloid deposit (original magnification ×100).

acterized by four internal homologous domains, which can be folded into a potential bivalent structure and may act as a bridge between cells expressing the appropriate ligand (Skonier et al. 1992, 1994). Pro501 is located in the third internal repeat and is conserved in humans, mice, chicks, and pigs. The mutation (Pro501Thr) we detected in LCDIIIA changes a nonpolar residue to a polar residue. Although the mechanism by which the Pro501Thr mutation leads to LCDIIIA is still unknown, proline is important in producing bends in a peptide chain. Therefore, it is possible that the tertiary structure of the mutant kerato-epithelin is deranged in LCDIIIA, leading to the formation of amyloidogenic intermediates.

Munier et al. (1997) identified four missense mutations at codons 124 and 555 of the βig-h3 gene, in four corneal dystrophies, and all four mutations occurred in a CpG dinucleotide of arginine codons. They postulated that the mutation R124 resulted in amyloidogenic intermediates. Our cases suggest that P501-mutated kerato-epithelin may also form amyloidogenic intermediates that precipitate in the cornea.

Acknowledgments

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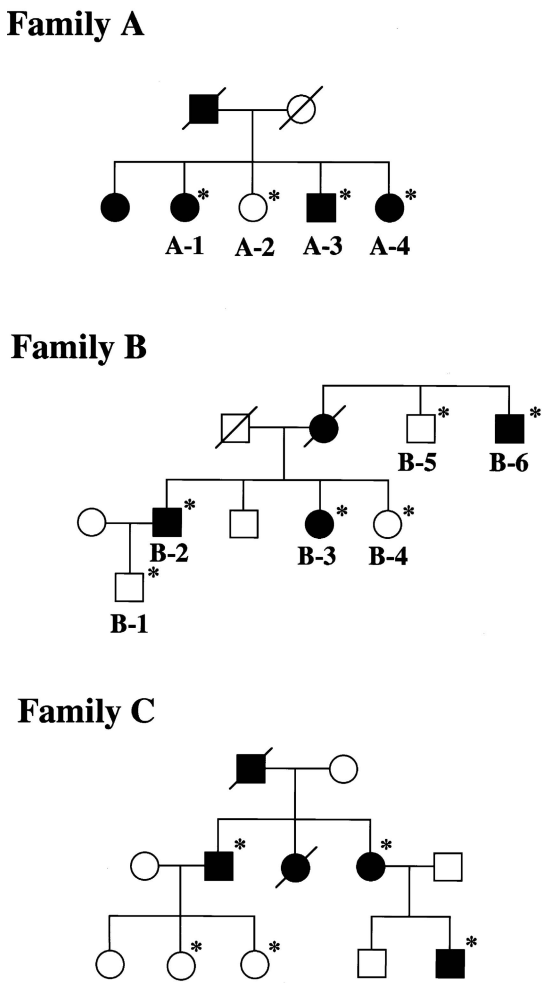

Figure 2 Pedigrees of families with LCD type IIIA. Asterisks (*) denote individuals whose leukocyte DNA was analyzed.

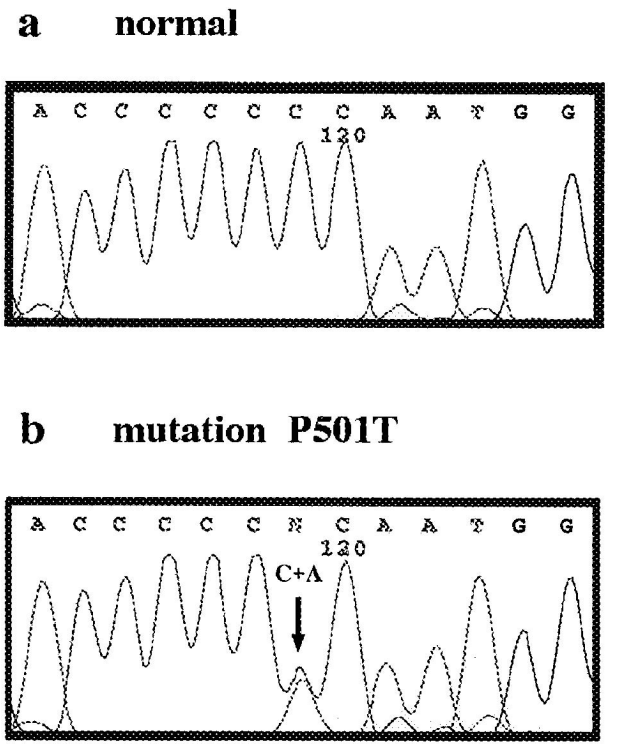

Figure 3 Direct sequencing of exon 11 of the βig-h3 gene. *a*, Unaffected control. *b*, Patient B-2, the proband of family B (fig. 2). Sequencing of the normal and mutant alleles of the patient identified the C→A transition (CCA→ACA) at position 1501, resulting in a proline-to-threonine substitution (Pro501Thr) in the protein. Other affected members had the same mutation. The nucleotide N indicates the presence of both C and A.

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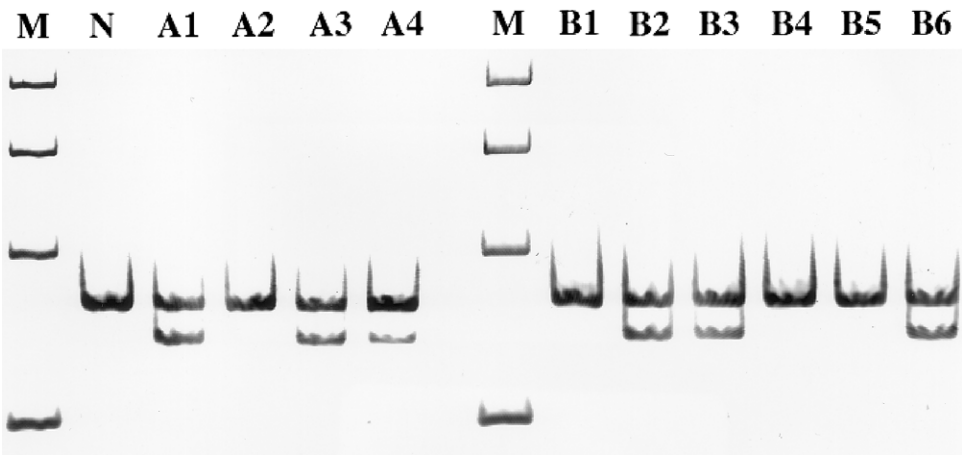

Figure 4 Cosegregation study by restriction-digestion analysis of exon 11. N = normal individual, for control; M = 100-bp ladder marker. The upper band (165 bp) represents the wild type, and the lower digested band (146 bp) represents the mutation Pro501Thr. All affected patients carry the mutation-specific lower band.

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