Exclusion of the Gelsolin Gene on 9q32-34 as the Cause of Familial Lattice Corneal Dystrophy Type I

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Summary

Familial lattice corneal dystrophy type I (LCD1) is a localized form of inherited amyloidosis limited to the corneal stroma. Recently the Finnish form of hereditary amyloidosis with lattice corneal dystrophy has been shown to be due to a mutation in the gelsolin gene (G654→A; Asp187→Asn). In this paper we exclude the gelsolin gene as the cause of the autosomal dominant form of isolated LCD1.

Introduction

Lattice corneal dystrophy type I (LCD1) is a fully penetrant autosomal dominant corneal dystrophy that primarily involves the stromal layer of the cornea (Waring et al. 1978; Isubota et al. 1987). The disease is characterized by recurrent corneal erosions causing pain, photophobia, and redness, and it usually progresses to severe visual impairment by the fourth to seventh decades of life. Although the underlying genetic abnormality and the pathogenesis of LCD1 are not known, some important observations have been made. Slit-lamp examination of affected individuals reveals a delicate interdigitating network of coalescing dots, dashes, and filaments, usually beginning in the central portion of the corneal stroma. Histological examination of affected corneas have confirmed the presence of amyloid consisting of small congophilic deposits with apple-green birefringence in polarized light (Klintworth 1971). Immunohistochemical analysis has not, to date, characterized the basic component of the amyloid fibril in LCD1 (Mondino et al. 1980; Gorevic et al. 1984).

The amyloidoses are a large heterogeneous group of both localized and systemic disorders that may be classified clinically and by histopathological identification of the protein subunit of the extracellular amyloid fibril (Benson and Wallace 1989; Benson 1991). In hereditary systemic amyloidosis, the most frequently encountered subunit protein is plasma prealbumin (transthyretin) (Benson 1991). In the subgroup of familial amyloidotic polyneuropathies, the Finnish syndrome (type IV FAP or FAF) is characterized by lattice corneal dystrophy in addition to progressive cranial neuropathy and systemic amyloidosis (Meretoja 1969; Boysen et al. 1979; Purcell et al. 1983; Darras et al. 1986). The slit-lamp appearance of thickened and radially oriented lines seen in FAF corneas differs from that seen in LCD1 (Meretoja 1972). The amyloidogenic protein in FAF has been identified as an internal degradation product of gelsolin (GSN), an actin-modulating protein (Ghiso et al. 1990; Maury 1990). A single amino-acid substitution at GSN residue 187 has been demonstrated in amyloid fibrils isolated from patients with FAF (Ghiso et al. 1990; Maury 1990). This amino acid change resulted from a single-nucleotide guanine-to-adenine transition at nucleotide position 654 (G654→A654) identified in genomic DNA, and it presumably represents the disease-causing mutation in FAF (Levy et al. 1990; Maury et al. 1990).

Herein we describe the results of a linkage analysis performed between the LCD1 gene (LCD1) and the GSN gene (GSN) by using a (GT)n polymorphism found within GSN. The population that formed the
basis for this study is a large Manitoba kindred of Belgian descent in which LCD1 is known to be segregating. Because a mutant GSN protein is involved in the pathogenesis of the lattice corneal dystrophy associated with FAF, it was important to evaluate GSN as a likely candidate gene for LCD1.

**Subjects and Methods**

**Family Studies**

The LCD1 kindred being studied is a large multigeneration Manitoba kindred of Belgian descent (fig. 1). This kindred was initially described by Robert Ramsay in 1958 (Ramsay 1958). The mode of transmission is consistent with autosomal dominant inheritance with full penetrance. Additional pedigree and clinical information will be reviewed elsewhere (S. Marles, unpublished data). All affected and at-risk individuals are now being followed by one ophthalmologist (M.B.E.). All affected individuals have been symptomatic and/or have demonstrated characteristic corneal changes on slit-lamp examination by age 25 years. There is neither known history nor objective signs of any associated systemic illness—specifically, neurological, cardiac, or renal. Sixteen individuals have received a total of 21 corneal transplants in the past decade. Characteristic histopathological changes of amyloid deposition have been confirmed in all corneas studied.

**DNA Methods**

DNA was extracted from heparinized blood samples obtained from 92 kindred members including 37 affected individuals, 23 unaffected individuals, 17 unaffected spouses, and 15 at-risk family members, as described elsewhere (Greenberg et al. 1987). Linkage between GSN and LCD1 was studied by using a highly polymorphic intronic microsatellite (GT)$_n$ repeat in

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**Figure 1** Manitoba pedigree with LCD1. □ = Unaffected male; ○ = unaffected female; ■ = male affected with LCD1; and ● = female affected with LCD1. A slash (/) indicates that the individual is deceased. An asterisk (*) indicates the portion of pedigree shown in fig. 2.
GSN. A 125–143-bp DNA fragment encompassing the (GT)$_n$ repeat was PCR amplified (Saiki et al. 1988) according to the following protocol: The PCR program included an initial denaturation at 94°C for 1.5 min; 25 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 40 s; and 72°C for 4 min 20 s. Each reaction was carried out in a total volume of 10 μl consisting of 2 μl dNTP mix (1 mM dATP, 1 mM dGTP, 1 mM dTTP, and 12.5 μM dCTP), 0.4 μl oligonucleotide primer-pair mix (100 ng/μl each), 0.08 μl *32P dCTP (NEG-013H; 3,000 Ci/mM; NEN) 1 μl 10 × reaction buffer (Perkin Elmer Cetus), 0.05 μl AmpliTaq (Perkin Elmer Cetus), 4.47 μl ddH$_2$O, and 2 μl genomic DNA (1:300 dilution of leukocyte DNA preparation). Two microliters of the PCR product was mixed with 3 μl of formamide stop solution, was heated at 80°C for 5 min, and was loaded on a 7 M urea, 6% polyacrylamide sequencing gel. After approximately 2 h at 2,000 V, the gels were dried and exposed to X-ray film for 1–24 h. The GSN alleles were designated 0–16, as described elsewhere for other (GT)$_n$ VNTR microsatellites (Kwitakowski et al. 1991). The allele with the fewest number of GT repeats seen was designated "0," and the other alleles were designated 2–16 according to the number of additional bases that their amplified fragments contained. The allele assignments were made without prior knowledge of disease status. To date, a total of 12 alleles with a PIC value of .76 and a heterozygosity of .70 have been described at the GSN locus (Kwitakowski and Perman 1990).

Linkage Analysis

Two-point LOD scores were calculated between GSN and LCD1 by using the Mark III computer program (Côte 1975). Inheritance in this family is consistent with autosomal dominant transmission with full penetrance. Thirty-two informative meioses from 10 nuclear families (3 paternal and 7 maternal) were scored for linkage. Only confirmed affected individuals or those unaffected individuals older than 25 years were included in the linkage analysis.

Results and Discussion

Figure 1 shows the pedigree of the participants in this study. There are 37 living individuals who are clinically affected with LCD1 as assessed by clinical and slit-lamp examinations. Figure 2 depicts the informative polymorphism in the length of amplified DNA in one affected nuclear family. This (GT)$_n$ polymorphism displays Mendelian codominant inheritance. The youngest daughter in this nuclear family is not

![Figure 1: Pedigree of the participants in the study.](image1)

![Figure 2: (GT)$_n$ repeat analysis in one nuclear family.](image2)
Table I

| Seperation Information | No. of Families | No. of Meioses | LOD Score for θ of |
|-------------------------|-----------------|---------------|--------------------|
|                         |                 |               | .05                | .10 | .20 | .30 | .40 |
| Paternal ................ | 3               | 10            | -1.34              | -0.60 | -0.04 | 0.13 | 0.12 |
| Maternal ...............  | 7               | 22            | -4.95              | -2.82 | -1.009 | -0.25 | 0.03 |
| Total ..................  | 10              | 32            | -6.29              | -3.42 | -1.049 | -0.12 | 0.15 |

included (fig. 2); since she is 18 years old and ophthalmologically normal, she cannot be scored in the linkage analysis. The results of two-point linkage analysis between the loci for LCD1 and GSN are shown in Table 1. The LOD score is -6.29 at a recombination fraction (θ) value of .05 and is -3.42 at θ = .10, for combined maternal and paternal meioses.

The genetic basis for LCD1, an autosomal dominant corneal stromal dystrophy, is unknown. A mutation in GSN appears to result directly in an amyloidogenic GSN molecule, a fragment of which polymerizes to amyloid fibrils that deposit in FAF (Levy et al. 1990; Maury 1990). Our study investigated GSN as a candidate locus for LCD1. Of 32 informative meioses scored for linkage, 11 obligate recombinants were seen (fig. 2 and data not shown). This results in strongly negative LOD scores between GSN and LCD1 (−6.29 at θ = .05). The genomic region region of GSN encompasses only 70 kb of DNA (Kwiatkowski et al. 1988), and our LOD score of −2.00 at a θ = .15 makes the possibility of 15% intragenic recombination virtually impossible. This genetic evidence thus excludes GSN as a candidate gene in our kindred. Our study also provides strong evidence that the LCD1 locus is not located within 10 cM of GSN. These results are further supported by our observations that immunohistochemical analysis with anti-GSN antibodies (Maury 1991) completely failed to stain the amyloid deposits in the corneas from our affected LCD1 patients (data not shown).

These results indicate that the pathogenesis of the corneal dystrophy seen in LCD1 is not due specifically to the GSN fragment but that other amyloidotic proteins can also deposit in the cornea. FAF is characterized by a systemic distribution of amyloid deposits in the cornea. FAF is characterized by a systemic distribution of amyloid deposits in addition to corneal deposits (Meretoja 1969; Purcell et al. 1983; Darras et al. 1986). LCD1, on the other hand, appears to have amyloid deposition localized solely to the stromal layer of the cornea (Klintworth 1971). It then follows that the localized nature of the amyloid deposition in LCD1 might be of a different origin than are the systemic deposits seen in FAF. A search for mutations in other candidate genes, including prealbumin (trans-thyretin) and apolipoprotein-AI, as well as the isolation and characterization of the corneal amyloid from LCD1 corneas, are in progress.

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