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Arg124Cys Mutation of the *βig-h3* Gene in a Japanese Family With Lattice Corneal Dystrophy Type I

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Abstract: To characterize severe lattice corneal dystrophy, we analyzed the *βig-h3* gene, clinical features, histological findings, and genotype-phenotype correlation in an affected Japanese family. Deoxyribonucleic acid was extracted from leukocytes in 16 members (12 affected and 4 unaffected) of a Japanese family with lattice corneal dystrophy type I. Exon 4 of the *βig-h3* gene was amplified and analyzed using molecular biological methods. Clinical and pathological data were also collected. We found a heterozygous point mutation that causes the disease phenotype. It was a single base-pair transition leading to an amino acid substitution (CGC→TGC, Arg124Cys). The phenotypic variation within families was not recognized. The affected members in the pedigree demonstrated severe visual disturbance in the third decade and required keratoplasty. Histopathological examination revealed amyloid deposits consisting of short and thin amyloid fibers and lattice corneal dystrophy type I. The heterozygous Arg124Cys mutation reported in Caucasian lattice corneal dystrophy caused severe lattice corneal dystrophy consisting of short and thin amyloid fibers in a Japanese family. Based on our study of many members of the family, we are able to construct the natural course of this disorder from its earliest clinical findings through its late manifestations. **Jpn J Ophthalmol 1998;42:450–455**

Key Words: *βig-h3*, high penetrance, lamellar keratoplasty, lattice corneal dystrophy, point mutation.

Introduction

Corneal dystrophies exhibit a hereditary pattern, are bilateral, and may be progressive. Lattice corneal dystrophy is an autosomal dominant corneal stromal dystrophy in which the affected patient suffers from painful bilateral recurrent corneal erosions, eventually causing marked reduction in vision. Lattice corneal dystrophy is clinically divided into three subtypes.[1–3] In type I lattice corneal dystrophy, small refractive lines, anterior stromal white dots, subepithelial ovoid or round opacities, and a faint central haze in the anterior stroma may appear in the first decade of life. Lattice corneal dystrophy type II is associated with a familial form of systemic amyloidosis. The corneal findings of lattice corneal dystrophy type III include broad lattice-like lines and diffuse subepithelial opacities, which become apparent after the age of 40, and good visual acuity. Although granular and lattice corneal dystrophies are usually considered independent entities, both clinically and histologically, recent reports revealed Avellino corneal dystrophy that had clinicopathologic features of both granular and lattice dystrophies.[4,5]

Four autosomal dominant corneal dystrophies including Reis-Bückler, lattice, granular, and Avellino corneal dystrophies were mapped to the long arm of chromosome 5,[6,7] and, recently, mutations of the *βig-h3* gene were reported.[8] Studies showed that Arg124Cys, Arg124His, Arg555Trp and Arg555Asn caused lattice type I, Avellino, granular (Graenow type I), and Reis-Bückler corneal dystrophy, respectively. We previously reported on two families with corneal dystrophy that histologically consists of short and thin amyloid fibers; clinically, type I lattice cor-

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neal dystrophy.[9] Based on our study of many members of the family described in this report, we are now able to construct the natural course of this disorder from its earliest clinical findings through its late manifestations. In addition, we characterize the causative genetic defect in this family and confirm cosegregation of the defect with the disease phenotype.

Materials and Methods

Family Study

The pedigree being studied is a large multigeneration "O" family of Japanese descent (Figure 1) that was initially described in 1978.[9] All individuals examined are now being followed in the Department of Ophthalmology, Juntendo University, Tokyo, and/or St. Luke's International Hospital, Tokyo.

Deoxyribonucleic Acid (DNA) Analysis

Genomic DNAs were extracted from leukocytes of peripheral blood collected, with informed consent, from 16 family members (12 affected and 4 unaffected). Approximately 200 ng of each member's DNA was used for 50 μL polymerase chain reaction (PCR). Exon 4 of the *βig-h3* gene was amplified using sense and antisense primers 5′CCCCAGAGGCCATCCCTCCT-3′ and 5′CCGGGCAGACGGAGGTCATC-3′. The PCR was carried out in a volume of 50 μL in a DNA Thermal Cycler (Perkin Elmer, Norwalk, CT, USA).[10] Each 50 μL PCR amplification contained the following final components: dNTPs (deoxyribonucleotide triphosphate), 0.2 mmol/L; Tris-HCL, 10 mmol/L, pH 8.3; 0.45% Triton X-100; $MgCl_2$, 15 mmol/L; KCl, 50 mmol/L; each primer, 25 pmol/L; and 1.25 U of *Taq* DNA polymerase. PCR conditions were 5 minutes at 94°C followed by 30 cycles at 94°C for 1 minute, 57°C for 1 minute, and 72°C for 1 minute, with a final extension step at 72°C for 10 minutes.

The PCR products from the family members described above were purified using the High Pure PCR Purification Kit (Boehringer Mannheim, GmbH, Germany) and were directly sequenced. Direct sequence was performed using DNA sequencing kit, Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer Applied Biosystems) by an automated DNA sequencer (Model 373A, Applied Biosystems) described previously.[11,12] Primers for se-

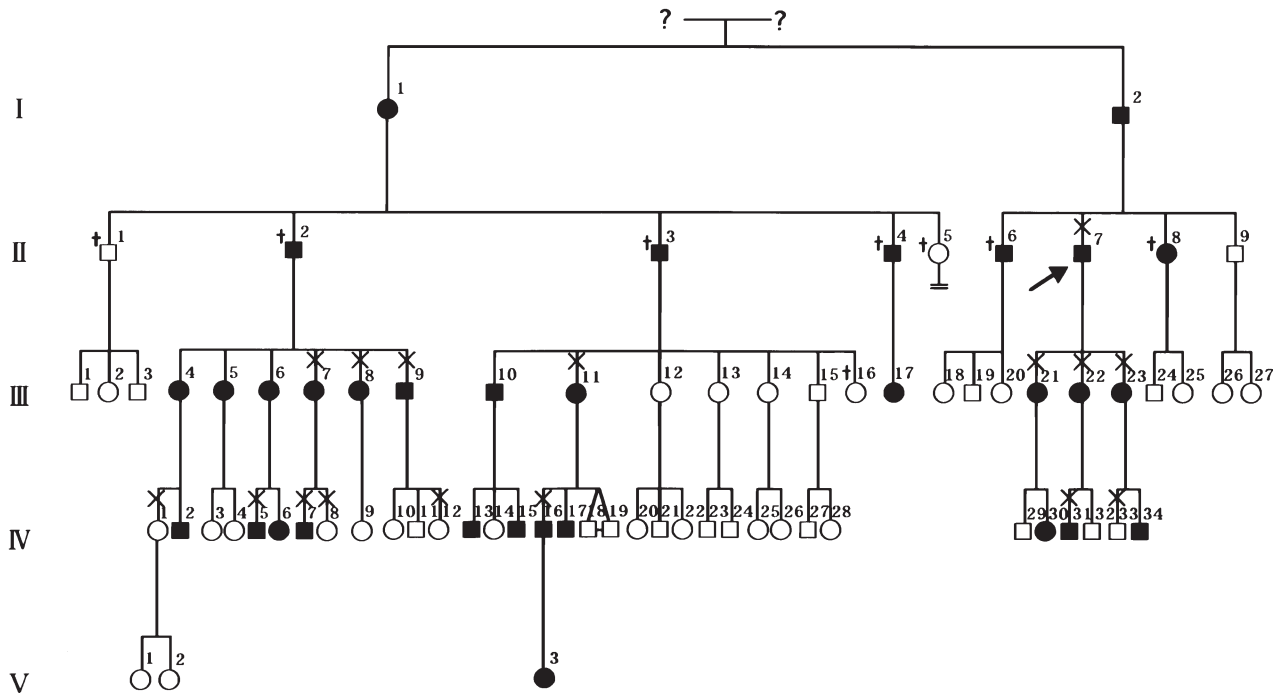

Figure 1. Pedigree that carries Arg124Cys mutation. Proband is patient II-7 (arrow). Affected females (●), affected males (■), family members who are clinically unaffected (○, □), patients who were examined (×), deceased (†). Disease is present in four consecutive generations, and father-to-son transmission occurs, which is consistent with autosomal dominant inheritance.

Jpn J Ophthalmol
Vol 42: 450–455, 1998

quence reaction were the same primers as for the PCR reaction. Sixteen DNA samples of the "O" family were sequenced on both sense and antisense strands.

Clinical Evaluation

Clinical details of the affected family members were obtained from the clinical records of both departments described above. Best corrected visual acuity was tested using an international chart. Most of the lamellar keratoplasties were performed using a 9-mm diameter graft, and the two penetrating keratoplasties for the proband, using a 7-mm diameter graft. Corneal buttons were fixed in 2.5% formaldehyde, dehydrated with serial alcohols, and embedded in paraffin. Sections, 5 μm thick, were stained with hematoxylin and eosin, PAS, and alkaline Congo red, using standard methods, and analyzed by light microscopy. Portions of corneal buttons were processed for transmission electron microscopy by standard methods. The embedded tissue was sectioned with a microtome, stained with lead citrate and uranium acetate, and analyzed by electron microscopy.

Results

Family Study

Four generations of this family had been positively diagnosed with lattice corneal dystrophy. The mode of transmission is consistent with autosomal dominant inheritance with 43% (32/75) incidence (almost full penetrance).

DNA Analysis

The direct sequencing of the *βig-h3* gene using the automated DNA sequencer revealed that 12 affected individuals showed the mutant pattern, whereas 4 unaffected showed the wild-type pattern. Figure 2 shows examples of affected (Patient III-8) and unaffected (Patient IV-1) individuals. The result was confirmed using both sense (S) and antisense (A) primers. The repetition of the sequencing confirmed the mutation. A single base-pair transition leading to an amino acid substitution (CGC→TGC, Arg124Cys) was detected in all 12 affected individuals and confirmed cosegregation of the mutation with the disease phenotype.

Clinical Evaluation

The family examined contained 12 affected individuals, aged 28 to 83 years (Table 1). The following selected case reports illustrate the clinical course of lattice corneal dystrophy in this family.

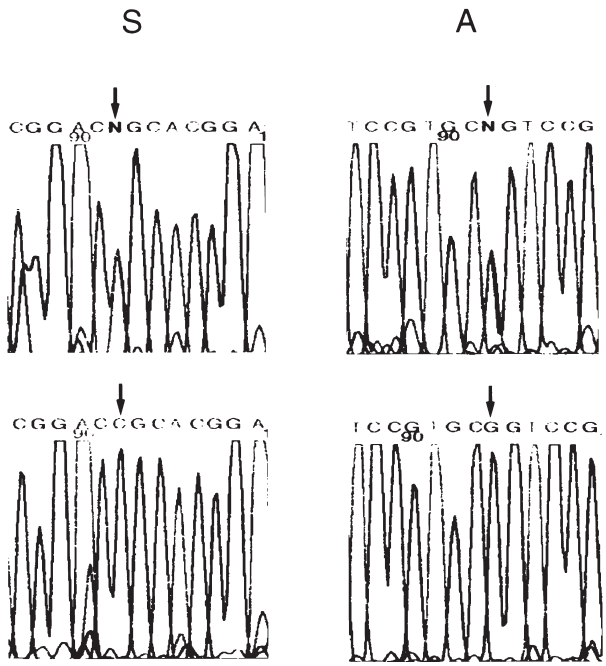

Figure 2. Result of direct sequencing around codon 124 of *βig-h3* gene from affected (III-8, top) and unaffected (IV-1, bottom) individuals using automated DNA sequencer. Polymerase chain reaction product was amplified with primers and directly sequenced by dye terminator method using sense (S) and antisense (A) primers as described in text. First base of codon 124 in affected family members showed both red (T) and violet (C) peaks (black [G] and green [A] peaks using (A) primer) resulting in a single base-pair transition leading to an amino acid substitution (CGC→TGC, Arg124Cys). No genetic defect was recognized in unaffected.

The proband (II-7) was 15 years old when he had recurrent corneal erosions. He first visited our hospital when he was 55 years old. His corrected visual acuity at that time was 0.1 OD and 0.06 OS. Slit-lamp examination of both corneas reveal diffuse opacity in the corneal epithelium and stroma. The small lattice-shaped opacity, a characteristic feature of lattice dystrophy, was observed by direct illumination and retroillumination. It seemed to be associated with the epithelial irregular surface. During the twenty-nine-year follow-up, the four surgical treatments shown in Table 1 were performed on both eyes. His corrected visual acuity is now 0.04 OD and hand movement OS.

The 31-year-old grandson (IV-5) of a cousin of the proband first presented at our hospital when he was 11 years old with no symptoms. His corrected visual acuity was 0.8 OD and 1.0 OS. Slit-lamp examination of both corneas revealed refractile stromal dots with-

Table 1. Clinical data of affected individuals

Patient No./ Sex/Age (Years)	Age at Onset (Year)	Length of Follow-Up (Years)	Surgical Treatment (Patient's Age When Performed)	Latest Corrected Visual Outcome
II-7/M/83	15	29	OD: LKP(58), PKP(79), EC(79), AK(81) OS: LKP(55), LKP(73), PKP(77), EC(79)[a]	OD: 0.04 OS: HM
III-7/F/54	15	16	OD: LKP(41) OS: LKP(44)	OD: 0.6 OS: 0.7
III-8/F/52	20	10	OD: LKP(42), LKP(52) OS: LKP(44), LKP(44)	OD: 0.1 OS: 0.7
III-9/M/49	10	21	OD: LKP(31), PT(32), LKP(39), LKP(44) OS: LKP(28)	OD: 0.5 OS: 1.0
III-11/F/61	17	21	OD: PKP(21), PKP(59) OS: LKP(40), PKP(53)	OD: 0.02 OS: 0.2
III-21/F/57	3	17	OD: LKP(39) OS: ND	OD: 0.6 OS: 0.7
III-22/F/54	3	15	OD: LKP(39) OS: LKP(53)	OD: 0.6 OS: 0.3
III-23/F/51	4	13	OD: ND OS: LKP(39)	OD: 0.05 OS: 0.5
IV-5/M/31	11	20	OD: ND OS: ND	OD: 0.5 OS: 0.8
IV-7/M/28	14	14	OD: ND OS: ND	OD: 0.3 OS: 0.5
IV-16/M/33	15	18	OD: ND OS: ND	OD: 1.2 OS: 0.5
IV-31/M/23	3	9	OD: ND OS: ND	OD: 0.8 OS: 0.6

AK: astigmatic keratotomy, EC: extracapsular cataract extraction with IOL implant, HM: hand motion, LKP: lamellar keratoplasty, ND: not done, PKP: penetrating keratoplasty, PT: pterygium resection.

[a]Patient hit his left eye and resuture of corneal graft was performed 7 months after original surgery.

out accompanying lattice lines and subepithelial opacity in the central cornea (Figure 3). With age, the fine refractile dots spread to involve the deeper and more peripheral layers of the stroma. At present, the small lattice-shaped opacity was observed by direct illumination and by retroillumination. His corrected visual acuity is now 0.5 OD and 1.0 OS.

The 52-year-old daughter (III-8) of a cousin of the proband first presented at our hospital when she was 42 years old, following the recurrence of corneal erosions that had first appeared when she was 20 years old. At our first examination, her corrected visual acuity was 0.1 OD and 0.1 OS. Slit-lamp examination of both corneas revealed diffuse opacity in the anterior third of the corneal stroma (Figure 4, top). The characteristic small lattice-shaped opacity already described was observed. During the 10-year follow-up, lamellar keratoplasty was performed twice on both eyes. Her corrected visual acuity is now 0.1 OD and 0.7 OS.

We have already reported the histopathologic features of this family.[9] A detailed study showed amyloid deposits consisting of short and thin amyloid fibers. Corneal buttons obtained from members (III-8 and III-22) of this family were studied histopathologically. The sections from both patients, after staining with Congo red, showed positive staining in stromal lesions with polarized light (Figure 4, bottom). Small vacuoles and irregularly shaped, electron-dense deposits, which measured up to about 5 μm in diameter, were observed in the stromal lesion. Electron-dense fibrils in an accumulation of about 80–100 Å indicated a tubular structure (data not shown).

Discussion

The phenotypic variations within a family were not recognized in this family. In this family, recurrent corneal erosion accompanied by pain, photophobia, and redness, usually occur during childhood. The recurrent erosions cause a serious problem to

Jpn J Ophthalmol
Vol 42: 450–455, 1998

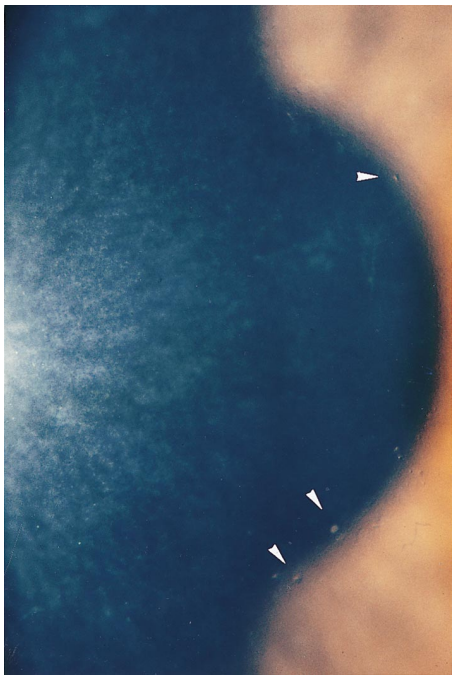

Figure 3. Early clinical features in patient IV-5 at 11 years of age. Both subepithelial opacity and refractile stromal dots (arrowheads) without accompanying lattice lines are seen in central cornea.

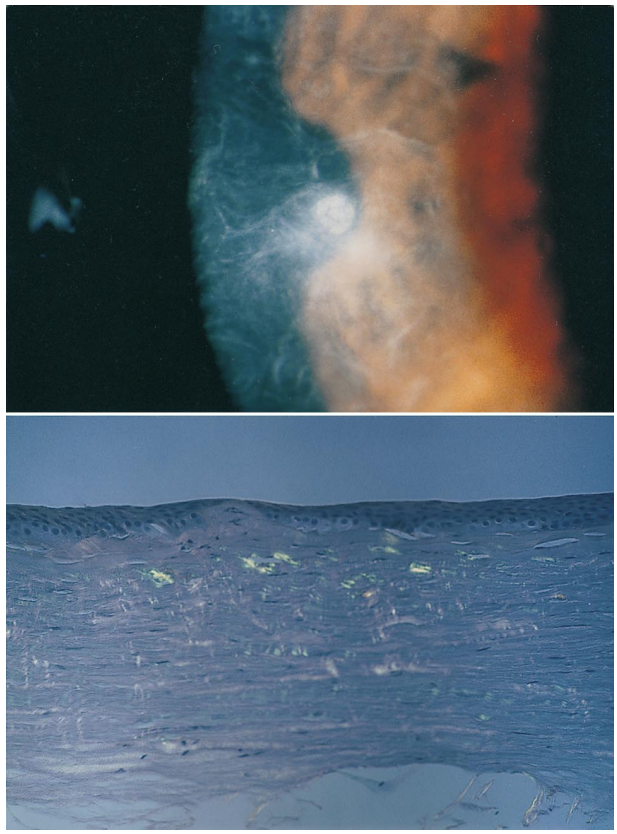

Figure 4. Clinical and histopathological features of patient III-8. Top: left eye at 42 years of age. Bottom: corneal button obtained from first lamellar keratoplasty of left eye. With polarized light, section stained with Congo red showed positive staining in stromal lesion.

the patient until the third decade. They complain of blurred vision and visual disturbance. Lamellar keratoplasty is performed and usually recurrence is observed following good visual acuity for several years after surgery.[13] Penetrating keratoplasty is chosen if recurrence follows several lamellar keratoplasties. The advantage of choosing lamellar keratoplasty for the first surgery is that we can repeat it several times. Also, it is technically simple and safe. However, the final visual outcome is slightly worse than with penetrating keratoplasty. Although we perform keratoplasty several times, because of recurrence the final visual outcome is poor.

Recent investigation revealed that various loci for corneal dystrophies have been identified on chromosome 1p,[14] 5q,[6,7] 16q,[15] and 20.[16,17] Causative mutations have been identified in the *LCAT* gene at 16q22.1, causing FISH-eye disease,[18] and in the gelsolin gene at 9q34, causing the Finnish form of hereditary amyloidosis with lattice corneal dystrophy (lattice corneal dystrophy type II).[19,20] Recently, mutations in the *βig-h3* gene causing Avellino, lattice type I, granular and Reis-Bückler corneal dystrophies, and in the cornea-specific keratin K3 or K12 gene causing Meesmann's corneal dystrophy, were reported.[8,21] Arg124Cys mutation of the *βig-h3* gene was reported in two Caucasian families with lattice corneal dystrophy type I.[8] The same mutation was detected in a different ethnic background, a Japanese family with lattice corneal dystrophy type I, and shows complete cosegregation of the mutation with the disease phenotype. This suggests that Arg124Cys mutation causes the lattice corneal dystrophy type I.

Lattice corneal dystrophy is generally divided into three subtypes. The classification had been determined mainly by clinical and pathological findings. We have already reported the clinical and histopathological features of Japanese patients with corneal dystrophies.[22] At present, the Japanese family presented here is classified into type I by the clinical and pathological findings.[9] Since clinical features of lattice corneal dystrophy are varied, clarification of the genotype for lattice corneal dystrophies will play a great role in establishing a new classification. The

βig-h3 gene mutation and the gelsolin gene mutation cause lattice type I, including our cases, and lattice type II, respectively. The identification of the causative genes for various lattice corneal dystrophies, including lattice corneal dystrophy type II, is also required to establish the exact classifications for lattice corneal dystrophy.

Although prenatal and postnatal DNA diagnoses are technically possible in this family, the clinical application of the DNA diagnosis is still disputable. The exon 4 of the *βig-h3* gene is amplified using DNA from peripheral blood by PCR amplification and directly sequenced to check codon 124 of the gene. If the Arg124Cys mutation is detected in an individual from this family at birth, it can be expected that he or she will develop severe corneal lattice dystrophy requiring several keratoplasties.

We employed PCR and direct sequence procedures using the automated DNA sequencer for all 16 individuals. The obvious heterozygous pattern was recognized in all attempts at sequencing in the 12 affected individuals; a normal pattern resulted in the 4 unaffected individuals. Since recent automated sequence procedure is sensitive enough to detect the heterozygous mutation pattern, the direct sequence instead of the single strand conformation polymorphism method,[23] which sometimes causes false-negative result, will be the first choice for the clinical diagnostic setting.

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References

1. Smolin G. Corneal dystrophies and degenerations. In: Smolin G, Thoft RA, eds. Cornea. Boston: Little Brown, 1994:499–533.
2. Hida T, Tsubota K, Kigasawa K, Murata H, Ogata T, Akiya S. Clinical features of a newly recognized type of lattice corneal dystrophy. Am J Ophthalmol 1987;104:241–8.
3. Hida T, Proia AD, Kigasawa K, et al. Histopathologic and immunochemical features of lattice corneal dystrophy type III. Am J Ophthalmol 1987;104:249–54.
4. Folberg R, Alfonso E, Croxatto JO, et al. Clinically atypical granular corneal dystrophy with pathologic features of lattice-like amyloid deposits, a study of three families. Ophthalmology 1988;95:46–51.
5. Holland EJ, Daya SM, Stone EM, et al. Avellino corneal dystrophy, clinical manifestations and natural history. Ophthalmology 1992;99:1564–8.
6. Stone EM, Mathers WD, Rosenwasser GOD, et al. Three autosomal dominant corneal dystrophy map to chromosome 5q. Nat Genet 1994;6:47–51.
7. Small KW, Mullen L, Barletta J, et al. Mapping of Reis-Bücklers' corneal dystrophy to chromosome 5q. Am J Ophthalmol 1996;121:384–90.
8. Munier FL, Korvatska E, Djemaï A, et al. Kerato-epithelin mutations in four 5p31-linked corneal dystrophies. Nat Genet 1997;15:247–51.
9. Kanai A, Tanaka M, Yamaguchi T, Nakajima A. Atypical lattice dystrophy of the cornea, a clinical and histological study. Doc Ophthalmol Proc Series 1978;20:181–92.
10. Saiki RK, Gelfand DN, Stoffel S. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 1988;239:487–91.
11. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 1977;74:5463–7.
12. Smith LM, Sanders JZ, Kaiser RJ, et al. Fluorescence detection in automated DNA sequence analysis. Nature 1986;321:674–9.
13. Yamaguchi T, Ohoshiro M, Kanai A, Nakajima A. Recurrence of corneal dystrophy and degeneration after corneal surgery. Proceedings of the Xth ICO Meeting, 1979:1677–80.
14. Shearman AM, Hudson TJ, Andresen JM, et al. The gene for Schnyder's crystalline corneal dystrophy maps to human chromosome 1p34.1-p36. Hum Mol Genet 1996;5:1667–72.
15. Vance JM, Jonasson F, Lennon F, et al. Linkage of a gene for macular corneal dystrophy to chromosome 16. Am J Hum Genet 1996;58:757–62.
16. Héon E, Mathers WD, Alward WLM. Linkage of posterior polymorphous corneal dystrophy to 20q11. Hum Mol Genet 1995;4:483–8.
17. Toma NMG, Ebenezer ND, Inglehearn CF, Plant C, Ficker LA, Bhattacharya SS. Linkage of congenital hereditary endothelial dystrophy to chromosome 20. Hum Mol Genet 1995;4:2395–8.
18. Skeretting, G, Prydz H. An amino acid exchange in exon 1 of the human lecithin: cholesterol acyltransferase (LCAT) gene is associated with fish eye disease. Biochem Biophys Res Commun 1992;182:583–7.
19. Gorevic PD, Monoz PC, Gorgone G, et al. Amyloidosis due to a mutation of the gelsolin gene in an American family with lattice corneal dystrophy type II. N Eng J Med 1991;325:1780–5.
20. de la Chapelle A, Tolvanen R, Boysen G, Santavy J, Bleeker-Wagemakers L, Kere J. Gelsolin-derived familial amyloidosis caused by asparagine or tyrosine substitution for aspartic acid at residue 187. Nat Genet 1992;2:157–60.
21. Irvine AD, Corden LD, Swensson O, et al. Mutations in cornea-specific keratin K3 or K12 genes cause Meesmann's corneal dystrophy. Nat Genet 1997;16:184–7.
22. Santo RM, Yamaguchi T, Kanai A, Okisaka S, Nakajima A. Clinical and histopathologic features of corneal dystrophies in Japan. Ophthalmology 1995;102:557–67.
23. Orita M, Suzuki Y, Sekiya T, Hayashi K. Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics 1989;5:874–9.