Unusual Phenotype of an Individual With the R124C Mutation in the TGFBI Gene

We describe here an unusual phenotype associated with the arginine-124→cysteine (R124C) mutation of the TGFBI gene. The proband was a 44-year-old woman who exhibited translucent lattice lines and circular central epithelial opacity in both corneas and who had a typical history of recurrent corneal erosions. She and members of her family harbored the R124C mutation of TGFBI, which has been implicated as a genetic change responsible for lattice corneal dystrophy (LCD) type I. We performed keratoplasty on her left eye and examined the removed tissue histopathologically. Light microscopy revealed deposition of mucopolysaccharide and disruption of the Bowman layer, but no Congo red–positive component was detected. Furthermore, apple-green dichroism was not apparent with polarized microscopy. Electron microscopy revealed numerous vacuoles within keratocytes as well as separated collagen fibrils, but no amyloid fibrils, in the stroma. The R124C mutation of TGFBI is thus not necessarily associated with amyloid deposition.

Point mutations of the TGFBI gene (also known as \(\beta\)ig-h3), which encodes keratoepithelin,\(^1\) have been shown to be responsible for corneal dystrophies. Mutation of codon 124 of TGFBI from arginine to cysteine (R124C), histidine (R124H),\(^2\) or leucine (R124L)\(^3\) has thus been associated with type I, Avellino dystrophy, and superficial granular corneal dystrophy, respectively. Although the mutation site is the same, it is possible to differentiate the R124C, R124H, and R124L mutations both clinically with a slitlamp microscope and pathologically.

Keratoepithelin is present in several human tissues, including the cornea. It forms fibrils and interacts with various extracellular matrix proteins, including fibronectin and collagen;\(^4\) these properties are thought to be related to the ability of the mutant protein to form amyloid deposits in corneal dystrophies. Keratoepithelin also plays a role in the adhesion and migration of dermal fibroblasts.\(^5\)

As far as we are aware, all reported cases of the R124C mutation of TGFBI have been diagnosed clinically as LCD type I, with deposition of amyloid in the cornea confirmed pathologically. We now present an unusual case of an individual with the R124C mutation of TGFBI without histopathologic evidence of amyloid deposition.

Report of a Case. A 44-year-old woman was referred to our hospital in October 1998 with complaints of pain in her left eye. In childhood, she had been diagnosed by her local ophthalmologist as having corneal dystrophy, and she had developed recurrent corneal erosions several times in both eyes that had also been treated by her local specialist. Medical information from her local ophthalmologist revealed that she had taken steroid eyedrops because the recurrent erosion was severe and inflammation persisted. At 20 years of age, she underwent trabeculotomy in her left eye as a result of steroid-induced glaucoma. She was referred to our cornea service because of an unusual pattern of healing of epithelial erosion. At the first visit, her visual acuity was 20/40 OD and 20/100 OS, and her intraocular pressure was 10 mm Hg OD and 15 mm Hg OS. Slitlamp examination revealed corneal epithelial and subepithelial opacity as well as fine translucent opacity in the stroma characterized by lattice lines in both eyes (Figure 1). On the basis of the slitlamp examination and her past history, we diagnosed the patient as having LCD. In January 1999, we performed penetrating keratoplasty on her left eye because of a decrease in left visual acuity to 20/600. No episodes of rejection or recurrent erosion have been apparent since the surgery, and the graft has remained clear (Figure 1). The visual acuity of her left eye is currently 20/15 with correction.

Figure 1. Slitlamp photographs of the right eye (A), left eye before surgery (B), and left eye after surgery (C) of the proband.
We obtained written informed consent for genetic analysis of TGFBI from the proband and 3 members of her family: her father, first son, and husband. This analysis was approved by the ethical committee of Yamaguchi University Hospital. We detected a heterozygous R124C mutation of TGFBI in the proband, her father, and her first son (Figure 2). We did not detect a mutation of TGFBI in the husband. We were not able to perform genetic analysis on other members of her family. Slitlamp examinations of her father, 1 of her 2 brothers, and her 2 sons were performed. The father, who died several years ago, had been diagnosed with LCD and underwent corneal transplantation in both eyes 2 decades ago at another hospital; no medical records of his surgeries were available to us. The slitlamp photographs reveal, however, that the grafts in both his eyes were clear and not edematous (Figure 3A). No translucent lines or other findings suggestive of corneal dystrophy were apparent in his own peripheral cornea tissue. The corneas of the proband's brother, however, exhibited typical translucent lines and central superficial opacity (Figure 3B). He had also experienced several episodes of recurrent erosion. We were not able to obtain informed consent for genetic analysis of the brother to confirm the presence of the mutation. The corneas of both the proband's sons exhibited translucent lines, but central superficial opacity was not apparent (Figure 3C and D). On the basis of the clinical findings for this family, we conclude that the corneal abnormality is inherited in an autosomal dominant manner. On the basis of the clinical findings and genetic analysis, we diagnosed the proband as having LCD type I.

Histopathologic examination by hematoxylin-eosin staining and light microscopy of the corneal specimen of the proband obtained during surgery revealed that the layered structure of the corneal epithelium was irregular, with prominent disarrangement of the basal cell layer (Figure 4A). The Bowman layer was defective in the central region of the cornea. In the same area, small numbers of infiltrated cells were present in the anterior portion of the stroma. Collagen fibers in the anterior stroma of the central cornea were separated, possibly as an artifact of fixation. We did not detect eosin-positive amorphous compo-

Figure 2. Pedigree of the proband and genetic analysis of TGFBI in the proband and her family. Closed symbols in the pedigree indicate individuals heterozygous for the R124C mutation of TGFBI; gray symbols indicate individuals suspected of harboring the mutation on the basis of clinical findings. Direct sequence analysis of exon 4 of TGFBI for the proband, her father, her eldest son, and her husband is shown below the pedigree; the first 3 of these individuals contained both C and T in the first position of codon 124.
The Descemet membrane and endothelial cells appeared intact without associated pathologic features. Congo red staining did not reveal any positive components in the corneal stroma (Figure 4B), and apple-green dichroism in the cornea was not detected by polarized light microscopy (Figure 4C). Mucopolysaccharide deposition was apparent in the anterior portion of the stroma but was restricted to the site of the defect in the Bowman layer, as revealed by staining with toluidine blue (pH 7.1) (Figure 4D). Electron microscopy revealed the presence of separated collagen fibrils in the stroma as well as numerous vacuoles indicative of lipid deposition in keratocytes; again, these vacuoles were apparent only at the site of the defect in the Bowman layer (Figure 4E and F). However, 8-nm to 10-nm nonbranching fibrils were not observed by electron microscopy. These pathological findings were thus not indicative of amyloid deposition. Indeed, the diagnosis provided by our pathologist (M.T.) was not amyloidosis of the cornea but healed corneal ulcer.

**Comment.** We have presented a case of an unusual phenotype associated with the R124C mutation of TGFBI. Although the proband exhibited clinical characteristics similar to those of LCD, amyloid deposition in the corneal stroma was not detected histopathologically. The definition of LCD type I is based on the presence of translucent lines and of amyloid, as revealed by Congo red staining or apple-green dichroism, in the anterior corneal stroma. The R124C mutation of TGFBI has previously been associated with LCD type I. The genetic, clinical, and pathological findings for the patient described here indicate that the phenotype associated with the R124C mutation may be varied.

The proband harbored a heterozygous R124C mutation in TGFBI. Her father and eldest son also harbored the same mutation. Furthermore, 1 of her brothers showed translucent lines and central epithelial opacity on slitlamp examination, findings typical of LCD type I. On the basis of the slitlamp examination of the proband and the pattern of inheritance of this condition in her family, we initially diagnosed her as having LCD type I. However, analysis of the corneal specimen obtained during surgery failed to detect amyloid deposition, precluding a diagnosis of LCD type I. The proband thus manifested an unusual phenotype associated with the R124C mutation of TGFBI.

The clinical characteristics of the patient included autosomal dominant inheritance, glasslike linear stromal opacity, epithelial-subepithelial opacity at the center of the cornea, and a past history of recurrent corneal erosions. On the basis of our clinical observations, we attempted to differentiate this case from gelatinous droplike dystrophy, Avellino dystrophy, and corneal leukoma associated with infec-
tious disease. In the proband, the surface of the corneal epithelium was relatively smooth, and lattice lines were apparent in the stroma. In contrast, in individuals with gelatinous droplike dystrophy, the surface of the corneal epithelium is irregular and bumpy and exhibits protrusions. In patients with Avelino dystrophy, the corneal opacities are isolated, each being circular and 1 to 2 mm in diameter. In contrast, in the present case, the corneal opacity was diffuse. The proband had no memory of a previous serious corneal infection such as herpes, measles, or treponemiasis. Our clinical findings thus excluded these 3 alternative diagnoses.

It is not known why the R124C mutation of TGFBI in the proband did not cause LCD type I. It is possible that an inhibitory mechanism prevented amyloid accumulation in the corneal stroma or that a mechanism for amyloid degradation was operative. Although it is thought that amyloid deposits in tissues do not disappear after they have formed, mouse astrocytes were recently shown to degrade amyloid-β.

In conclusion, we have described a case of an unusual phenotype associated with the R124C mutation of TGFBI. The proband exhibited clinical characteristics of LCD type I but showed no amyloid deposition. The proband also did not appear to suffer from a different type of corneal dystrophy. Although molecular genetics has demonstrated the relation between specific gene mutations and hereditary diseases, extensive clinical and pathological validation is still required to verify that a particular mutation is really responsible for disease pathogenesis.

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Figure 4. Light microscopic (A through D) and electron microscopic (E and F) examinations of the surgically removed cornea of the proband. Hematoxylin-eosin staining, Congo red staining, polarized light microscopy, and toluidine blue (pH 7.1) staining are shown in A through D, respectively. Insets in A and D provide corresponding higher-magnification views.