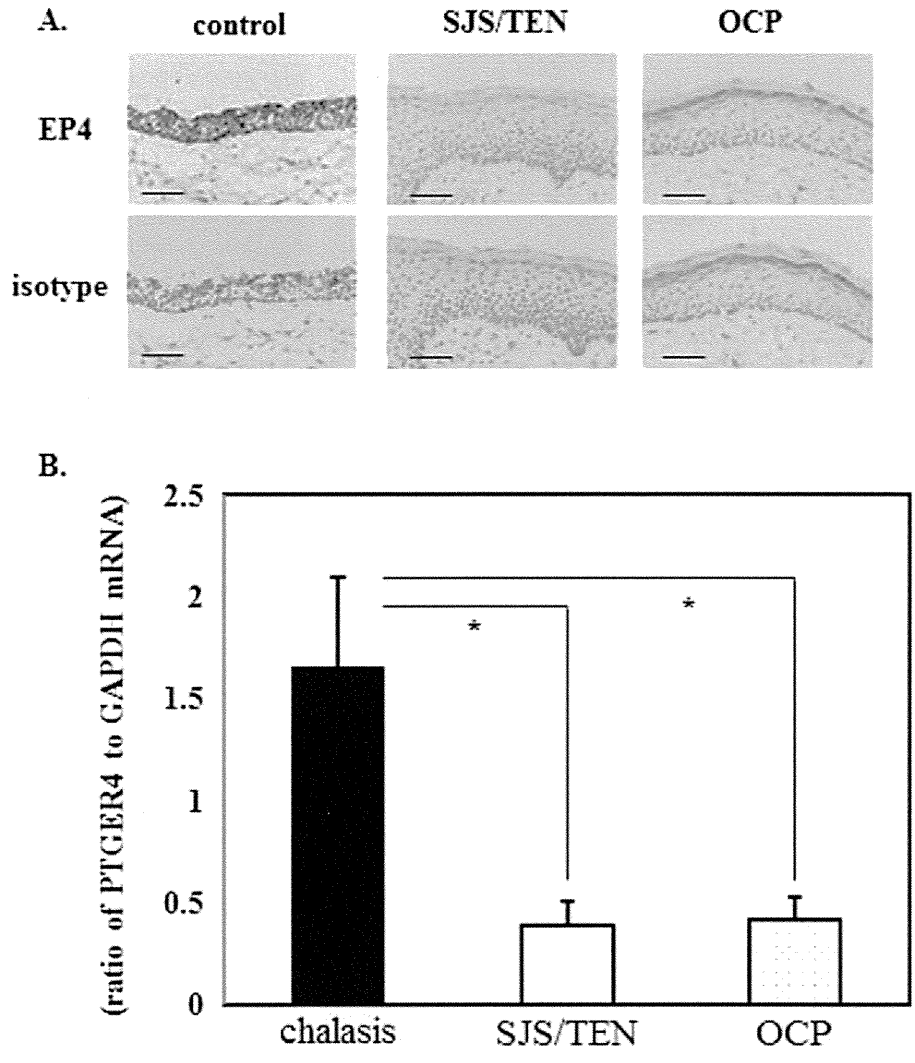

Figure 1 The expression of *PTGER4* mRNA in conjunctival tissues from patients with Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN), ocular cicatricial pemphigoid (OCP) and the controls. (A) Representative findings of EP4 immunoreactivity in each group (control, SJS/TEN, OCP). (B) Expression of *PTGER4* mRNA in human conjunctival tissues (*p<0.05).

in two it was slightly lower than in the control and in the remaining patient it was not detected. There was no EP4 immunoreactivity in conjunctival epithelium from two patients with subacute SJS/TEN (figure 2D), a patient with severe GVHD (figure 2E) as same as patients with chronic SJS/TEN or OCP.[8]

We found that, as in normal human conjunctival epithelium, EP4 is expressed in conjunctival epithelium from patients with chemical eye burn. On the other hand, EP4 immunoreactivity was not detected in conjunctival epithelium from patients with SJS/TEN, OCP or severe GVHD. We did not detect EP4 protein in cells infiltrating subconjunctival tissues in any of the human conjunctival tissues we examined.

DISCUSSION

Elsewhere we reported the expression of EP4 in normal human conjunctival epithelium and its down-regulation in conjunctival epithelium from patients with SJS/TEN and OCP.[8] Here we confirmed that in conjunctival tissues from SJS/TEN and OCP patients its mRNA expression was significantly down-regulated, and we also document that EP4 is expressed normally in conjunctival epithelium from patients with severe chemical eye burn which, like SJS/TEN and OCP, is a devastating ocular surface disorder.

On the ocular surface of patients with severe chemical eye burn, conjunctival invasion into the cornea may occur due to the stem cell deficiency of corneal epithelial cells. This results in devastating ocular surface disorders similar to OCP and SJS/TEN. However, in the conjunctiva of patients with severe chemical eye burns, EP4 expression was not down-regulated.

In patients with Mooren's ulcer, an ocular surface inflammatory disease, the expression of EP4 protein varied; in some patients it was down-regulated. In patients in the subacute stage of SJS/TEN with ocular surface inflammation, the expression of EP4 protein was remarkably down-regulated.

Our results suggest that it is possible that EP4 in conjunctival epithelium might contribute the ocular surface homeostasis, while the EP4 may not necessarily be down-regulated in all devastating ocular surface disorders.

Kabashima *et al*[7] reported that in mice, EP4 deficiency impaired mucosal barrier function and induced

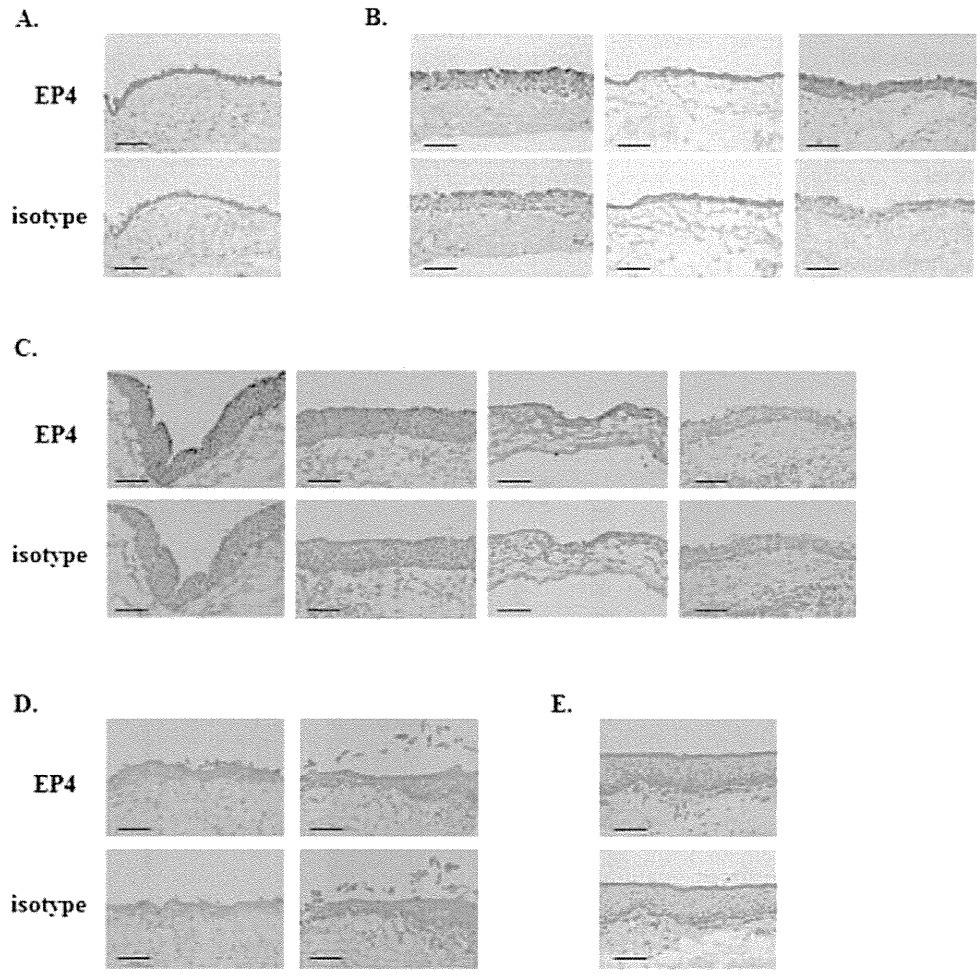

Figure 2 Immunohistological analysis of prostaglandin E receptor subtype EP4 in conjunctival epithelium of patients with ocular surface diseases. (A) Nearly normal conjunctival tissues from patients with conjunctivochalasis. (B) Conjunctival tissues from patients with chemical eye burn requiring ocular surface reconstruction. (C) Inflammatory conjunctival tissues from patients with active Mooren's ulcer requiring resection of the inflammatory conjunctiva. (D) Conjunctival tissues from Stevens-Johnson syndrome/toxic epidermal necrolysis patients in the subacute stage. (E) Conjunctival tissues from a patient with severe graft versus host disease. Each scale bar represents 100 μm.

the aggregation of lymphocytes and neutrophils in the colon, and that the administration of an EP4-selective agonist to wild-type mice ameliorated severe colitis. In mice treated with an EP4-selective antagonist the recovery from colitis was suppressed, leading them to conclude that EP4 maintains intestinal homeostasis by preserving mucosal integrity and down-regulating the immune response. On the other hand, Yao *et al*[9] found that PGE_2 acting on its receptor EP4 on T cells and dendritic cells not only facilitated T helper 1 (T_H1) cell differentiation but also amplified interleukin-23-mediated T_H17-cell expansion in vitro. The administration of an EP4-selective antagonist to mice with experimental autoimmune encephalomyelitis or contact hypersensitivity decreased the accumulation of both T_H1 and T_H17 cells in regional lymph nodes and suppressed disease progression. Based on these observations they concluded that PGE_2-EP4 signalling promotes immune inflammation.

In human conjunctival tissues EP4 protein was expressed in epithelial cells but not in cells infiltrating subconjunctival tissues. We posit that the down-regulation of EP4 in conjunctival epithelium is associated with the ocular surface inflammation seen in patients with OCP, SJS/TEN and Mooren's ulcer.

On the other hand, elsewhere we reported that although EP3 and EP2 agonists suppressed the production of CCL5, CXCL11 and CCL20 in response to polyI:C stimulation, these chemokines were not suppressed by the EP4 agonist in human conjunctival epithelial cells.[5] Studies are underway in our laboratory to elucidate the function of EP4 in conjunctival epithelial cells.

In summary, EP4 is expressed not only in normal conjunctival epithelium but also in conjunctival epithelium from patients with chemical eye burns and some patients with Mooren's ulcer. On the other hand, it is strongly down-regulated in conjunctival epithelium from patients with OCP and chronic SJS/TEN and subacute SJS/TEN.

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Contributors All the authors substantially contributed to the conception and design, acquisition of data, analysis and interpretation of data, drafting the article or revising it critically for important intellectual content and final approval of the version to be published.

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Atypical Continuous Keratitis in a Case of Rheumatoid Arthritis Accompanying Severe Scleritis

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Purpose: Rheumatoid arthritis (RA) often presents with ocular complications: typically dry eye, peripheral corneal ulcer, and scleritis. We report for the first time a case of severe scleritis with RA, accompanying atypical continuous keratitis, which apparently differs from typical peripheral ulcerative keratitis (PUK).

Methods: Observational case report.

Results: A 68-year-old woman with RA presented at our hospital complaining of worsening arthritis accompanying ocular injection and discharge. On examination, nodular scleritis and peripheral corneal infiltration were noted. In addition to administering topical steroid and antibiotics, cyclosporine and an oral steroid were added because of the patient's worsening scleritis. Despite gradual improvement of the scleritis, the efficacy of the additional treatments was limited. Four months after initial treatment, the patient presented with uveitis, thought to be caused by a herpetic virus. Antivirus treatment was effective for the uveitis, but atypical continuous keratitis suddenly appeared. The keratitis was located from 4-o'clock to 10-o'clock positions continuously in the midperipheral cornea and apparently differed from herpetic keratitis or PUK as typically seen in RA cases. Immune reaction was suspected, and the keratitis improved within 2 weeks. After that, the introduction of an anti–tumor necrosis factor α drug (infliximab) completely resolved the severe scleritis and there was no recurrence of ocular inflammation.

Conclusion: As is shown in this case, RA can present with atypical continuous keratitis, thought to be a manifestation of an immunologic reaction other than PUK. In addition, although immunosuppressants are often used for the treatment of RA with scleritis, the efficacy is limited. Infliximab may be a useful treatment for treatment-resistant scleritis.

Key Words: rheumatoid arthritis, scleritis, peripheral ulcerative keratitis, uvetis, infliximab

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Rheumatoid arthritis (RA) is a chronic progressive autoimmune disease characterized by a polyarticular synovitis. RA often presents with ocular complications caused by immunocomplex deposits: typically dry eye, peripheral ulcerative keratitis (PUK), and scleritis. Moreover, RA causes a wide variety of ocular lesions, including uveitis and corneal impairment in the clinical course.[1]

Scleritis is one of the severe ocular manifestations of RA and is associated with systemic inflammation.[2,3] As compared to patients with idiopathic scleritis, RA-associated scleritis is sometimes resistant to treatments.[4] In addition, RA presents with various corneal lesions associated with the occurrence of scleritis.[5,6] PUK is a severe inflammatory disease that characteristically involves the peripheral cornea. The clinical presentation of PUK is characteristically a noninfiltrating ulcer at the periphery of the cornea that is often contiguous with adjacent scleritis.[7]

Conventional treatment of RA consists of the administration of systemic corticosteroids and immunosuppressants. Methotrexate is widely used as the first-line treatment; however, methotrexate monotherapy often fails to reduce or eradicate the inflammation. A combination with other immunosuppressants, such as cyclophosphamide, cyclosporine A, and azathioprine, is often used to lower the disease activity.[4,8] In addition, surgical intervention by keratoepithelioplasty is commonly chosen for treating the severe peripheral corneal ulcer.[9] However, very little published data exist regarding treatments for RA-related ocular inflammation resistant to conventional treatments. Tumor necrosis factor α (TNF-α) is an inflammatory cytokine, and agents blocking its action have proven to be effective in treating RA. Reports are few, but some case studies have reported the effective treatment of severe scleritis.[10,11]

In this present study, we report for the first time a case of severe scleritis with RA accompanying atypical continuous keratitis that apparently differs from typical PUK, which ultimately was effectively treated by the use of an anti-TNF drug.

CASE REPORT

A 68-year-old woman was referred to our hospital presenting with conjunctival injection and ocular discharge in her OD and worsening of arthralgia. She had been diagnosed with RA over the previous 15 years and had been treated with prednisolone (5 mg/d) and methotrexate (6 mg/wk). Her best-corrected visual acuity was 20/32 OD and 20/20 OS. In her OD, nodular scleritis at the upper and lower sclera and peripheral corneal infiltration at the 3-o'clock and 9-o'clock positions were noted (Fig. 1). There were no inflammatory cells in the anterior

chamber and vitreous cavity. A fundus examination showed normal macula, peripheral retina, disk, and vessels in the OD.

The patient was initially treated with topical betamethasone and levofloxacin but was found to be resistant to those treatments. One month after the initial treatment, oral steroids and cyclosporine (50 mg) were added to the treatment. As a result, the scleritis and peripheral corneal infiltration showed gradual improvement, yet the efficacy was limited. Four months after the initial treatment, she presented with anterior uveitis with mutton fat keratic precipitates with pigment and ocular hypertension (Fig. 2). From these findings, an accompanying herpetic uveitis was highly suspected. Thus, antiherpetic drugs were administered and the anterior uveitis responded well and improved immediately. After that, and most interestingly, an atypical continuous keratitis appeared, which apparently differs from typical PUK. The lesion was located from the 4-o'clock to 10-o'clock positions continuously in the midperipheral cornea, with diffuse superficial punctate keratitis (SPK) (Fig. 3), and we continued to administer antivirus drugs and topical steroids. Two weeks later, the atypical keratitis gradually disappeared, yet conjunctival invasion and hurricane keratopathy were temporarily noted. Subsequently, administration of an anti–TNF-α drug (infliximab) was initiated because of the persistence of ocular and systemic inflammation. As a result, both scleritis and infiltrative keratitis were completely improved, and there was no recurrence of ocular diseases (Fig. 4).

DISCUSSION

The ophthalmologic manifestations of RA are dry eye, PUK, scleritis, and other ocular complications.[1] RA also presents with various corneal impairments. It is reported that the scleritis is often accompanied by corneal lesions and that the activity of infiltrative keratitis correlates with that of the scleritis.[5,6] In addition to severe scleritis, this case presented for the first time a unique corneal complication, one that is clearly different from typical PUK.

In the present case, the continuous keratitis suddenly appeared in the midperipheral cornea in the course of treatment for uveitis, thought to be caused by a herpetic virus. Considering the shape and the location of the epithelial lesions, an immunologic mechanism was highly suspected. Although the accompanying diffuse SPK, the subsequent hurricane keratopathy, and the effect of the reduced antivirus drug may support the indication of a drug-induced mechanism, the shape and location of the epithelial lesions were atypical for drug-induced corneal damage. Herpetic keratitis was also suspected, but the SPK surrounding the ulcer and the effect of the reduced antivirus drug were incompatible with that hypothesis.

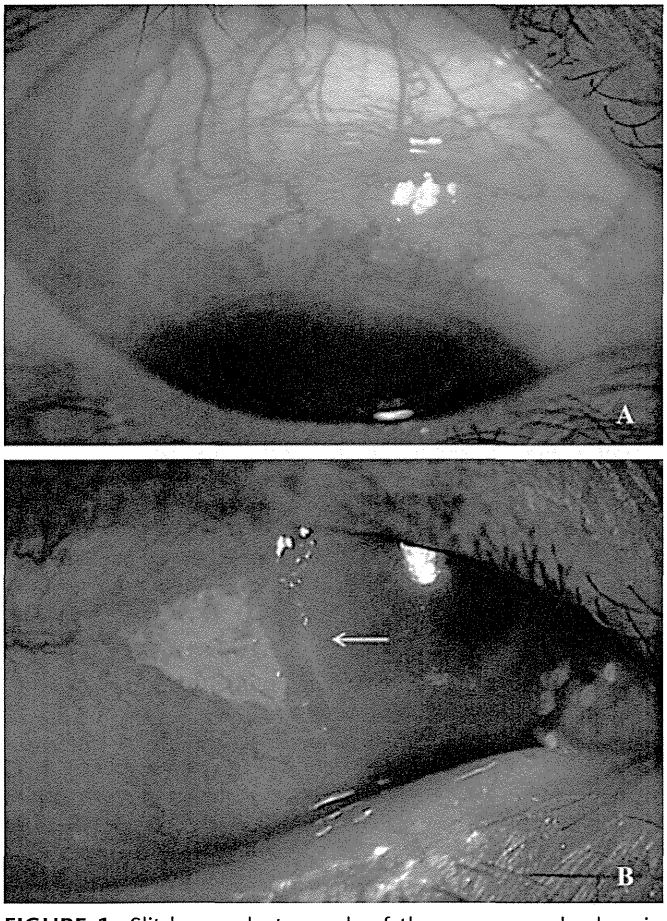

FIGURE 1. Slit-lamp photograph of the cornea and sclera in diffuse illumination showing nodular scleritis at the upper sclera (A) and peripheral corneal infiltration at the 3-o'clock and 9-o'clock positions (white arrow) (B).

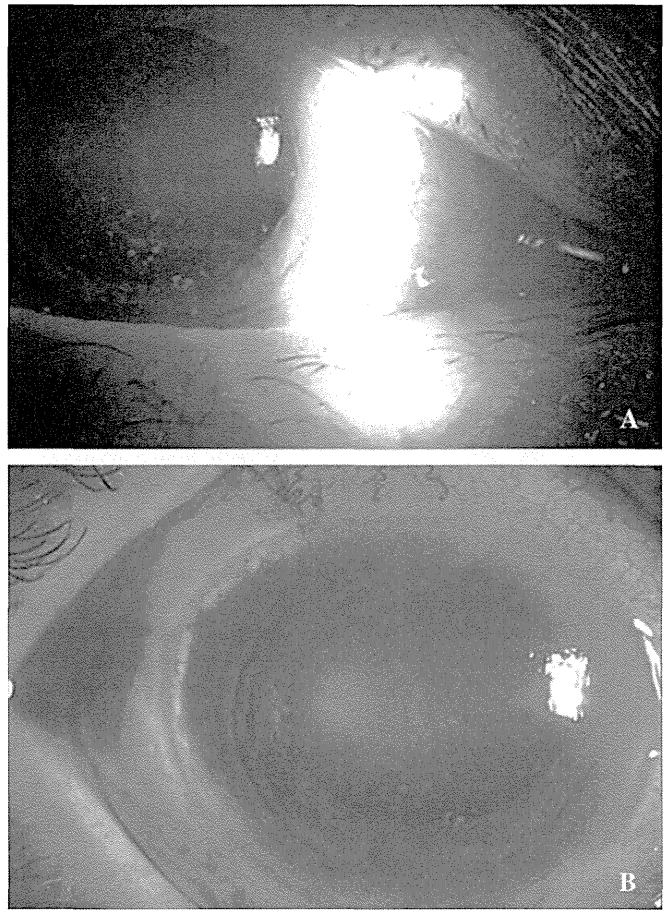

FIGURE 2. A, Slit-lamp photograph of the anterior chamber with scleral scattering showing anterior uveitis with mutton fat keratic precipitates with pigment. B, Slit-lamp photograph of the cornea showing the residual nodular scleritis and peripheral corneal infiltration.

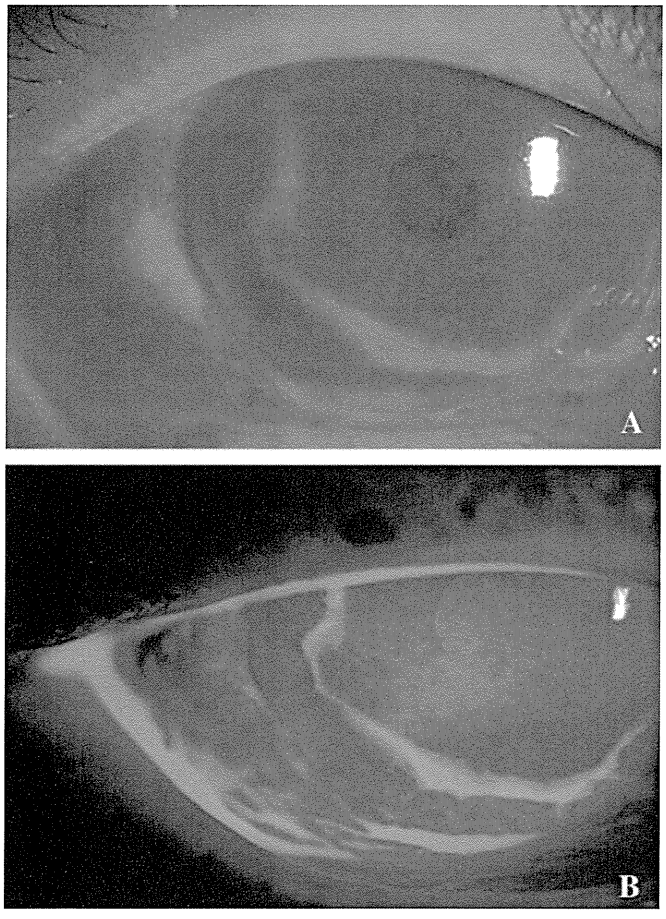

FIGURE 3. A, Slit-lamp photograph of the cornea showing continuous corneal epithelial defect, located from the 4-o'clock to 10-o'clock positions in the midperipheral cornea. B, Slit-lamp photograph of the cornea with fluorescein stain showing diffuse SPK and the corneal epithelial defect.

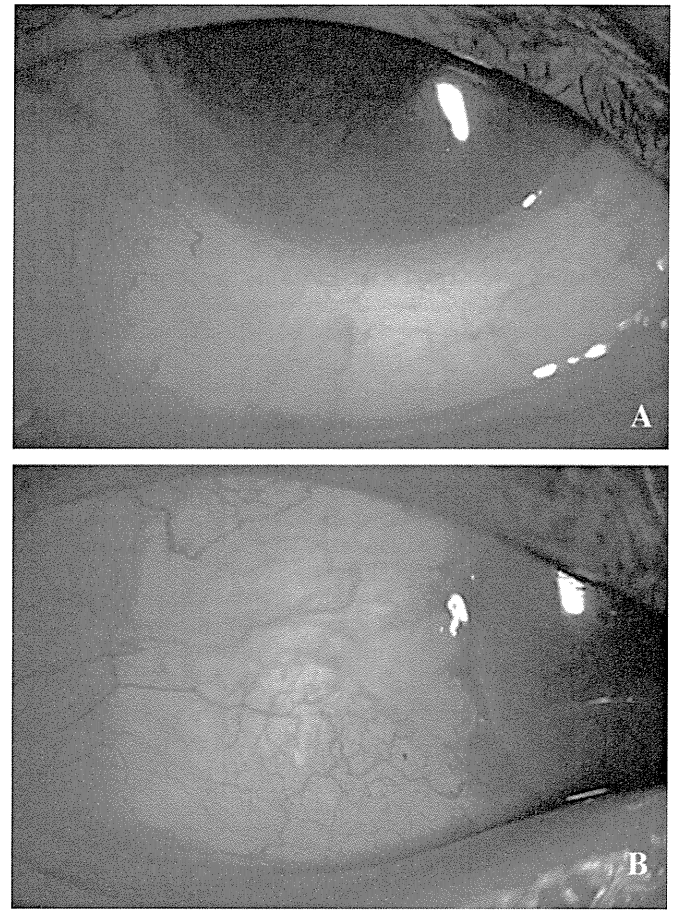

FIGURE 4. A, B, Slit-lamp photograph of the cornea and sclera in diffuse illumination showing the complete improvement of the scleritis and peripheral corneal infiltration after the administration of infliximab.

The standard treatment for RA-associated scleritis is the administration of topical betamethasone, but RA is often unresponsive to topical treatments. Previous reports have shown that cyclosporine is effective for treating severe scleritis; however, side effects that limit the effect of that treatment frequently occurred in elderly patients.[12–14] In the case presented in this study, we added cyclosporine and an oral steroid because of the disease's resistance to topical treatments. The activity of scleritis and peripheral corneal infiltration improved gradually, but the efficacy was limited.

The anti–TNF-α monoclonal antibody infliximab is widely used for the treatment of RA.[15] Previous reports suggested that infliximab is effective for the treatment of ocular inflammation associated with RA, especially refractory scleritis.[10,16] In this present case, infliximab was initiated after the administration of cyclosporine because ocular activity and systemic inflammation remained. Considering the fact that both the scleritis and the peripheral corneal infiltration were completely resolved without any side effects, infliximab may prove to be the optimal treatment option in refractory cases of RA-associated scleritis and corneal ulcer, especially in elderly patients.

In conclusion, RA can present with atypical continuous keratitis that is thought to be caused by an immunologic mechanism, as is shown in this case. The pathophysiology is complicated because of the modification of the disease by its long clinical course and the various drugs that are administered for treatment. In addition, although immunosuppressants are often used for the treatment of RA with severe scleritis, the efficacy of those drugs is limited and side effects can frequently occur. Infliximab could be considered a treatment choice in patients who are found to be resistant to topical or conventional treatments.

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A Study of Host Corneal Endothelial Cells After Non-Descemet Stripping Automated Endothelial Keratoplasty

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Purpose: To determine the short-term fate of the host endothelium and Descemet membrane after non-Descemet stripping automated endothelial keratoplasty (nDSAEK).

Methods: Eight unilateral DSAEK (n = 4) or nDSAEK (n = 4) surgeries were performed in the right eyes of 8 rabbits. Corneal transparency and thickness were followed-up by slit-lamp microscopy, and 2 weeks postoperatively, corneas were evaluated by immunohistochemistry and transmission electron microscopy.

Results: Corneas remained clear after both DSAEK and nDSAEK. One week after DSAEK, the stroma-to-stroma surgical interface was identifiable as a zone of fibrotic tissue a few microns thick, whereas in the nDSAEK group, the recipient corneal endothelium and Descemet membrane were clearly visible at the graft–host interface. The retained endothelial cells were positive for Na^+/K^+-ATPase but assumed a markedly different morphology from healthy endothelial cells, with cell processes extending into the graft stroma or engulfing strands of irregularly dissected grafted stromal tissue where they occasionally appeared to compartmentalize the transplanted matrix and became detached from the underlying Descemet membrane.

Conclusions: Host endothelial cells 2 weeks after nDSAEK express markers of pump function, but appear to be morphologically altered, occasionally detaching from the adjacent Descemet membrane, extending into the graft stroma or engulfing strands of the grafted stroma at the interface. The short-term persistence and subsequent phenotypical alternation of residual endothelial cells, aligned to structural changes to Descemet membrane, might influence graft adherence after nDSAEK.

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Key Words: nDSAEK, DSAEK, host endothelium, adhesion, pump function

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Corneal endothelial disorders such as Fuchs endothelial dystrophy or pseudophakic bullous keratopathy lead to irreversible corneal endothelial dysfunction. Penetrating keratoplasty has been widely performed for the restoration of endothelial function. However, this has several potential adverse effects such as the occurrence of irregular astigmatism, suture-induced problems, and fragility against trauma. Alternative methods for replacing the endothelium have been developed lately and include posterior lamellar keratoplasty, deep lamellar endothelial keratoplasty, Descemet stripping endothelial keratoplasty, Descemet stripping automated endothelial keratoplasty (DSAEK), and Descemet membrane endothelial keratoplasty.[1–4]

Recently, a new modified form of DSAEK has been reported, which has been termed non-Descemet stripping automated endothelial keratoplasty (nDSAEK).[5] This procedure differs from DSAEK in that the host Descemet membrane and endothelium are not surgically removed before the introduction of the posterior lamellar graft. nDSAEK has been shown to be efficient for the treatment of endothelial dysfunction not associated with guttata, with rapid visual recovery and minimal induced astigmatism.[5] In contrast, donor dislocation has been reported after nDSAEK,[5] and subclinical corneal abnormalities have been observed by laser confocal microscopy.[6] We hypothesize that the continued presence of the host endothelium and Descemet membrane may adversely affect the adherence of the posterior graft tissue. The aim of this study was to understand more fully the change of function and anatomy of the residual endothelial cells in the immediate post-nDSAEK period in a rabbit model.

MATERIALS AND METHODS

Surgical Procedure

Japanese white rabbits (female, 9–10 weeks old, 2–3 kg body weight; Shimizu Laboratory Supplies Co, Ltd, Kyoto, Japan) were used as an animal model for corneal endothelial

transplantation and treated in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. The experimental procedures were approved by the Animal Care and Use Committee of Kyoto Prefectural University of Medicine (Approval No. 12-12).

Eight corneas of 4 rabbits, euthanized by injection with pentobarbital sodium (130 mg/kg; Kyoritsu, Tokyo, Japan), were used as donor material, with preparation of donor lenticules immediately preceding the DSAEK or nDSAEK procedures. First, a donor corneoscleral button was placed on a Barron artificial anterior chamber (Katena Products, Denville, NJ), after which saline was infused into the chamber. For each button, a corneal endothelial graft, 100 to 200 μm thick and 8 mm in diameter, was dissected manually using a DALK spatula set (Dutch Ophthalmic Research Center, Zuidland, the Netherlands) and separated by dermal punch using the technique described by Melles et al.[7] These 8 graft tissues were then implanted unilaterally into the right eyes of 8 rabbits as described in the following, 4 using the DSAEK technique and 4 using nDSAEK.

One week before the DSAEK and nDSAEK surgeries, lensectomies and peripheral iridectomies were performed in the right eyes of the 8 rabbits. At surgery, recipient rabbits were anesthetized by intramuscular injection of a mixture of ketamine hydrochloride (70.4 mg/kg; Sankyo, Tokyo, Japan), xylazine (11.8 mg/kg; Bayer, Munich, Germany), and topical oxybuprocaine. Pupils were expanded with topical tropicamide. Surgery was performed on the right eye of each rabbit using the modified technique described by Price et al.[3] First, for DSAEK, a 3-mm limbal-corneal incision was made and the endothelium/Descemet membrane was mechanically scraped using a modified Price-Sinskey hook (ASICO, Westmont, IL) while infusing air into the anterior chamber through a 25-gauge cannula. The scraped area measured at least 9 mm in diameter, and the denuded area was confirmed by 0.04% trypan blue staining during surgery. Descemet membrane scraping was not done in the nDSAEK group. Next, a Busin glide (Moria, Doylestown, PA) was loaded with the donor tissue, oriented so that its endothelial side faced the anterior chamber. This was coated with Viscoat (Alcon, Fort Worth, TX) to protect the cells from physical damage. The graft was pulled into the Busin glide opening, and a 25-gauge anterior capsular forceps (Inami, Tokyo, Japan) that entered the opposite side of the anterior chamber through a small incision was used to grasp the graft. The inserted graft was then attached to the recipient cornea by air injection, and the incision was closed with 10-0 nylon sutures. After surgery, all animals received 0.3% ofloxacin ointment and were maintained with the right side of the face facing downward for 30 to 60 minutes. At days 1, 3, 5, 7, and 14 after surgery, corneal transparency was assessed by the use of a slit-lamp microscope.

Immunohistochemistry

Two weeks after surgery, animals were killed by injection with pentobarbital sodium (130 mg/kg; Kyoritsu), and 4% paraformaldehyde was perfused into the anterior chamber and applied dropwise to the ocular surface for 5 to 10 minutes. Corneoscleral buttons were then excised and immersed in 20% sucrose overnight at 4°C and embedded in optimal cutting temperature compound at −80°C. Sections (10 μm thick) were cut, fixed in 4% formaldehyde for 5 minutes at room temperature, permeabilized for 5 minutes in phosphate-buffered saline (PBS) containing 0.5% Triton X-100, washed, and incubated for 60 minutes with 1% bovine serum albumin. This was followed by overnight incubation at 4°C with 1:100 diluted mouse anti-Na^+/K^+-ATPase antibody (Anti-Na+/K+ ATPase α-1, clone C464-6; Millipore, Billerica, MA) and 3 washes in PBS. Sections were then incubated at room temperature with 1:2000 diluted Alexa Fluor 488–conjugated goat antimouse IgG (Invitrogen Corporation) and washed 3 times. They were subsequently washed with PBS in the dark, mounted on glass slides with antifading mounting medium containing 4′,6-diamidino-2-phenylindole (DAPI) (VECTASHIELD; Vector Laboratories, Burlingame, CA) and inspected with a fluorescence microscope (Olympus, Tokyo, Japan).

Transmission Electron Microscopy

For light and transmission electron microscopy, excised corneas, which had first been perfused in situ for 5 to 10 minutes with 4% paraformaldehyde as was done for the immunohistochemistry preparation, were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M Sorensen buffer, pH 7.2 to 7.4, for 2 to 3 hours at room temperature. After several washes in the buffer and postfixation with 1% osmium tetroxide, they were dehydrated and embedded in Araldite resin. Semithin sections (1 μm thick) were stained with toluidine blue for inspection at the light microscope level, whereas ultrathin sections (~90 nm thick) were collected on uncoated copper grids for study by transmission electron microscopy. Contrasting of ultrathin sections was with phosphotungstic acid and aqueous uranyl acetate (1 and 12 minutes, respectively), followed by Reynolds lead citrate (5 minutes) with washes in between. Stained sections were examined in a JEOL 1010 transmission electron microscope (JEOL, Tokyo, Japan), equipped with a Gatan ORIUS SC1000 CCD camera.

RESULTS

Slit-Lamp Microscopy

Slit-lamp examinations of the 8 operated eyes revealed that the corneas were clear in both the nDSAEK and the DSAEK groups at postoperative day 7 (Fig. 1). In the nDSAEK group, 1 lamellar graft had become dislocated the day after surgery, but it readhered to the center of the cornea after the injection of air into the anterior chamber. Slit-lamp examination revealed that graft adhesion was good in both groups. However, after nDSAEK, folds appeared and became progressively more noticeable in number and size at the graft edge in all 4 cases (Fig. 2).

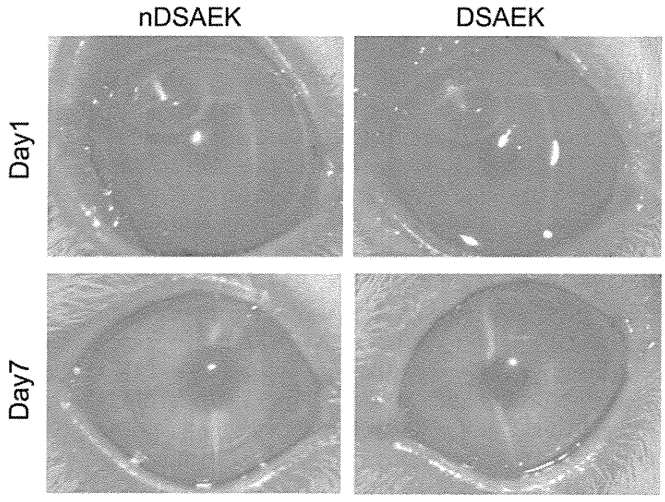

FIGURE 1. Slit-lamp photographs 1 and 7 days after surgery, indicating cloudiness at 1 day with a return to clarity by 7 days after both DSAEK and nDSAEK.

Immunohistochemistry

Histological sections of corneas healing after DSAEK indicated a well-adhered graft with a thin and fairly indistinct stroma-to-stroma interface (Fig. 3). Histology of post-nDSAEK tissue, in contrast, showed the clear retention of residual Descemet membrane and endothelium at the surgical interface, sandwiched between host stroma and graft stroma (Fig. 3). The recipient corneal endothelial cells retained in the cornea after nDSAEK were also identified on histochemistry by the nuclear stain, DAPI, which revealed a line of positive cells at the junction between the host and graft tissues 14 days after surgery (Fig. 4). Immunohistochemistry, moreover, suggested that the sandwiched host endothelial cells retained a lot of cellular function because they were positive for Na^+/K^+-ATPase protein (Fig. 4).

Transmission Electron Microscopy

The lamellar graft in nDSAEK adhered well to the residual endothelium/Descemet layer; nevertheless, at the electron microscopic level, distinct phenotypic and morphological changes in the endothelial cells and Descemet membrane were evident (Fig. 5A, B). Notably, the endothelial cells often appeared to extend cell processes into the adjacent grafted stroma, resulting in partial compartmentalization of the matrix, or to engulf loose strands on the irregular surface of grafted stroma, which might include some phagocytic activity (Figs. 5A, B). Duplication of Descemet membrane was also seen, with the membrane present at the apical and basal sides of the host endothelium (Fig. 6A). Residual endothelial cells also sometimes became detached from the host Descemet membrane, with a layer of stromal tissue that resembled fibrotic tissue lying between the cells and Descemet membrane (Fig. 6B). In these areas of graft–host interface, portions of duplicated, or split and invaded, Descemet membrane could also often be seen in close association with the detached host endothelium (Fig. 6B).

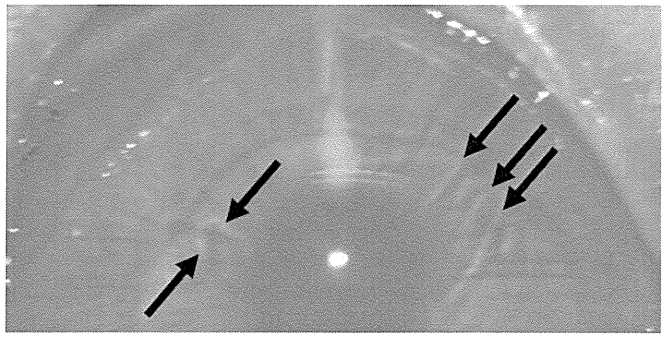

FIGURE 2. A slit-lamp photograph of a representative cornea in the nDSAEK group, 7 days after surgery. The folds seen at the edge of the graft (arrows) are typical of all 4 nDSAEK surgeries and increased in number and size throughout the observation period.

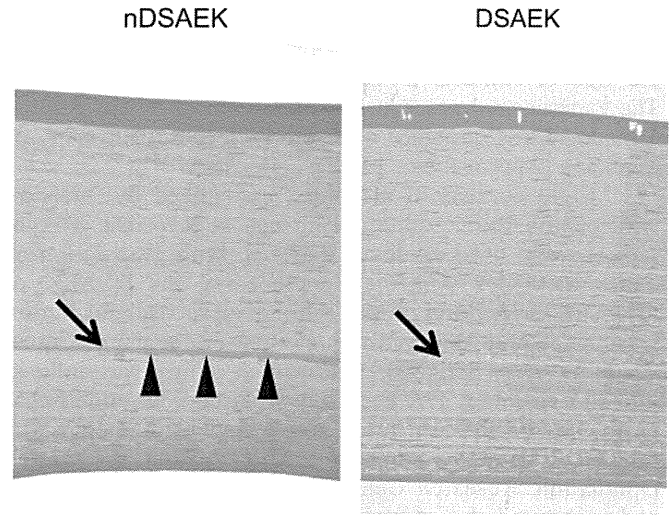

FIGURE 3. Light micrographs of DSAEK and nDSAEK corneas 7 days after surgery taken from semithin sections of resin-embedded tissue stained with 1% toluidine blue. The graft–host interface is indicated by an arrow, with the host Descemet membrane and endothelium clearly visible in nDSAEK sections (arrowheads). Bar = 50 μm.

DISCUSSION

nDSAEK is a contemporary modification of the gamut of endothelial keratoplasties, and although clinical outcomes are promising, there is a dearth of fundamental knowledge about the postoperative fate of the residual endothelium and Descemet membrane. This knowledge is important because it is suspected that the good adherence of the graft interface after DSAEK—where the host endothelium and Descemet membrane are removed—is, much like after laser in situ keratomileusis, a result of direct stroma-to-stroma contact, and we hypothesize that the retained endothelial cells and Descemet membrane might have some effect on this adhesion.

Our investigations reveal that, 2 weeks after nDSAEK, endothelial cells persist at the boundary between host and graft and also express Na^+/K^+-ATPase protein. Often, these

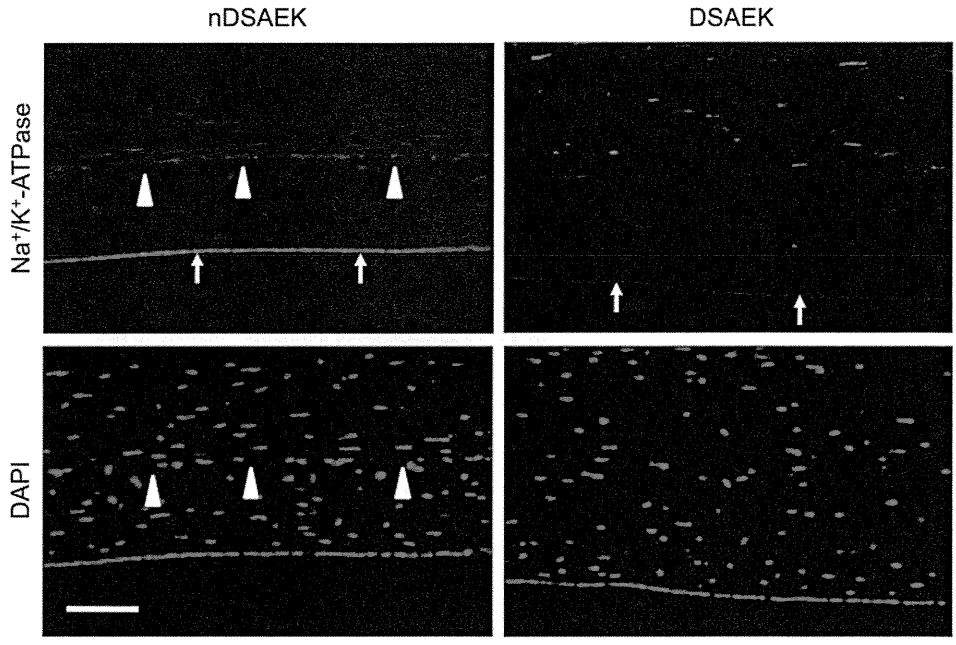

FIGURE 4. Function-related protein expression in the cornea in nDSAEK and DSAEK, 14 days after surgery. Some unexplained focal positivity was seen throughout the stroma in all corneas studied, and in DSAEK and nDSAEK corneas, a single layer of immunolabeled donor endothelial cells were observed with Na^+/K^+-ATPase (arrows). The graft–host interface was not identifiable by immunohistochemistry in DSAEK corneas, but in nDSAEK corneas, the retained monolayer of host corneal endothelial cells expressed Na^+/K^+-ATPase. These were also stained with the nuclear stain, DAPI, which formed a discontinuous line at the graft–host interface (arrowheads). Bar = 100 μm.

cells are closely apposed to the residual host Descemet membrane and form a monolayer not unlike that in a healthy endothelium. On occasion, however, the endothelial cells assume an abnormal phenotype, with cell processes extending into the graft stroma or engulfing loose strands of the dissected stromal matrix. Deposition of fibrotic tissue between the host endothelium and residual Descemet membrane was also found, and we speculate that phenotypic changes in the host endothelium represent the early stages of cellular transformation under stress[8,9] or some phagocytic activity of the endothelial cells. In the normal adult cornea, the endothelium lays down collagen type IV at its distal surface, which constitutes the posterior nonbanded portion of Descemet membrane. It might be the case that the endothelium deposits collagen type IV at its proximal surface also, only for this to be removed by the fluid dynamics of the aqueous humor. If this is so, it might explain the presence of duplicated basement membrane–like material adjacent to the proximal side of the host endothelium.

Immunohistochemistry indicates that retained endothelial cells 2 weeks after nDSAEK display a partial endothelial phenotype as evidenced by Na^+/K^+-ATPase protein expression. This leads us to consider the potential metabolic response of the sandwiched endothelial cells and the likelihood of any pump function. The corneal stroma will swell rapidly if bathed in an aqueous medium,[10] a phenomenon that

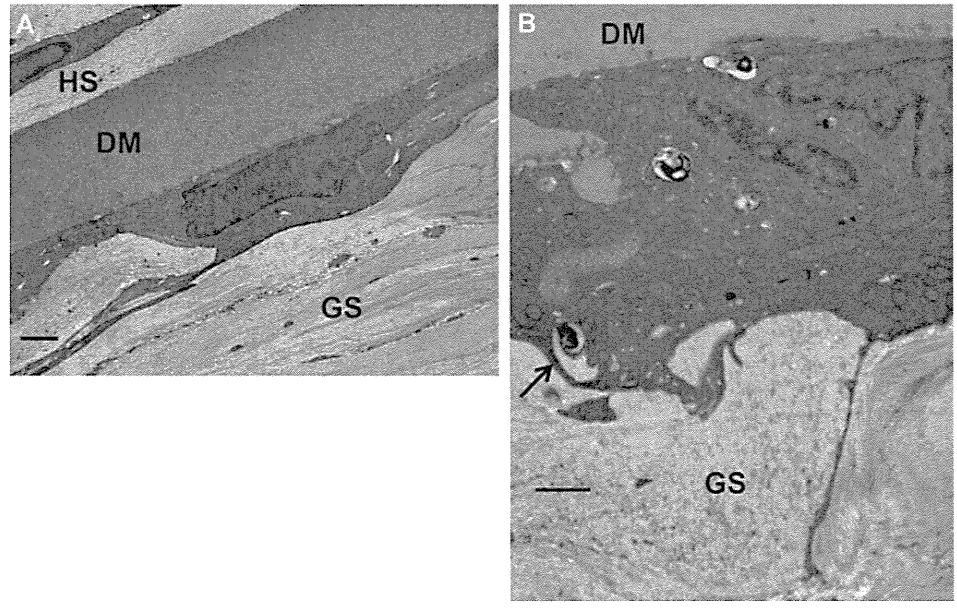

FIGURE 5. A, An electron micrograph of residual host Descemet membrane (DM) and a well-adhered endothelium after nDSAEK. Bar = 2.5 μm. B, An electron micrograph of residual host DM and endothelium (arrow) after nDSAEK. Bar = 500 nm. Endothelial cells appear to extend into graft stroma or engulf loose connective tissue strands on the surface of the transplanted tissue. GS, graft stroma; HS, host stroma.

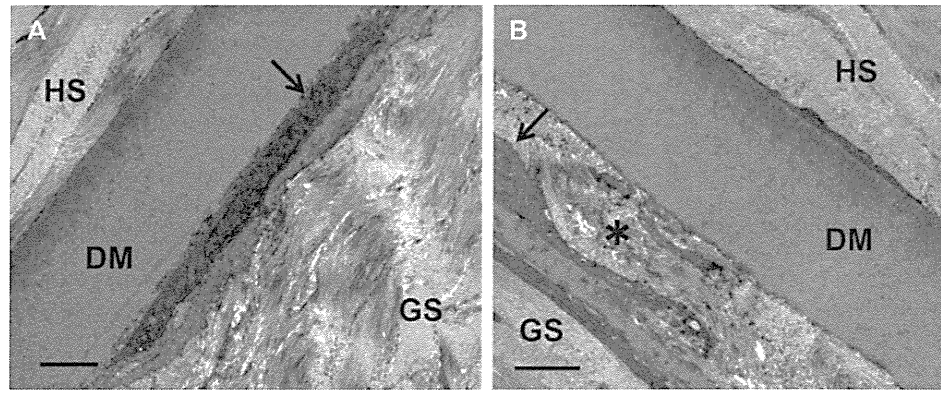

FIGURE 6. A, An electron micrograph of residual host Descemet membrane (DM) and a well-adhered endothelium (arrow) sandwiched between host stroma (HS) and graft stroma (GS) after nDSAEK. Bar = 2 μm. B, An electron micrograph of residual host DM and endothelium sandwiched between HS and GS after nDSAEK. The endothelium (arrow) has become detached from DM, and a fibrotic stromal matrix (indicated by an asterisk) exists between the endothelium and DM. Bar = 2 μm.

is due predominantly to the presence of collagen-bound sulfated proteoglycans[11] and chloride anions that are electrostatically associated with the matrix in a transient manner.[12,13] This swelling tendency is neutralized in the physiologically normal cornea by the continuous removal of bicarbonate ions from the corneal stroma by the metabolically active endothelial pump.[14] The presence of a monolayer of residual host endothelial cells after nDSAEK, aligned with immunohistochemical evidence of Na^+/K^+-ATPase protein expression, prompts us to consider the likelihood of a dual bicarbonate pump in these corneas, with functionality in both the new grafted endothelium and the trapped host endothelium. Physiological reasoning and a consideration of most of the key aspects of endothelial metabolism indicate no reason why the sandwiched endothelium in the early stages after nDSAEK could not function as an active bicarbonate pump. However, we are reminded of a series of experiments (S. A. Hodson, PhD, personal communication, August 2011), which indicated that endothelial pump function examined in vitro was stopped when the circulation of fluid bathing the apical face of the endothelium was temporarily ceased, only to start up again once fluid circulation was resumed. This was attributed to the nonremoval and consequent build up of lactic acid near the endothelium, something that would likely occur in the graft stroma adjacent to the residual endothelium in nDSAEK. Based on this reasoning, aligned to the longer term morphological changes and transformation of the trapped endothelial cells in nDSAEK, we suspect little if any functionality in the retained host endothelium.

nDSAEK is a useful surgery that can lead to favorable clinical outcomes, as has been reported by other investigators. It appears that in the early postoperative period, in rabbits at least, healing is accompanied by the close apposition between graft stroma and the apical surface of host endothelial cells that retain some pump function. We also note that the series of phenomena reported here may not be restricted to nDSAEK but might occur in DSAEK too at the corneal periphery if the lateral extent of the graft material overlaps a smaller debrided area.

ACKNOWLEDGMENT

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Clinical Outcomes of Phototherapeutic Keratectomy in Eyes With Thiel-Behnke Corneal Dystrophy

OSAMU HIEDA, SATOSHI KAWASAKI, KOUICHI WAKIMASU, KENTA YAMASAKI, TSUTOMU INATOMI, AND SHIGERU KINOSHITA

• PURPOSE: To investigate the functional and morphologic midterm outcome of phototherapeutic keratectomy (PTK) for Thiel-Behnke corneal dystrophy diagnosed by gene-mutation analysis.

• DESIGN: Retrospective, single-center clinical study.

• METHODS: Between July 2001 and May 2010, 10 consecutive PTKs were performed in 10 eyes of 5 patients (2 male, 3 female; mean age: 55 ± 13 years) with superficially accentuated opacities caused by Thiel-Behnke corneal dystrophy and were followed up for at least 12 months (range: 12–108 months). Main outcome measures included (1) best-corrected visual acuity (BCVA), (2) uncorrected visual acuity (UCVA), (3) spherical equivalent, and (4) recurrence rate. The probability of recurrence of Thiel-Behnke corneal dystrophy after PTK was calculated using the Kaplan-Meier method for survival analysis.

• RESULTS: The p.Arg555Gln mutation was found within the *TGFBI* gene in all 5 patients. Average logarithm of minimal angle of resolution (logMAR) BCVA change was −0.55 ± 0.26. Average logarithm UCVA change was −0.54 ± 0.31. In 5 of the 10 eyes, recurrence of central superficial opacification was clinically identified during the follow-up periods, and in 4 of those 5 eyes, the level of the recurrence was so significant that the visual acuity was reduced more than 2 lines. The maximum follow-up period of the 1 eye without significant post-PTK recurrence was 108 months.

• CONCLUSIONS: PTK is a successful therapy for Thiel-Behnke corneal dystrophy, and results in midterm stable visual acuity and corneal transparency. Unlike in Reis-Bücklers corneal dystrophy cases, PTK delays the need for more invasive surgical intervention in Thiel-Behnke corneal dystrophy. (Am J Ophthalmol 2013;155: 66–72. © 2013 by Elsevier Inc. All rights reserved.)

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THIEL-BEHNKE CORNEAL DYSTROPHY,[1] ALSO KNOWN as "honeycomb" corneal dystrophy,[2] is an autosomal dominant inheritable disease and was described as corneal dystrophy of the Bowman layer and superficial stroma type II (CDB II, OMIM#602082) in a report by Küchle and associates.[3] Recent molecular biological analysis has revealed that this dystrophy is caused by the missense mutation (p.Arg555Gln) of the human transforming growth factor beta–induced (*TGFBI*) gene.[2,4–7]

Characteristic bilateral, subepithelial corneal opacities, frequently accompanied by recurrent corneal erosions, normally appear in Thiel-Behnke corneal dystrophy patients between the ages of 10 and 20 years. This disease runs a slow progressive course, with painful erosive episodes and gradual deterioration of vision.[2–4] The treatment modalities for this disease include superficial keratectomy, lamellar keratoplasty,[3] penetrating keratoplasty (PKP),[8] and phototherapeutic keratectomy (PTK).[9–12]

In Japan, the Ministry of Health, Labour and Welfare approved the medical use of 193-nm argon-fluoride excimer laser devices for PTK procedures in 2000. Since then, PTK has been applied for the treatment of various types of corneal diseases, including inheritable corneal dystrophies,[2,12–17] band keratopathy,[10,18–20] recurrent corneal erosion,[15,21,22] certain types of degenerative corneal diseases (eg, Salzmann's degeneration),[10,20,23] and bullous keratopathy.[24,25] The PTK procedure is generally thought to produce the best results when it is used for the ablation of corneal opacity restricted to the anterior stroma of the cornea. When PTK is performed on patients who have passed strict diagnostic criteria, the satisfaction level in relation to the results of this procedure is reportedly very high.[26]

The purpose of this retrospective, single-center clinical study was to investigate the functional and morphologic midterm outcome of PTK performed in multiple Thiel-Behnke corneal dystrophy patients who were strictly diagnosed through the use of gene mutation analysis.

METHODS

• STUDY POPULATION: This study involved 10 consecutive PTKs performed in 10 eyes (5 right eyes and 5 left eyes) of 5 patients (2 male and 3 female) to treat superficially accentuated opacities that were clinically and

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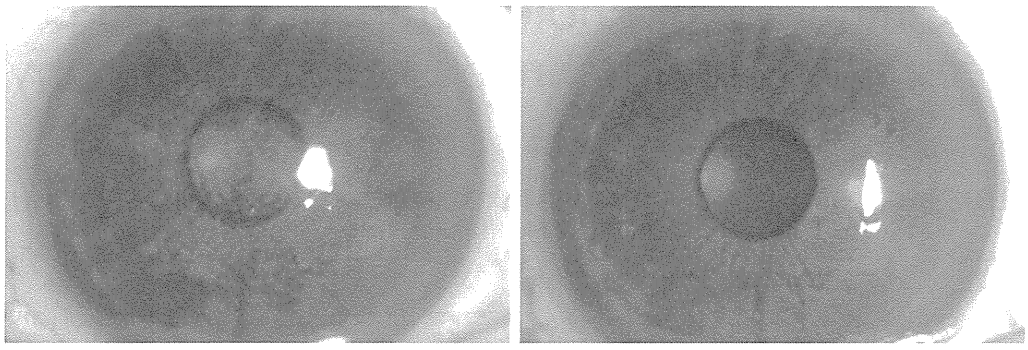

FIGURE 1. Slit-lamp microscopy images of the left eye of a 57-year-old woman with Thiel-Behnke corneal dystrophy (Patient 1) (Left) before and (Right) 1 month after undergoing phototherapeutic keratectomy surgery. Note that the superficial corneal opacities visible around the axial area of the cornea in the left-hand image (presurgery) are no longer visible in the right-hand image (postsurgery).

TABLE. Summarized Clinical Information of Eyes With Thiel-Behnke Corneal Dystrophy Before and After Phototherapeutic Keratectomy

				BCVA (logMAR)			(Month)	
Patient[a]	Age (y)	Sex	Eye	Preoperative	Postoperative[b]	Calculated Ablation (μm)	T1	T2
1	57	F	R	0.52	−0.18	113	1	108
1	65	F	L	0.70	0.10	100	12	12
2	32	M	R	0.22	−0.08	100	3	108
2	32	M	L	0.15	−0.08	110	3	108
3	57	F	R	0.52	0.05	114	24	96
3	57	F	L	1.22	0.15	120	3	96
4	66	M	R	0.70	−0.08	120	12	18
4	66	M	L	0.70	0.22	121	12	18
5	61	F	R	0.52	0.15	140	6	18
5	61	F	L	0.52	0.10	125	3	18

BCVA = best-corrected visual acuity; F = female; L = left; logMAR = logarithm of minimal angle of resolution; M = male; R = right; T1 = mean time before achieving the best overall BCVA after surgery; T2 = follow-up period.

[a]Patients 2, 3, and 5 were blood relatives.

[b]Postoperative BCVA denotes the best overall BCVA after surgery.

genetically diagnosed as Thiel-Behnke corneal dystrophy between July 13, 2001 and May 14, 2010. Only the patients who were followed up for at least 12 months after the PTK surgery were enrolled in this study. The mean age of the patients was 55 ± 13 years (range: 32–66 years). None of the enrolled patients had any previous history of corneal surgery. The PTK surgery was performed for the patient's visual rehabilitation at the time when the patient complained of decreased vision or when the patient's best-corrected visual acuity (BCVA) had become worse than logMAR 0.15. Each of the 5 patients had experienced painful erosive episodes prior to undergoing the surgery. Three of the 5 patients were members of the same pedigree.

• MOLECULAR ANALYSIS: Peripheral blood samples were collected from all 5 patients after they had received a complete, detailed explanation of the study protocols. DNA was extracted from the peripheral blood lymphocytes using a commercially available kit (DNeasy Blood & Tissue Kit; QIAGEN GmbH, Hilden, Germany). Exons 4, 11, 12, and 13 of the *TGFBI* gene, as well as their flanking introns, were amplified by polymerase chain reaction (PCR) and directly sequenced on both strands using previously published primers.[27]

• INTERVENTIONAL PROCEDURE: PTK was performed by the use of 1 of 3 commercially available 193-nm excimer laser devices, each produced by a different company. Five eyes were treated using the EC-5000 excimer laser (NIDEK Co Ltd, Gamagori, Japan), 3 eyes were treated using the VISX S4IR excimer laser (Abbott Medical Optics Inc, Abbot Park, Illinois, USA), and the remaining 2 eyes were treated using the Technolas T-217z Zyoptix laser system (Bausch & Lomb, Rochester, New York, USA). In all 10 eyes, the epithelium was removed directly by the excimer laser, and the ablation continued

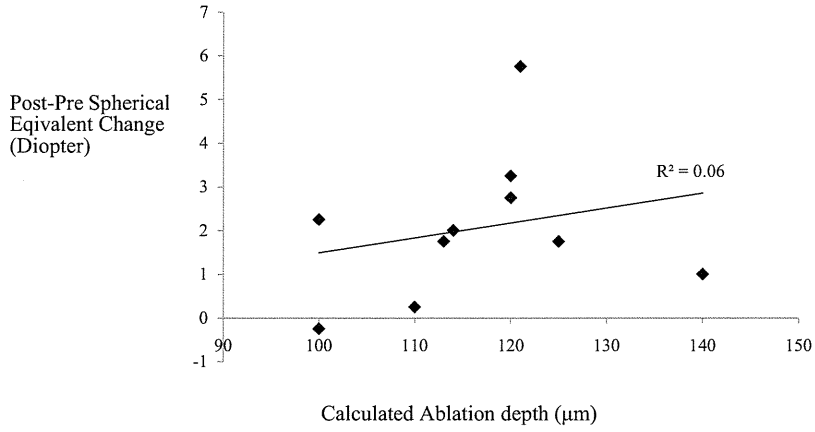

FIGURE 2. A scattergram demonstrating the relationship between the spherical equivalent changes (between preoperative and postoperative) and the calculated ablation depth of phototherapeutic keratectomy surgery in eyes with Thiel-Behnke corneal dystrophy. Although the spherical equivalent change seems to have a tendency to increase with the increase of ablation depth, no statistically significant ($R^2 = 0.06$) association was found between the spherical equivalent changes and the calculated ablation depth.

into the corneal stroma until approximately 50 μm of the stromal ablation was performed. During the surgery, the cornea of each eye was examined by use of the microscope equipped in the excimer laser devices under sclerotic scattering illumination using a vitrectomy-endoilluminator placed on the limbus.[28] When deemed necessary, additional ablations were performed to remove the bulk of the pathologic opacity from the visual axis. For all 10 eyes, the ablation was performed until the majority of opacities were removed, thus resulting in a mean calculated total ablation depth (including epithelium and stroma) of 116 ± 12 μm (range: 100–140 μm). Masking fluid was not used. Postoperatively, all 5 patients were initially administrated 0.1% fluorometholone (FLUMETHOLON; Santen Pharmaceutical Co, Ltd, Osaka, Japan) and 1.5% levofloxacin hydrate (CRAVIT; Santen Pharmaceutical Co, Ltd) 4 times daily, with a tapering-off of the dosage over 12 weeks. Each patient was instructed to continually wear a soft contact lens on the operated cornea until the epithelial defect had closed.

• MAIN OUTCOME MEASURES: In this present study, main outcome measures including BCVA, uncorrected visual acuity (UCVA), spherical equivalent (SE), and recurrence of Thiel-Behnke corneal dystrophy were assessed.

• CLINICAL DEFINITION FOR RECURRENCE OF THIEL-BEHNKE CORNEAL DYSTORPHY POST–PHOTOTHERAPEUTIC KERATECTOMY: The recurrence of Thiel-Behnke corneal dystrophy was considered significant when slit-lamp examination showed signs of increased central opacification of the superficial cornea that were also associated with significant visual loss (a 2-line or more loss of BCVA) according to the previous study.[15] The probability of recurrence of Thiel-Behnke corneal dystrophy after PTK surgery was calculated using the Kaplan-Meier method for survival analysis.[12,14,15,17]

• STATISTICAL ANALYSIS: For analysis of the results, Excel Tokei 2002 statistics software (SSRI Co Ltd, Tokyo, Japan) was used. Differences between paired samples were analyzed with the paired *t* test. A probability value of $<.05$ was considered statistically significant.

RESULTS

THE P.ARG555GLN MUTATION WAS FOUND WITHIN THE *TGFBI* gene in all 5 patients. In all 5 patients, the superficial corneal opacities were successfully removed from the corneal visual axis (Figure 1). In all 10 eyes, epithelial defects closed within 3 to 5 days and visual acuity gradually improved in 3 to 24 months after the surgery (Table.). The mean follow-up period was 60 ± 46 months (range: 12–108 months). Postoperatively, all 5 patients requested to have PTK surgery performed to their contralateral eyes, thus indicating that they were satisfied with the results of the initial PTK surgery.

A 2-line or more increase in BCVA was found in all of the 10 enrolled eyes after the PTK surgery. The mean logMAR BCVA improved from 0.57 ± 0.29 preoperatively to the overall best of 0.04 ± 0.13 postoperatively, which was statistically significant ($P < .001$). The mean UCVA also significantly improved, from logMAR 0.84 ± 0.34 preoperatively to the overall best of logMAR 0.30 ± 0.26 postoperatively ($P < .01$). The average logMAR UCVA change was −0.54 ± 0.31.

The mean SE significantly increased from −1.63 ± 2.74 diopters (D) preoperatively to 0.43 ± 2.13 D postoperatively, which was statistically significant ($P < .01$). The

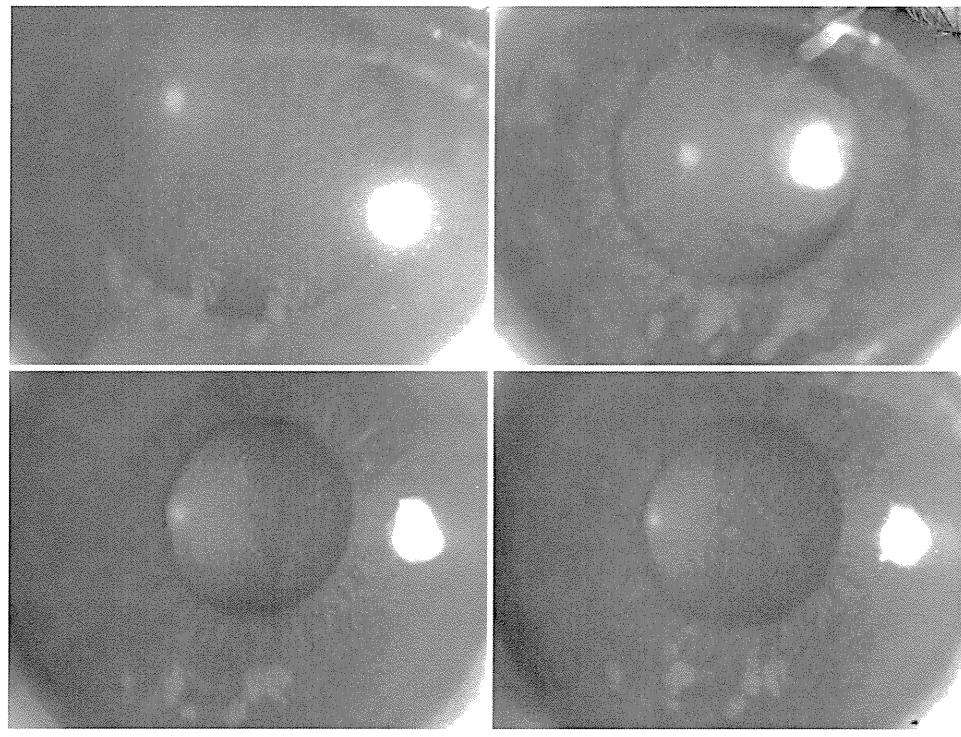

FIGURE 3. Slit-lamp microscopy images of the right eye of 32-year-old man with Thiel-Behnke corneal dystrophy (Patient 2) (Top left) before phototherapeutic keratectomy, (Top right), 3 years after phototherapeutic keratectomy, (Bottom left) 5 years after phototherapeutic keratectomy, and (Bottom right) 8.5 years after phototherapeutic keratectomy. The degree of the opacification at the final follow-up visit (Bottom right) is almost the same as that at 3 years after the phototherapeutic keratectomy surgery (Top right).

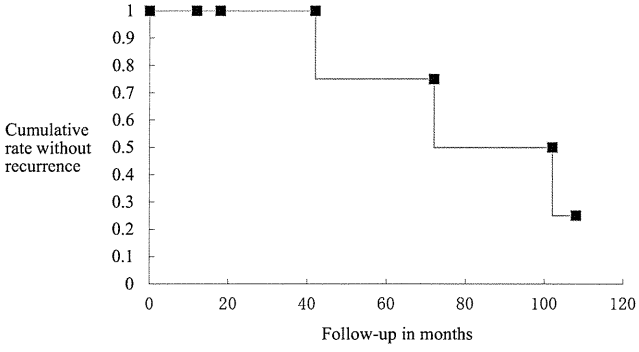

FIGURE 4. Line chart demonstrating the cumulative survival rate for the recurrence of Thiel-Behnke corneal dystrophy after phototherapeutic keratectomy analyzed by Kaplan-Meier survival analysis. The recurrence of Thiel-Behnke corneal dystrophy was defined by significant visual decrease with a 2-line or more loss of best-corrected visual acuity (BCVA) caused by the increased corneal opacification resulting from Thiel-Behnke corneal dystrophy.

average refractive change was +2.05 ± 1.68 D (range: −0.25 to +5.75 D). There was no apparent difference in the SE changes in relation to the type of excimer laser device used or to the calculated ablation depths (Figure 2).

Postoperative complications such as infection, delay in epithelial healing, or stromal haze were not noticed in any of the 10 eyes, and none of the patients experienced any postoperative painful erosive episode. During the follow-up period, 5 of the 10 eyes experienced recurrence of the central superficial opacification. One of those 5 eyes (the right eye of Patient 2) had only a 1-line decrease of visual acuity at 108 months postoperatively (Figure 3). However, in 4 of those 5 eyes, the degree of recurrence was significant enough to lead to a decrease in visual acuity of more than 2 lines, yet none of those patients requested a PTK reoperation at their final follow-up visit. The earliest significant recurrence (the right eye of Patient 3) was observed after 42 months (Figures 4 and 5). The remaining 5 of the 10 eyes exhibited no apparent signs of recurrence, possibly because the follow-up period for those eyes was not very long.

DISCUSSION

IN THE PRESENT STUDY, PTK SURGERY FOR THIEL-BEHNKE corneal dystrophy was found to result in the significant midterm improvement of visual acuity for all patients. During the follow-up period, a gradual recurrence of central superficial opacification was observed, yet the level of the opacification was not severe enough to lead to a significant decrease in visual acuity for at least 42 months after surgery.

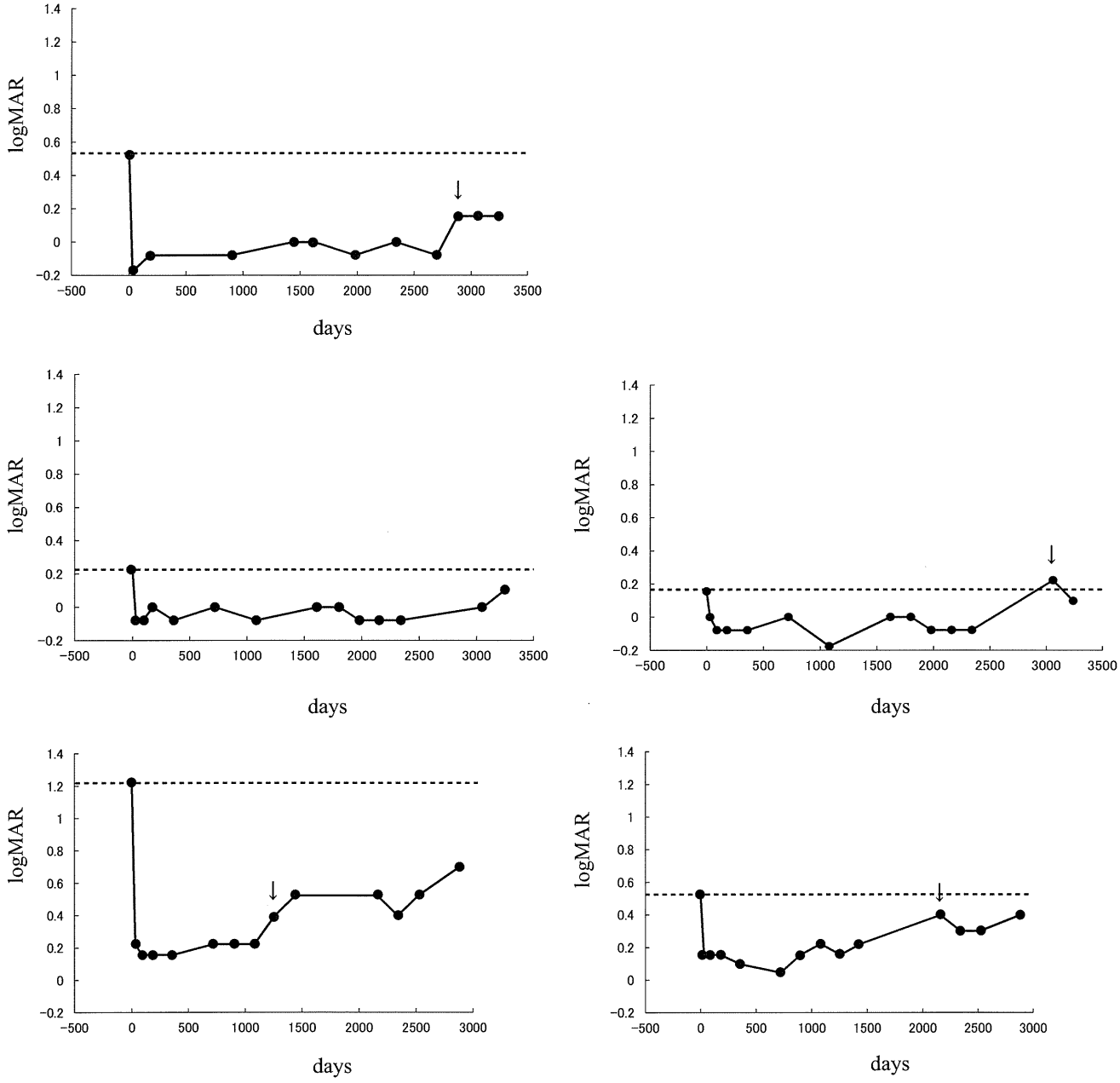

FIGURE 5. Polygonal line graphs denoting the time course of logMAR best-corrected visual acuity (BCVA) in 5 eyes of 3 Thiel-Behnke corneal dystrophy patients followed up for over 96 months after phototherapeutic keratectomy surgery. (Top) Right eye of a 57-year-old woman (Patient 1). (Middle left) Right eye of a 32-year-old man (Patient 2). (Middle right) Left eye of a 32-year-old man (Patient 2). (Bottom left) Right eye of a 57-year-old woman (Patient 3). (Bottom right) Left eye of a 57-year-old woman (Patient 3). Solid circles indicate the time points of the patients' follow-up visits. Arrows indicate the time points when significant recurrences were observed. Broken lines indicate the preoperative logMAR best-corrected visual acuity (BCVA). The recurrence of Thiel-Behnke corneal dystrophy was defined by significant visual decrease with a 2-line or more loss of BCVA caused by increased corneal opacification resulting from Thiel-Behnke corneal dystrophy. logMAR = logarithm of minimal angle of resolution.

To date, 3 previous studies have reported a successful clinical outcome in Thiel-Behnke corneal dystrophy patients after undergoing PTK surgery. The first study enrolled 6 eyes of 5 patients who were diagnosed with Thiel-Behnke corneal dystrophy just from the clinical appearance of their corneas and not by genetic analysis.[9] The second study enrolled 8 eyes of 4 patients who were clinically diagnosed with Thiel-Behnke corneal dystrophy but who had a genomic linkage to chromosome 10, which is therefore thought to be a distinct pathologic entity of the typical Thiel-Behnke corneal dystrophy bearing the p.Arg555Gln mutation within the *TGFBI* gene.[11] The third study enrolled 1 eye of 1 patient who had the p.Arg555Gln mutation within the *TGFBI* gene and who

underwent the PTK procedure on a grafted cornea that had undergone PKP.[12]

Reis-Bücklers corneal dystrophy[29] is a bilateral and autosomal dominant inheritable disease. This disease is clinically characterized by corneal opacities in a "geographic" pattern at the level of the Bowman layer, frequently associated with the episode of recurrent painful corneal erosion,[2] and has been referred to as corneal dystrophy of the Bowman layer and the superficial stroma type I (CDB1, OMIM#60847).[3] Because of the similarity of opacity pattern and opacity depth observed in Thiel-Behnke corneal dystrophy and Reis-Bücklers corneal dystrophy, considerable clinical confusion may still exist in distinguishing between these 2 diseases. Although the opacity pattern of Thiel-Behnke corneal dystrophy is reported to be apparently different from that of Reis-Bücklers corneal dystrophy, the opacity patterns of the 2 corneal dystrophies can appear to ordinary ophthalmologists to be quite similar, thus possibly resulting in considerable clinical confusion in discriminating between these 2 dystrophies. In the 1980s, Thiel-Behnke corneal dystrophy was considered to be a special type of Reis-Bücklers corneal dystrophy. These 2 corneal dystrophies of the Bowman layer were reported to be distinguishable through electron microscopy examination of the patient's corneal tissue. However, since a patient's corneal tissue is normally unavailable, it is often difficult to distinguish between these 2 dystrophies.[30] The only clinically identifiable difference between Thiel-Behnke corneal dystrophy and Reis-Bücklers corneal dystrophy is that Reis-Bücklers corneal dystrophy is clinically characterized by disease onset occurring in the patient at a younger age, the severe degree of corneal opacity, and a worse deterioration of vision compared to Thiel-Behnke corneal dystrophy.[2–4] Recent molecular biological analysis has revealed that Reis-Bücklers corneal dystrophy is caused by a mutation (p.Arg124Leu)[7] of the *TGFBI* gene that is distinct from that in Thiel-Behnke corneal dystrophy.

It should be noted that the early reports[19,20] of PTK for Reis-Bücklers corneal dystrophy possibly included Thiel-Behnke corneal dystrophy cases. In general, there is almost always a recurrence of Reis-Bücklers corneal dystrophy after PTK,[15] and even after PKP,[8] and the recurrence occurs earlier and with a more severe degree of disease compared with other *TGFBI*-related corneal dystrophies. However, despite the prominent similarities in several clinical attributes found in Thiel-Behnke corneal dystrophy and Reis-Bücklers corneal dystrophy, Thiel-Behnke corneal dystrophy patients demonstrated a relatively slow postoperative rate of recurrence after undergoing PTK surgery, as is shown in the findings of this present study. This finding may be the result of the difference in the molecular character of the TGFBI proteins that reflect each distinct mutation site. Thus, the simple PTK procedure appears to be an insufficient treatment for Reis-Bücklers corneal dystrophy. In a previous clinical trial, the usefulness of a topical administration of mitomycin C was assessed in Reis-Bücklers corneal dystrophy patients, and it was found to have a beneficial effect on preventing recurrence after PTK surgery.[31] Thus, it is very important to perform gene mutation analysis against the *TGBFI* gene to definitively discriminate Thiel-Behnke corneal dystrophy from Reis-Bücklers corneal dystrophy in order to make a more precise prediction of postoperative prognosis, as well as to consider the possible additional treatments, such as the administration of mitomycin C, that will be needed when patients are diagnosed as having Reis-Bücklers corneal dystrophy.

In conclusion, the findings of the present study show that PTK is a successful therapy for Thiel-Behnke corneal dystrophy. It should be noted that this study was retrospective and that the sample size was not very large. In addition, *TGFBI* corneal dystrophies sometimes demonstrate varying degrees of severity even with the same gene mutation. Thus, it is difficult to make a generalized statement as to the efficacy of PTK surgery for Thiel-Behnke corneal dystrophies from the limited results presented in this study. However, and to the best of our knowledge, this is the first report to demonstrate the midterm clinical outcome of PTK surgery for Thiel-Behnke corneal dystrophy patients who were diagnosed strictly by gene mutation analysis to have the p.Arg555Gln mutation and who had not undergone any surgery to their corneas prior to the PTK surgery. We hope that a randomized controlled trial will be conducted in the future in order to better understand the more precise clinical course of Thiel-Behnke corneal dystrophy after PTK surgery.

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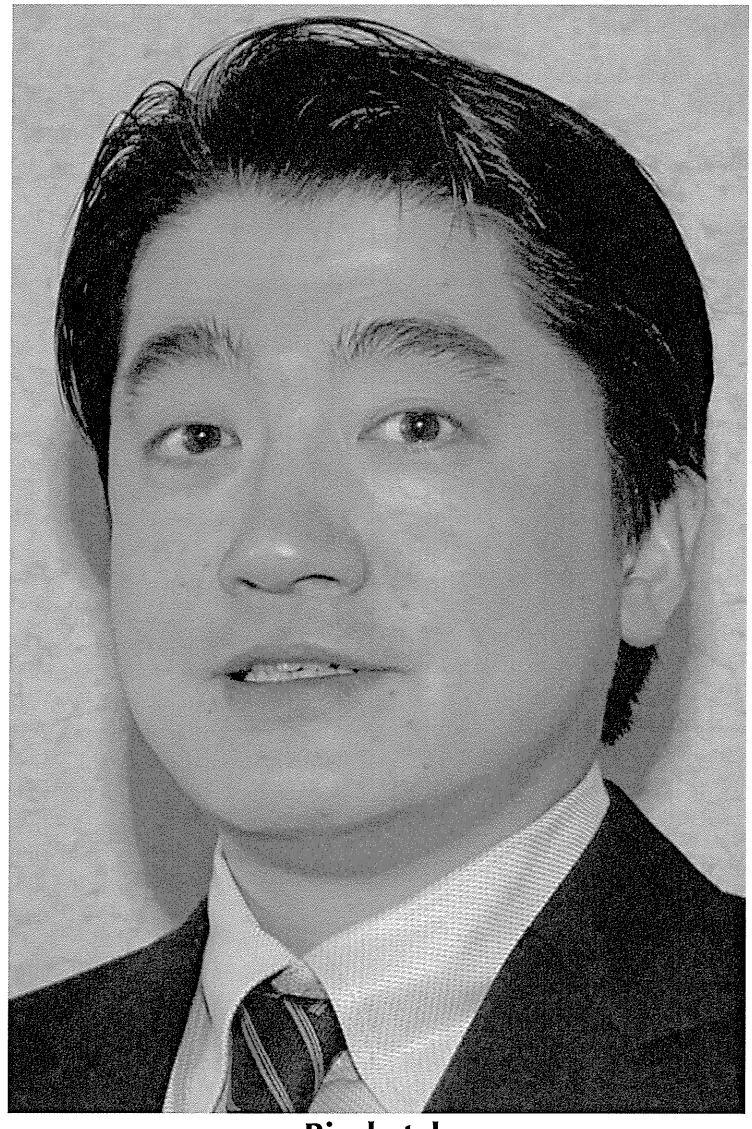

Biosketch

Osamu Hieda, MD, is a staff doctor at the Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan, where he received his clinical training and corneal refractive surgery fellowship. Dr Hieda's current interests include laser surgery for the treatment of corneal dystrophies.