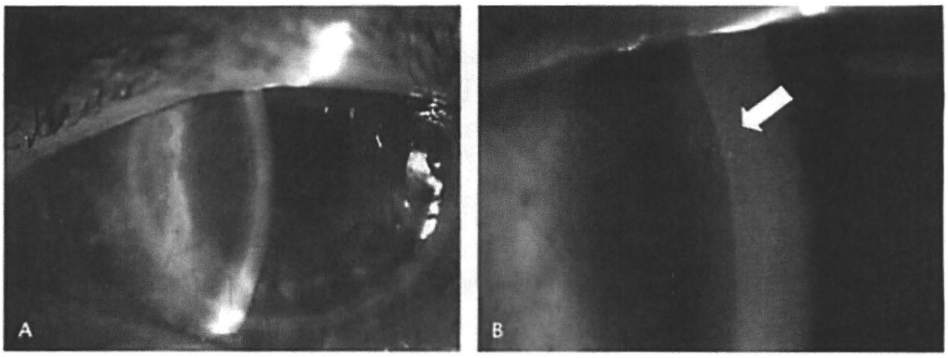

FIGURE 1. Slit-lamp examination at the onset of CMV endotheliitis. The corneal stroma is edematous in the upper temporal area (A), and accompanied by coin-shaped KPs (B, arrow).

agents were required to control the elevated IOP. Fundoscopic examination indicated neither retinitis nor extensive glaucomatous disc damage. Because of the suspicion of CMV endotheliitis, an anterior chamber tap was performed. Polymerase chain reaction (PCR) analysis detected CMV-DNA in the aqueous humor but not herpes simplex virus and varicella zoster virus DNAs. The patient's serum antibody titer for CMV was 23.1 (immunoglobulin G) and 0.32 (immunoglobulin M). The patient received 5 mg/kg ganciclovir intravenously twice per day over a period of 10 days with topical corticosteroids (0.1% betamethasone, 3 times per day) and an antibiotic (levofloxacin 0.5%, 3 times per day) eyedrop. No systemic side effects were found except mild pancytopenia. After ganciclovir treatment, his IOP was well controlled and the characteristic KPs were resolved. However, because of irreversible endothelial destruction and deficiency, the graft resulted in bullous keratopathy.

In May 2008, we performed the fourth PKP and administered intravenous ganciclovir for 10 days postoperatively. We prescribed systemic (hydrocortisone 300 mg, intraoperatively, and prednisolone starting at a dose of 20 mg/d, postoperatively) and topical (0.1% betamethasone, 5 times per day) corticosteroids with an antibiotic (levofloxacin 0.5%, 5 times per day, postoperatively) around the operation. We did not administer any topical cyclosporine. At 12 months after the final PKP, the graft has remained clear and no recurrence has been noted at the present time. His best-corrected visual acuity has improved (0.9 + 1.5 diopters, cylinder = −3.5 diopters, Ax20°), and his IOP remained within the normal range.

Histological and DNA Analyses of the Failed Corneal Graft

During the fourth PKP procedure, the failed graft was obtained and subjected to further PCR and histopathological examinations. The excised failed graft was homogenized, and PCR was performed using CMV-specific primers including intermediate early region and late antigenic region as has been reported.[8,9] CMV-DNA was detected in the whole corneal button of the failed graft, with the specificity confirmed by a Southern hybridization analysis (Figs. 2A, B). Histological examinations (Hematoxylin–Eosin staining) indicated that there was little inflammatory change in either the epithelial or stromal layer. In addition, preservation of the structures was noted in both layers apart from mild stromal edema (Figs. 3A, B).

DISCUSSION

To the best of our knowledge, this is the first report of direct evidence of CMV infection of the cornea. In previous studies of CMV endotheliitis, PCR to detect viral DNA was performed using aqueous humor and local CMV-specific antibody production was documented also in aqueous humor with a clinical response to ganciclovir in these patients.[2–5] Although these evidences collected so far raise the possibility of CMV infections in cornea, the findings did not provide any conclusive evidence that CMV was responsible for corneal infections. In this article, our PCR analysis did show that the corneal endotheliitis could be traced to the CMV-DNA within the cornea.

Ocular human CMV infection can appear as various clinical modalities, including primary infection, reinfection with new strains, reactivation of a latent infection, or as a persistent infection. In our case, 2 possibilities of infection routes are speculated. First, mononuclear cells or myeloid progenitor cells can harbor the CMV in a latent form after the primary infection during childhood[10,11] and carry them to cornea via the bloodstream of iris and ciliary body under the

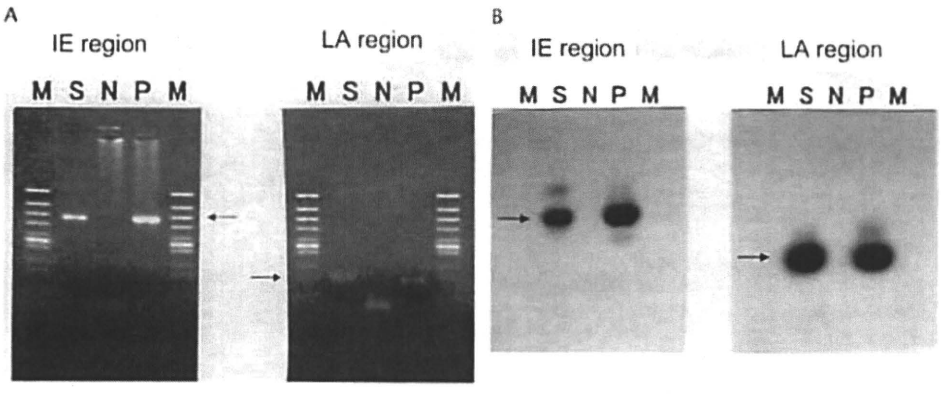

FIGURE 2. A, CMV-DNA of the excised corneal button was determined by PCR using CMV-specific primers of intermediate early (IE) region and late antigenic (LA) region. B, Southern hybridization analysis results confirmed the specificity of the amplified CMV-DNA. Lane M, molecular marker; lane S, DNA extracted from the excised corneal button; lane N, negative control; lane P, positive control.

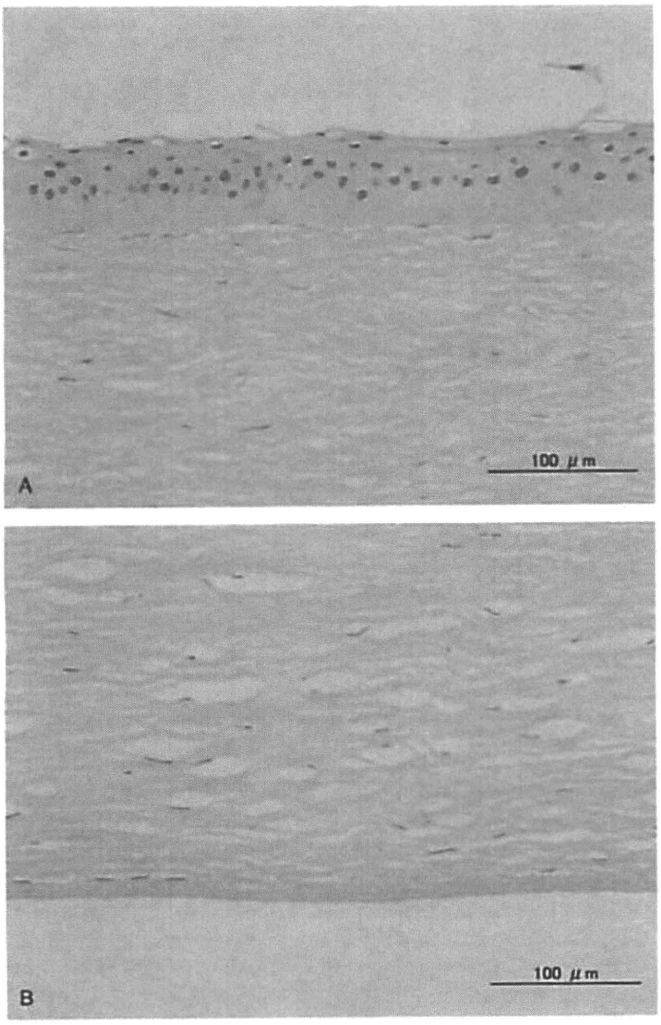

FIGURE 3. Histopathological findings by H–E staining. A, Both the stromal and epithelial layers exhibited little inflammation. B, Although edema was very slight within the stroma, it increased in the deeper portion of the stroma close to endothelium.

immunosuppressed conditions. Second, donor corneas of repeated PKPs can be a source of CMV inoculation as has been reported.[12] We cannot determine the exact infection route, however, the onset of CMV endotheliitis of our case might be in August 2007 when typical clinical findings of CMV endotheliitis such as corneal KPs in coin-shaped region with stromal edema first appeared. The characteristic KPs were found with the progression of corneal edema. This might be the indicator of CMV infection to endothelial cells.

There was no remarkable inflammatory change in both the epithelial and stromal layers by histological analyses of the failed corneal button. Considering with the fact that CMV-DNA was positive in the excised failed graft, this suggests that the endothelial cells are the primary targets of the CMV infection. The reports of cytomegalic transformation of corneal endothelial cells in CMV panuveitis[1] and owl's eye morphology in corneal endothelial cells in CMV endotheliitis[7] are in agreement with our finding that CMV exists within the endothelium and could be a direct causative agent of infectious endotheliitis. Our observation also supports the nomenclature of CMV corneal endotheliitis made by Koizumi et al.[2]

It is not clear why CMV-DNA could be detected in the cornea after the antiviral therapy using ganciclovir in our case. PCR can only confirm the presence of the fragment of CMV-DNA in the cornea and cannot determine whether the replication competence is retained or not. Detected CMV-DNA might be the fragment of the organism. The previous report[7] showed rapid disappearance of owl's eye lesion and negative PCR of aqueous humor after ganciclovir treatment. However, once recurrence of inflammation occurs after the cessation of ganciclovir, CMV-DNA can be detected in aqueous humor again.[13] Therefore, CMV might be latently harbored within the eye after ganciclovir treatment. And immunosuppression by steroid and cyclosporine might trigger the CMV endotheliitis as was shown in our case.

In conclusion, we have revealed the direct evidence that corneal endothelial cells could be the target of CMV infection.

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In Vivo Confocal Microscopic Evidence of Keratopathy in Patients with Pseudoexfoliation Syndrome

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ABSTRACT

Purpose: To measure the density of cells in different layers of the cornea, and to determine whether morphological changes of the subbasal corneal nerve plexus are present in eyes with the pseudoexfoliation (PEX) syndrome.

Methods: Twenty-seven patients with unilateral PEX syndrome and 27 normal controls were investigated. All eyes underwent corneal sensitivity measurements with the Cochet-Bonnet esthesiometer and in vivo confocal microscopy with the Heidelberg Retina Tomograph II-Rostock Cornea Module. The densities of the epithelial, stromal, and endothelial cells were measured. The density and tortuosity of the subbasal corneal nerve plexus were also evaluated.

Results: Eyes with the PEX syndrome had significantly lower cell densities in the basal epithelium ($P = 0.003$), anterior stroma ($P = 0.007$), intermediate stroma ($P = 0.009$), posterior stroma ($P = 0.012$), and endothelium ($P < 0.0001$) than in the corresponding layers of normal eyes. PEX eyes also had lower subbasal nerve densities and greater tortuosity of the nerves than normal eyes. The fellow eyes of patients with PEX also had significantly lower densities of the basal epithelial and endothelial cells than the normal eyes. The corneal sensitivity was significantly decreased in PEX eyes, and this was significantly correlated with the decrease of basal epithelial cell and subbasal nerve densities.

Conclusions: Our results have shed light on understanding the pathogenesis of decreased corneal sensitivity in eyes with PEX syndrome. The PEX syndrome is probably a binocular condition in which the keratopathy of the fellow eye also needs to be alerted to.

The pseudoexfoliation (PEX) syndrome is a common age-related disorder of the extracellular matrix and is frequently associated with severe chronic secondary open angle glaucoma and cataract.[1-3] The prevalence of PEX syndrome varies widely in different racial and ethnic population. In addition, the prevalence of PEX is dependent on the age and gender distribution of the population examined; the clinical criteria used to diagnose PEX; and the ability of the examiner to detect early stages and more subtle signs of PEX. For example, the highest rates in studies of persons > 60-years-of age have been reported to be about 25% in Iceland and over 20% in Finland.[3,4] The ocular manifestation of the PEX syndrome is the production and progressive accumulation of abnormal extracellular fibrillar material in almost all of the inner wall tissues of the anterior segment of the eye. This characteristic alteration predisposes the eye to a broad spectrum of intraocular complications including phacodonesis and lens subluxation, angle closure glaucoma, melanin dispersions, poor mydriasis, blood-aqueous barrier dysfunction, posterior synechiae, and other related complications.[1-3]

The PEX syndrome is associated with corneal endotheliopathy, and this has been suggested to be the cause of the so-called atypical non-guttata Fuchs endothelial dystrophy.[5,6] PEX endotheliopathy is mostly bilateral but often asymmetrical, and is a slowly progressing disease of the corneal endothelium. It can lead to early corneal endothelial cell decompensation, which can then induce severe bullous keratopathy, a vision-threatening disorder.

The clinical signs of the eyes with the PEX syndrome include decreased corneal sensitivity, thinning of the central corneal thickness, and impaired tear film stability.[7-9] However, the underlying cause of these clinical findings has not been well investigated, which may be because objective and accurate in vivo examination techniques are not available.

Recent advances in imaging technology have improved the ability of these instruments to

diagnose different ocular diseases. The Rostock Cornea Module (RCM) consisting of a contact lens system attached to the Heidelberg Retina Tomograph (II) is such an instrument. It utilizes laser scanning technology to investigate the cornea at a cellular level, and structures such as the subbasal nerve plexus, which cannot be seen by slit-lamp microscopy, can be clearly seen.[10,11]

In vivo confocal microscopy (IVCM) was used by Martone et al to examine one eye with the PEX syndrome,[12] and non-contact IVCM method was used to study eyes with PEX, PEX suspects, and normal eyes by Sbeity et al.[13] However, there has not been a detailed and quantitative study of the morphological changes in the cornea of eyes with the PEX syndrome.

Thus, the purpose of this study was to examine the underlying pathogenesis of PEX keratopathy and to obtain evidence to explain the clinical findings such as the decreased corneal sensitivities observed in patients with PEX syndrome. To accomplish this, we used IVCM to determine the cell densities in different corneal layers of eyes with the PEX syndrome and their clinically unaffected fellow eyes. These findings were compared to those of normal control eyes. The nerve densities in the subbasal layer were also analyzed and their relationship with the alterations of clinical corneal sensitivity were analyzed.

METHODS

Subjects

We studied 27 patients diagnosed with unilateral PEX syndrome. There were 16 men and 11 women with a mean age of 74.4 ± 6.3 years and a range of 65 to 90 years. In all eyes, exfoliation material (XFM) was seen at the pupillary border and/or on the anterior lens capsule by slit-lamp microscopy. Eyes with the PEX syndrome were placed in the PEX group, and the clinically normal fellow eyes were placed in the PEX fellow eye group. Age- and gender-

matched normal subjects, 16 men and 11 women with a mean age of 72.7 ± 6.5 years and a range of 61 to 92 years were also studied. One eye from the normal control group was randomly selected and used in the statistical analyses. The exclusion criteria included eyes with Stevens-Johnson syndrome, lymphoma, sarcoidosis, corneal dystrophy, injury and inflammation, any systemic therapy with drugs with known corneal toxicity, eyes being treated with topical anti-glaucoma drugs, steroids or NSAIDs, eyes of contact lens wearers, eyes with previous ocular surgeries, and eyes with other ophthalmic diseases.

The procedures used conformed to the tenets of the Declaration of Helsinki. An informed consent was obtained from all subjects after an explanation of the nature and possible consequences of the procedures. The protocol used was approved by the Ethics Committee of Ehime University School of Medicine.

Corneal sensitivity measurements

Measurement of the corneal sensitivity was performed with a Cochet-Bonnet nylon thread esthesiometer as described.[14] The examination was begun with a 60-mm length of nylon filament applied perpendicularly to the central cornea, and the tests were continued by shortening the filament by 5 mm each time until the subject felt the contact of the filament. Each subject was measured twice with a between test interval of at least 5 minutes, and the average of two measurements was used for the statistical analyses.

In vivo confocal microscopy (IVCM)

IVCM was performed on all subjects with the Rostock Corneal Module of the Heidelberg Retina Tomograph II (HRTII-RCM; Heidelberg Engineering, Heidelberg, Germany). After topical anesthesia with 0.4% oxybuprocaine (Santen Pharmaceuticals, Osaka, Japan), the

subject was positioned in the chin and forehead holder and instructed to look straight ahead at a target to make sure that the central cornea was scanned. The objective of the microscope was an immersion lens (magnification x63; Zeiss, Chester, VA) covered by a polymethylmethacrylate cap (TomoCap, Heidelberg Engineering). Comfort gel (Baush & Lomb, Berlin, Germany) was used to couple the applanating lens cap to the cornea. The TomoCap was applanated onto the center of the cornea by adjusting the controller, and in vivo digital images of the cornea were seen on the monitor screen. When the first layer of superficial epithelial cells was seen, the digital micrometer gauge was set to zero and then a sequence images were recorded as the focal plane was gradually moved toward the endothelium. Each subject was scanned three times with an interval of at least 15 minutes.

The laser source of the HRT-II RCM is a diode laser with a wavelength of 670 nm. Two-dimensional images consisting of 384 x 384 pixels covering an area of 400 x 400 µm were recorded. The digital resolution was 1.04 µm/pixel transversally and 2 µm/pixel longitudinally as stated by the manufacturer.

Image analyses

The central corneal images of all subjects were taken and three best-focused images from the superficial epithelium, basal epithelium, subbasal nerve plexus, anterior stroma, intermediate stroma, posterior stroma, and endothelium were selected for analyses. The selected images were randomly presented to two masked observers (XZ, SO) for evaluation. All data are presented as averages of three images.

Cell density analyses

The morphological characteristics and densities in the different layers of the cornea in the PEX and PEX fellow eyes were assessed and compared with those of normal controls. The superficial epithelial cells were identified as polygonal cells with clearly visible cell borders, bright cytoplasm, and dark nuclei. The basal epithelial cells were identified as the layer just above the amorphous appearing Bowman membrane. The basal cells had bright borders, a uniform shape, and non-homogenous cytoplasm. The anterior stroma was identified as the first layer immediately beneath Bowman membrane, and the posterior stroma was identified as the layer just anterior to Descemet membrane and the endothelium. The intermediate stroma was defined as the layer halfway between the anterior and posterior stroma.[15] The corneal endothelium consisted of a monolayer of regularly arranged hexagonal cells with dark borders and bright reflecting cytoplasm.

After selecting a frame of the image, and manually marking the cells inside the frame (>50 cells), the cell densities were calculated automatically by the software installed in the instrument. The cells partially contained in the area analyzed were counted only along the upper and right margins. The results are expressed in cells/mm^2.

Analyses of subbasal nerve plexus

The subbasal nerve plexus layer is located between Bowman membrane and the basal epithelial layer through which numerous nerve fibers pass. The density and tortuosity of the subbasal nerve plexus was analyzed as described.[14,16] Two parameters were analyzed: the long nerve fiber density (LNFD) was determined by dividing the number of long nerves by the image area (0.16 mm^2); and the nerve branch density (NBD) was determined by dividing the total number of long nerves and their branches by the image area. Nerve tortuosity was graded

into 4 degrees; Grade 1 = approximately straight nerves and Grade 4 = very tortuous nerves with significant convolutions throughout their course.[16]

Statistical analyses

Data were analyzed with the JMP version 8.0 for Windows statistical software (SAS Japan Inc., Tokyo, Japan). All data are expressed as the means ± standard deviations (SDs). The differences of cell densities between PEX eyes and normal controls or between PEX fellow eyes and normal controls were evaluated with 2-tailed Student's *t* tests. The differences of cell densities between PEX eyes and their fellow eyes were evaluated by paired *t* tests. The Wilcoxon rank sum test was used to compare the values of corneal sensitivity, LNFD, NBD, and the nerve tortuosity between PEX patients and normal controls. Spearman's correlation was used to determine the correlation among the parameters of basal epithelial cell density, subbasal nerve density, and corneal sensitivity. A probability level of $P < 0.05$ was considered statistically significant.

RESULTS

The mean age was not significantly different between patients with PEX and normal controls (2-tailed Student's *t* tests, $P = 0.725$). The eyes with PEX showed typical whitish exfoliation material on the pupillary border and/or on the anterior lens capsule on slit-lamp examination. Pigmented keratoprecipitates and slight folding of Descemet membrane were also detected in some of the patients. The fellow eyes of PEX eyes and normal control eyes appeared normal by slit-lamp microscopy.

Corneal sensitivity

The mean corneal sensitivity was 47.8 ± 5.6 mm for the PEX eyes and 53.7 ± 4.9 mm for the PEX fellow eyes. This difference was significant ($P = 0.005$; Wilcoxon rank sum test). The mean corneal sensitivity was 55.6 ± 4.7 mm for the normal control subjects, and the cornea of eyes with PEX were significantly less sensitive than normal control eyes ($P < 0.0001$). The difference in corneal sensitivity between PEX fellow eyes and normal controls was not significant ($P = 0.378$).

Cell densities

The density of the corneal superficial epithelial cells was 872.6 ± 95.3 cells/mm^2 and that for the basal epithelial cells was 4829.7±462.1 cells/mm^2 in the PEX eyes. The densities for the corresponding layers in the PEX fellow eyes were 910.4 ± 80.8 cells/mm^2 and 4996.7 ± 438.7 cells/mm^2, and the densities for the normal control eyes were 886.4 ± 101.7 cells/mm^2 and 5446.4 ± 639.9 cells/mm^2. The density of the basal epithelial cells was significantly lower for the PEX eyes and the PEX fellow eyes than that of the control eyes ($P = 0.003$ and $P = 0.015$, respectively; 2-tailed Student's *t* tests; Fig. 1). The difference in the density of the basal epithelial cells between the PEX eyes and the PEX fellow eyes was not significant ($P = 0.589$; paired *t* test). The differences in the densities of the superficial epithelial cells among the 3 experimental groups were also not significant (Fig. 1).

The densities of the cells in the three stromal layers of the PEX eyes, PEX fellow eyes, and normal control eyes are shown in Figure 2. Compared to normal controls, the cell densities of PEX eyes were significantly lower in all three layers of the stroma (anterior stroma, $P = 0.007$; intermediate stroma, $P = 0.009$; posterior stroma, $P = 0.012$; 2-tailed Student's *t*

tests). The densities in these three stromal layers in the PEX fellow eyes were also lower but the decrease was not significant ($P = 0.196$; $P = 0.261$; $P = 0.08$; respectively; Fig. 2).

The endothelial cell densities were 2240.7±236.6 cells/mm^2, 2386.6±200.8 cells/mm^2, and 2738.7±233.2 cells/mm^2 for PEX eyes, PEX fellow eyes, and normal eyes, respectively. The difference between the PEX eyes and normal controls was significant ($P < 0.0001$; 2-tailed Student's *t* test; Fig 1) and between the PEX fellow eyes and normal controls was also significant ($P = 0.001$). The difference in the endothelial cell density between the PEX and PEX fellow eyes was not significant ($P = 0.754$; paired *t* test).

There was a higher degree of pleomorphism and polymegethism in the PEX eyes than in the control eyes. The coefficient of variation of the cell area (CV) was 45.2%±8.7% and the percentage of hexagonal cells (HEX) in the PEX eyes was 30.5%±10.3%. Both values are significantly different than that of normal control eyes (CV, 30.6±5.6%, $P = 0.016$; HEX, 50.3± 6.8%, $P = 0.008$; 2-tailed Student's *t* test). The PEX fellow eyes also showed similar tendency of increased pleomorphism and polymegethism, but the differences were not statistically significant.

Subbasal nerve plexus

The LNFD and NBD were significantly decreased in the PEX eyes (17.4 ± 6.3 and 32.2 ± 8.3 nerves/mm^2, respectively) compared to that in the normal controls (35.9±8.2 and 72.2±8.8 nerves/mm^2; $P < 0.0001$ and $P < 0.0001$, respectively; Wilcoxon rank sum test; Fig. 3). The PEX fellow eyes also had decreased LNFD and NBD, however these changes were not significantly different from that of the controls (31.5±7.8 and 69.9±9.4 nerves/mm^2; $P = 0.093$ and $P = 0.301$).

Confocal images of PEX eyes showed extremely tortuous nerve fibers, thinning of the nerves, short nerve sprouts, fewer branches from the main nerve trunk, and highly reflective inflammatory infiltrates in close vicinity of the subbasal nerves. Representative confocal images of the three groups are shown in Figure 4. In the PEX eyes, 85.2% (23 of 27 eyes) had grade ≥3 subbasal nerve tortuosity, and the degree of tortuosity in the PEX eyes was significantly higher than that of the controls, (3.2 ± 0.7 *vs.* 1.6 ± 0.6; P <0.0001; Wilcoxon rank sum test). The degree of tortuosity in the PEX fellow eyes was also greater than that of normal controls although the difference was not significant (2.1 ± 0.9 *vs.* 1.6 ± 0.6, P = 0.054).

It was also our impression that the PEX eyes had more inflammatory cells, including dendritic cells, infiltrating the subbasal cell layer and anterior stroma, and these changes were more severe in eyes with decreased subbasal nerve densities and lower corneal sensitivities (Fig. 4).

Correlation between corneal sensitivity and subbasal nerve density and basal epithelial cell density

Spearman's correlation analyses showed that there was a significant positive correlation between corneal sensitivity and the subbasal nerve densities (LNFD, r = 0.764; P <0.0001; NBD, r = 0.634; P <0.0001; Spearman correlation coefficient). The corneal sensitivity was also significantly and positively correlated with the basal epithelial cell density and significantly and negatively correlated with the subbasal nerve tortuosity (Table 1).

Confocal microscopic detection of hyperreflective material

IVCM showed hyperreflective material, probably XFM, in the subbasal epithelial layer and/or the anterior stroma of 22 of the 27 PEX eyes (81.5%). The hyperreflective material was also

observed abundantly in the endothelium of all PEX eyes. In the PEX fellow eyes, 5 of 27 (18.5%) eyes showed hyperreflective deposits in the subbasal epithelial layer or anterior stroma and 51.9% eyes (14 of 27) had endothelial surface deposits of hyperreflective material. In sharp contrast, none of the normal eyes showed hyperreflective material in the subbasal epithelial or anterior stromal layers and only 2 eyes (7.4%) had a small amount of hyperreflective material on the endothelial surface (Figs. 5 and 6).

DISCUSSION

The manifestations of the PEX syndrome in the anterior segment are widely known to affect intraocular surgery with poor mydriasis and intensive postoperative inflammation. The fact that aggregates of XFM can be identified in autopsy specimens of the heart, lung, liver, kidney, and other organs in patients with ocular PEX suggests that the ocular PEX syndrome is part of a general systemic disorder.[1-3, 17] In fact, the PEX syndrome has been reported to be associated with cardiovascular diseases, chronic cerebral disorders, Alzheimer disease, and acute cerebrovascular events.[1-3] Two single nucleotide polymorphisms in the lysyl oxidase-like 1 (*LOXL1*) gene have been recently identified as strong genetic risk factors for the PEX syndrome and PEX glaucoma.[18]

IVCM with the Rostock Corneal Module provides a new imaging method which allows rapid, noninvasive, high-resolution, and microstructural examination of the cornea.[10,11] There are only 2 studies that used IVCM to study the cornea of patients with the PEX syndrome. Martone et al.[12] reported the findings in one case, and they reported that ICVM can detect hyperreflective deposits and dendritic cells infiltrating the basal epithelial cell layer. Fibrillar subepithelial structures were found and the endothelial layer showed cellular anomalies. In a

prospective observational case series, Sbeity et al.[13] used non-contact IVCM to detect XFM on the lens surface and corneal endothelium of PEX eyes and their fellow eyes.

Our study was the first to use IVCM to investigate the cell densities in different layers of the cornea and to determine the alterations of subbasal nerve density and tortuosity in PEX and PEX fellow eyes. Our results showed a significant decrease in the densities of the corneal endothelial cells in PEX eyes and their fellow eyes which is in agreement with earlier observations by specular microscopy.[8,19,20] In addition, the clear confocal images allowed us to detect pleomorphisms and polymegethisms of the endothelial cells. All of the PEX eyes and 51.9% of the PEX fellow eyes showed deposits of hyperreflective material in the endothelium indicative of either pigment granules or XFM. In agreement with Sbeity et al, we believe that the pleomorphic and irregular deposits found on the corneal endothelium most likely represent XFM rather than pigment granules that are round and uniform in size.[13] In addition, a number of patients who had no visible pigment keratoprecipitates on slit-lamp microscopy were found to have abundant large and irregular hyperreflective deposits on the endothelium in the confocal images.

The PEX syndrome associated corneal endotheliopathy has been suggested to be due to one or a combination of the following alterations; hypoxic changes in the anterior chamber, accumulation of extracellular matrix, fibroblastic changes of the endothelium, and increased concentration of TGF-β.[1-3] Our confocal microscopic findings suggest that the XFM, possibly at different stages of the normal course of PEX, may be deposited on the endothelium or migrate from the endothelial cells that undergoes fibroblastic changes. Our findings also showed that the hyperreflective materials are found not only on the endothelium of the PEX eyes but also in their fellow eyes indicating that the fellow eyes might be at a preclinical stage of the PEX syndrome. A bilateral decrease in the endothelial cell counts and morphologic

alterations of endothelium support the idea that PEX is a binocular and systemic abnormality. Patients with unilateral PEX syndrome may have asymmetric manifestation of this slowly progressing disease.

One clinical significance of our finding is that the decreased stromal cell densities observed by IVCM could possibly explain the report that the central cornea of PEX eyes is thinner than that of normal subjects.[8] The pathogenesis of the decrease of stromal cell density in PEX eyes needs future study. Because XFM deposits were simultaneously observed in the anterior stroma of PEX eyes, we suggest that the XFM may be somehow causative for this alteration, for example, in a way of inducing apoptosis of the keratocytes. Other pathogenic factors such as altered levels of cytokines or chemokines in the cornea could also be responsible and this definitely warrants future investigation. In addition, the PEX fellow eyes also had lower cell counts in the stroma although the difference was not statistically significant. We suggest that the cause of the binocular differences in our cases may be because the two eyes were at different stages of the PEX process, and the PEX fellow eyes may be still at a preclinical stage of the PEX syndrome.

Other important findings were found in the subbasal nerve plexus. Our results showed that the subbasal nerve density was significantly lower and the nerves were mostly tortuous with beading and thinning in the PEX eyes than in normal controls. Interestingly, the PEX fellow eyes also had similar alterations although the changes were not significant. These findings support the idea that the PEX syndrome is a binocular abnormality that is expressed in both eyes but to different degrees. The important clinical significance of our study is that our correlation analyses showed that the decreased subbasal nerve density and increased tortuosity were significantly correlated with the decreased corneal sensitivity. These results provide evidence, for the first time, that the cause of the decreased corneal sensitivity in eyes

with PEX syndrome is the decreased subbasal nerve density. For patients with the PEX syndrome, it would be practical and feasible to examine the corneal sensitivity to assess the severity of PEX keratopathy, and perhaps in predicting the progression of the PEX syndrome. In addition, detection of the morphological changes in cell densities and subbasal nerve abnormalities by IVCM in the fellow eyes indicates that it is a sensitive tool for the diagnosis of preclinical stage of the PEX syndrome. Our findings showed that PEX keratopathy could develop before any clinically visible XFM deposits are detected on the lens capsule or iris. If these findings are confirmed, then the keratopathy may be the first event of the ocular complications of the PEX syndrome. These findings also indicate that clinically unaffected fellow eyes of patient with PEX syndrome are probably at risk of developing PEX syndrome and more frequent ophthalmologic examinations are necessary.

This study has increased our understanding of the keratopathy of this most likely systemic abnormality. Whether the alternations of the subbasal corneal nerves are primary or secondary changes of the disease needs to be determined. Because of the increase in the elastic microfibril components, and imbalances in the matrix metalloproteinases (MMPs) and tissue inhibitors of MMP in eyes with the PEX syndrome, there is an accumulation of PEX fibrils in the tissues.[1-3] Our findings that XFM deposits were frequently observed close to the subbasal epithelial layer or anterior stroma support the idea that besides an abnormal aggregation of elastic microfibrils into exfoliation fibers (the elastic microfibril hypothesis),[1-3, 21] other extracellular matrix components, such as basement membrane components may possibly interact and become incorporated into the composite XFM (the basement membrane hypothesis).[2,3] In addition, our observation of an infiltration of dendritic cells in close vicinity of the subbasal nerve plexus layer indicates the possibility that accumulation of extracellular XFM may induce inflammatory responses which then recruit antigen presenting cells such as

immunocompetent dendritic cells. This excessive deposition of XFM and infiltration of dendritic cells may play a role in the neuropathy of the subbasal nerve plexus resulting in decreased corneal sensitivity in patients with the PEX syndrome.

Some limitations exist for this study. First, the IVCM scans a very small area of the cornea, which may generate biases among different portions of scanning of different groups. As mentioned, efforts were taken to scan the center of the cornea of all subjects. In addition, we also confirmed our findings by scanning the mid-peripheral and peripheral portion of the cornea (data not shown).

Second, the IVCM images may not represent the true histological changes of the cornea. By applying the same criteria for image evaluation, we can conclude that the differences between the studied groups were still detected. Furthermore, it was our impression that there are fewer keratocytes seen in the stroma of corneal specimens obtained from penetrating keratoplasty patients with the PEX syndrome.

Future investigations, including a thorough and quantitative analysis of the exfoliation material by confocal imaging are needed. Also, the correlations between IVCM findings with endothelial barrier function should be determined. If the confocal findings can provide clues for preclinical stage of endothelial barrier dysfunction of the cornea in PEX syndrome, the clinical significance can be used in designing an early treatment protocol.

In summary, our study demonstrated that eyes with the PEX syndrome have decreased cell densities in the cornea. The subbasal nerve density was also significantly decreased, and this was significantly correlated with clinically decreased corneal sensitivity. Our study shed light on understanding the cause of impaired corneal sensitivity in patient with PEX syndrome. The PEX syndrome is probably a bilateral event in which the keratopathy of the fellow eye also needs to be alerted to.

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