Quantitative Regional Differences in Corneal Endothelial Abnormalities in the Central and Peripheral Zones in Fuchs' Endothelial Corneal Dystrophy

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Submitted: February 26, 2014
Accepted: July 12, 2014

Citation: Fujimoto H, Maeda N, Soma T, et al. Quantitative regional differences in corneal endothelial abnormalities in the central and peripheral zones in Fuchs' endothelial corneal dystrophy. *Invest Ophthalmol Vis Sci*. 2014;55:5090–5098. DOI:10.1167/iovs.14-14249

Purpose. To quantitate the regional corneal differences in endothelial abnormalities in Fuchs' endothelial corneal dystrophy at multiple sites, including the peripheral zone.

Methods. Forty-one eyes of 23 patients with Fuchs' endothelial corneal dystrophy were studied at Osaka University Hospital. The sizes of the areas of degeneration resulting from guttae were measured using a new noncontact specular microscope in the central cornea, the paracentral zone 0.6 mm from the center, and the peripheral zone 3.7 mm peripheral to the center.

Results. The percentages of the images covered by the abnormal areas were 71% ± 36% in the center, 68% ± 35% in the paracentral zone, and 33% ± 36% in the peripheral zone. The values in the peripheral zone were significantly ($P < 0.001$) smaller than in the center and paracentral zones. The percentage of the abnormal area in the peripheral zone was correlated significantly ($P < 0.001$, $R^2 = 0.452$) with the disease grade in advanced cases, whereas those in the center or paracentral zones were not. Among the areas of the peripheral zone, the abnormal inferotemporal areas were significantly ($P < 0.001$) larger than superonasally.

Conclusions. In Fuchs' endothelial corneal dystrophy, the corneal endothelium is damaged more severely in the center and paracentral zones than in the peripheral zone, and peripheral measurement can objectively grade the disease. In the peripheral zones, the inferotemporal endothelium is damaged more severely. These findings might provide a new understanding of the disease mechanisms.

Keywords: Fuchs' endothelial corneal dystrophy, endothelium, specular microscopy, peripheral

Fuchs' endothelial corneal dystrophy is a progressive corneal endothelial dysfunction that causes irreversible damage to the endothelial cells and corneal edema.[1–5] The disease is characterized by degeneration of the endothelial cell layer, which synthesizes an abnormal basement membrane. Focal endothelial excrescences (guttae) are considered the hallmark of the disease.

In the early disease stage, abnormal endothelial changes are thought to occur initially in the central zone and spread peripherally.[6] To assess the stage, the disease is graded according to the slit-lamp findings based on the confluence and area of guttae at the central corneal zone.[7]

The endothelial damage induces increased corneal thickness. A clinical study suggested that in Fuchs' endothelial corneal dystrophy the central cornea swells more than the peripheral cornea.[1,8] The peripheral cornea remains relatively unaffected except in the final stage.

Disease progression leads to corneal stromal edema as a result of decompensation of the endothelial pump function. This bullous keratopathy is irreversible and results in visual impairment.[4] Keratoplasty currently is the only option available to improve vision in cases with disease progression, with penetrating keratoplasty the conventional surgical treatment for Fuchs' endothelial corneal dystrophy. Recently, Descemet stripping automated endothelial keratoplasty (DSAEK)[9–12] and Descemet membrane endothelial keratoplasty (DMEK)[13,14] have been developed. As a result, an increased number of surgical treatments are being performed at an earlier disease stage. To determine if surgery is indicated in the early disease stage, evaluation of visual function affected by guttae is important. Guttae in the central and paracentral zones, especially inside the entrance pupil, can cause visual impairment. To comprehensively access the visual impairment caused by guttae, quantification of guttae in the paracentral, peripheral, and central cornea is needed.

Little is known quantitatively about localization of the abnormality, however. To investigate the regional corneal differences in the abnormality, we evaluated the corneal endothelium at multiple sites, including the periphery, using a new noncontact specular microscope (CEM-530; Nidek Co., Ltd., Gamagori, Japan) to study the peripheral zone 3.7 mm from the corneal center and the paracentral zone 0.6 mm from the center in addition to the corneal center.

Materials and Methods

Patients

Patients with Fuchs' endothelial corneal dystrophy were recruited from among patients at Osaka University Hospital,

Osaka, Japan. Forty-one eyes of 23 patients (17 women, 6 men) evaluated between June 2012 and May 2013 were included. The institutional review board of Osaka University Hospital approved the study, which adhered to the tenets of the Declaration of Helsinki.

Corneal specialists diagnosed Fuchs' endothelial corneal dystrophy based on the presence of bilateral guttae in eyes without a history of intraocular surgery, contact lens wear, or other corneal diseases. Cases with pseudoguttae[15,16] and pseudoexfoliation syndrome were excluded.

Corneas with Fuchs' endothelial corneal dystrophy were graded using a modified scale ranging from 1 to 6 based on the confluence and area of guttae and the presence of edema (Table 1).[7,17] Cases with stromal edema (e.g., grade 6 dystrophy on the Krachmer grading scale) were excluded because no specular microscopy images were obtainable.

Specular Microscopy

A non–contact-type specular microscope (CEM-530; Nidek Co., Ltd.) was used. The captured images are 0.55 mm high and 0.25 mm wide. We studied six peripheral points for every two clock hours 27 degrees from the center and paracentral eight points 5 degrees from the center for every 1.5 clock hours (Fig. 1). Twenty-seven degrees corresponded to 3.7 mm from the center in eyes with a K of 43.5 diopters [D], 3.8 mm in eyes with a K of 42.5 D, and 3.6 mm in eyes with a K of 44.5 D. Five degrees correspond to 0.63 mm from the center in eyes with a K of 43.5 D, 0.65 mm in eyes with a K of 42.5 D, and 0.61 mm in eyes with a K of 44.5 D. Because there were no large variations in the distance from the center according to corneal curvature, the peripheral zones were referred to as 3.7 mm from the center and the paracentral zones were referred to as 0.6 mm from the center (Fig. 1). A total of 15 corneal points were examined. In one specular microscopy shot, 16 consecutive pictures were obtained and the examiners selected one image of every location studied. The imaging point was controlled by patient fixation, namely, locations were determined based on the patient's primary line of sight and not the vertex normal. The area measured was confirmed by a monitor camera.

Image Analysis of Abnormal Areas

All analyses, image processing, and statistical analyses were performed using MATLAB (The MathWorks, Inc., Natick, MA). To define the areas of abnormal endothelial degradation resulting from guttae, specular microscopy images were binarized, which requires homogenized brightness. However, specular microscopy uses a horizontally tilted slit light, and the specular reflection is recorded. Because of the tilted slit light, the original specular microscopy images usually have horizontally different brightness levels of the pixels. To correct this, each pixel was homogenized by adding or subtracting the same vertical value so that the average vertical pixel values were equal in one image (Supplementary Fig. S1).

To separate the guttae from the rest of the image, a thresholding algorithm was used. Areas of abnormal degeneration due to guttae were defined as areas with brightness under a fixed threshold determined by visual inspection of each corrected image (Fig. 2) by an examiner other than the specular microscopy examiner. During analysis, the image position in the cornea was randomized and the thresholds determined by a researcher who was not the specular microscopy examiner. Fifteen images were analyzed for each eye. The percentage of the pixels covered by the abnormal area to all pixels in the image was calculated for every specular microscopy image (percentage of the abnormal area). This analysis was performed at 15 points in each eye centrally and 0.6 and 3.7 mm peripherally.

Statistical Analysis

The percentages of the abnormal area variables were compared using generalized estimating equation models to account for a possible correlation between fellow eyes of the same subject.[18,19] The Kolmogorov-Smirnov test, Kruskal-Wallis ANOVA test, and Tukey HSD post hoc test were performed to compare the percentages of the abnormal areas for one eye. Correlations between the percentages of the abnormal area and corneal thickness or Krachmer grading scale variables were illustrated using Pearson correlation coefficients, and the levels of significance of the correlations were determined using generalized estimating equation models. P less than 0.05 was considered significant for all analyses.

Quantification of Guttae Localization in the Central, Paracentral, and Peripheral Zones

The percentages of the abnormal areas to the total area were compared between the corneal center, paracentral zone, and the peripheral zone using generalized estimating equation models. The same analysis also was performed in every eye for every Krachmer grade.

For each eye, the percentages of the abnormal areas were compared among nine zones in the center and paracentral zones and six zones in the peripheral zone using the Kolmogorov-Smirnov test. The same statistical comparison also was performed for the sum of the data from all 41 eyes using generalized estimating equation models.

Quantification of Circumferential Differences of Guttae Distribution Among Six Points in the Periphery

The areas of damage among the six points for every two clock hours 3.7 mm from the center were evaluated. We determined if the percentages of the abnormal areas were affected by a specific location among the six points 3.7 mm from the center using generalized estimating equation models. To examine the location of the abnormalities, the percentages of the abnormal areas were fitted by a least-square approximation plane. The square of an error between an estimated plane and the true value were calculated for six points 3.7 mm from the center of every eye. The plane was determined by the least-square method to minimize the average of all square errors.[20] The direction of inclination of the slope of the least-square approximation plane was determined automatically by this fit using the linear least-square method. Projections of the percentage of the abnormal area in the direction of the inclination were analyzed using regression analysis. To consider enantiomorphism,[21] the horizontally reversed values of the right eyes and the left eye were added.

Quantification of Correlation Between Corneal Thickness and the Percentages of the Abnormal Areas

The corneal thicknesses were measured by specular microscopy. The correlation between the corneal thickness and the percentage of the abnormal areas was examined for the sum of the data from all 41 eyes except for the points at which the corneal thickness values were not obtained by specular microscopy.

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Table 1. Clinical Grading of Fuchs' Endothelial Corneal Dystrophy

Grade	Central or Paracentral Guttae	Edema
1	≤12 scattered guttae, nonconfluent	No
2	>12 scattered guttae, nonconfluent	No
3	1–2 mm (widest diameter), confluent guttae	No
4	2–5 mm (widest diameter), confluent guttae	No
5	>5 mm (widest diameter), confluent guttae	No
6	>5 mm, confluent guttae	Stromal or epithelial edema

Fuchs' endothelial corneal dystrophy corneas were graded based on the number, confluence, and area of the guttae and on the presence or absence of corneal edema. This grading scale is based on the scale devised by Krachmer et al.[7]

Results

The patient ages ranged from 30 to 80 years (mean ± SD, 62 ± 12).

Specular microscopy images were studied in 13 corneas of eight patients with grade 2 disease on the Krachmer grading scale, 8 corneas of six patients with grade 3 disease, 16 corneas of 11 patients with grade 4 disease, and 4 corneas of three subjects with grade 5 disease (Tables 1, 2; Supplementary Table S1).

Comparison of Corneal Endothelial Damage Severity Among Zones

The abnormal areas in the central and the paracentral zones 0.6 mm from the center tended to be large, whereas the abnormal area in the peripheral zone 3.7 mm from the center remained small in every grade (Table 2; Figs. 1, 3). As the grade of the Krachmer scale increased, the percentage of the abnormal area increased in all areas of all zones (Fig. 4). Interestingly, the percentage of the abnormal area at 3.7 mm stayed relatively low (Fig. 4). In every grade, the percentages of the abnormal areas at 3.7 mm were significantly ($P < 0.001$, generalized estimating equation models) smaller compared with the central and 0.6 mm paracentral zones (Fig. 4). However, in every grade, the percentages of the abnormal area at the 0.6 mm paracentral zone did not differ significantly ($P \geq 0.05$, generalized estimating equation models) compared with the center.

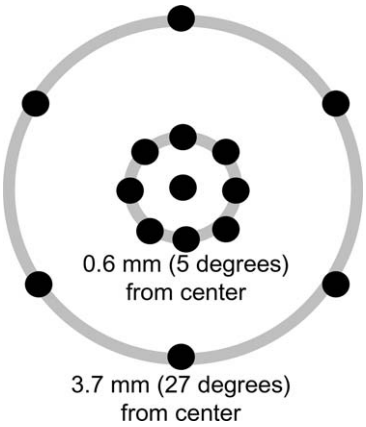

Figure 1. Locations examined by specular microscopy include the central zone and eight points 5 degrees from the center (0.6 mm from center in an eye with a K of 43.5 diopters [D]), and six points 27 degrees from the center (3.7 mm from center in an eye with a K of 43.5 D). The exact distance from the corneal center differs depending on the corneal base curve.

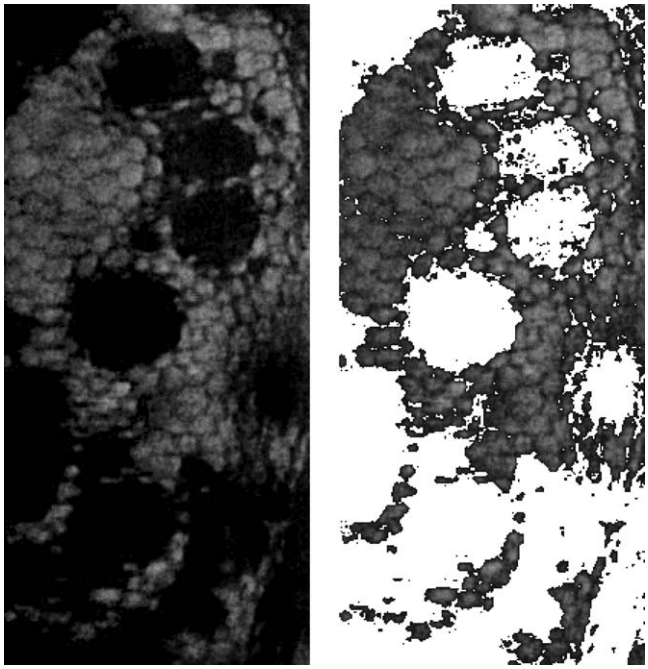

Figure 2. Detection of abnormal areas. The size of the abnormal area with guttae is measured. Areas in which guttae are detected are highlighted in white in the image on the right.

The percentage differences among the nine zones in the center and paracentral zones and six zones in the peripheral zone were examined in each eye. Because no significant differences in the percentage of the abnormal area were seen between one central image and eight images of the 0.6-mm paracentral zone (Fig. 4), nine images of the central and paracentral zones and six images of the 3.7 mm peripheral zone were evaluated statistically in each eye. The abnormal areas in the central and paracentral zones were significantly ($P < 0.05$, Kolmogorov-Smirnov test) larger than in the zone 3.7 mm from the center (Fig. 3) in 36 of 41 eyes. No significant difference was seen in the remaining five eyes ($P \geq 0.05$, Kolmogorov-Smirnov test). Of these five cases, four eyes had grade 4 and one eye had grade 2 disease. In no cases were the abnormal areas in the center and paracentral zones significantly smaller than those 3.7 mm from the center.

When the data from the 41 eyes were totaled, the abnormal areas in the central and paracentral zones represented 68% ± 35% of the total area, and those 3.7 mm from the center represented 33% ± 36% of the total area. The difference reached significance ($P = 2.9 \times 10^{-13}$, generalized estimating equation models).

Abnormal Area in the Peripheral Zone and Objective Grading

In every grade of Fuchs' endothelial corneal dystrophy, the peripheral zone endothelium was less damaged than in the central or paracentral zones. In advanced cases (grades 3 to 5), the central and paracentral endothelium was almost covered completely by guttae and the abnormal area was nearly 100%. Specular microscopy measurement of the corneal center cannot distinguish cases in these grades. However, in every grade the percentages of the abnormal areas in the periphery were smaller

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TABLE 2. Percentages of the Sizes of the Abnormal Areas Compared With the Total Size of the Specular Microscopy Images in the Central, Paracentral, and Peripheral Zones

Position (Clock Hour)	% of Abnormal Area	Central Zone	Paracentral Zone							Peripheral Zone						
			Superior (12)	Superonasal (10.5)	Nasal (9)	Inferonasal (7.5)	Inferior (6)	Inferotemporal (4.5)	Temporal (3)	Superotemporal (1.5)	Superior (12)	Superonasal (10)	Inferonasal (8)	Inferior (6)	Inferotemporal (4)	Superotemporal (2)
Total (grades 2–5)		71 ± 36	65 ± 35	62 ± 36	63 ± 38	65 ± 38	68 ± 38	75 ± 33	74 ± 33	73 ± 31	20 ± 28	25 ± 31	32 ± 37	40 ± 40	41 ± 37	37 ± 38
Grade 2		25 ± 22	22 ± 18	22 ± 17	18 ± 14	19 ± 17	20 ± 14	35 ± 31	36 ± 27	37 ± 24	1 ± 2	1 ± 2.	3 ± 7	6 ± 13	10 ± 12	4 ± 10
Grade 3		92 ± 16	77 ± 24	72 ± 26	69 ± 30	83 ± 20	89 ± 14	92 ± 7	95 ± 7	82 ± 21	5 ± 9	8 ± 11	24 ± 22	20 ± 21	36 ± 24	18 ± 20
Grade 4		94 ± 1	85 ± 18	80 ± 27	86 ± 21	83 ± 28	85 ± 25	91 ± 16	88 ± 20	89 ± 16	31 ± 28	44 ± 34	46 ± 37	60 ± 37	58 ± 40	58 ± 32
Grade 5		100 ± 0	100 ± 0	100 ± 0	100 ± 0	96 ± 8	100 ± 0	97 ± 4	97 ± 5	99 ± 2	45 ± 38	42 ± 18	83 ± 34	95 ± 9	86 ± 18	87 ± 13

The percentages of the abnormal areas examined at 15 points (1 in the central zone, 8 in the paracentral zone, and 6 in the peripheral zone) examined are expressed as the means ± SDs. The Krachmer grading scale values also are shown.

compared with the central and paracentral zones (Fig. 4) and differed depending on the grade. The central guttae in grade 2 is nonconfluent and scattered (Table 1). Clinically, it is easy to separate grade 2 from grades 3 to 5 with confluent central guttae not only by specular microscopy but also by slit-lamp examination. Therefore, we examined the correlation between grades limited to 3 to 5 without 2 and the percentage of the abnormal area. In grades 3 to 5 in the peripheral zones 3.7 mm from the center, the percentages of the abnormal areas differed and were correlated significantly by grade ($P = 5.3 \times 10^{-5}$, $R^2 = 0.452$, generalized estimating equation models) (Fig. 5). However, the percentages in the central and paracentral zones did not differ significantly depending on grade ($P \geq 0.05$, $R^2 = 0.097$ and 0.105, respectively, generalized estimating equation models) (Fig. 5). These results indicated that measurement of an abnormal area of 3.7 mm in the peripheral zone can be objective for grading advanced cases of this disease with grades 3 to 5, which are difficult to distinguish by central specular observation, whereas measurements of the central or paracentral zones cannot.

Circumferential Location of Damage in the Peripheral Zones

The degrees of abnormality among six points 3.7 mm from the center were examined to address whether the degree or disease severity circumferentially varied depending on the location in the peripheral zone. As the grade of the Krachmer scale increased, the percentage of the abnormal area increased at all locations in the peripheral zone (Fig. 6). With every grade, the percentages of the abnormal areas tended to be large inferiorly (Fig. 6). A significant difference ($P = 1.0 \times 10^{-4}$, generalized estimating equation models) was observed among six points in the peripheral zones in 41 eyes.

The percentages of the abnormal areas at three superior points were significantly ($P = 0.0054$, generalized estimating equation models) smaller than at three inferior points. Moreover, the percentages of the abnormal areas at two temporal points (superotemporal and inferotemporal) were significantly ($P = 0.025$, generalized estimating equation models) larger than at two nasal points (superonasal and inferonasal), which indicated that the corneal endothelial cells degenerated more severely inferotemporally. The abnormal areas in the inferotemporal zone were consistently large among the six points 3.7 mm from the center (Fig. 7). However, no significant difference ($P \geq 0.05$, generalized estimating equation models) was seen among eight points in the paracentral zone.

We also evaluated whether abnormalities were oriented in a specific direction among the six points 3.7 mm from the center. To calculate the direction quantitatively and evaluate the most severe positions among the peripheral zones, the percentages of the abnormal areas of endothelial abnormality were fitted by a plane using the least-square method (Supplementary Fig. S2). The percentages of the abnormal areas were well fitted by a simple plane and exhibited no bimodal or nonlinear pattern of distribution. The direction of inclination of the plane was inferotemporal (–54.7 degrees in the left eye) (Fig. 7). The result indicated that the inferotemporal direction tended to be damaged severely among the six points 3.7 mm from the center. In addition, projection of the percentages of the abnormal areas to the tilting direction of the plane showed that the abnormal areas at the inferotemporal site were significantly ($P = 1.0 \times 10^{-4}$, $R^2 = 0.136$, generalized estimating equation models) larger than at the superonasal site (Fig. 7). The percentages of abnormal areas at two inferotemporal points (4 and 6 o'clock) were significantly ($P = 1.5 \times 10^{-4}$, generalized estimating equation models) larger than at two superonasal points (10 and 12 o'clock).

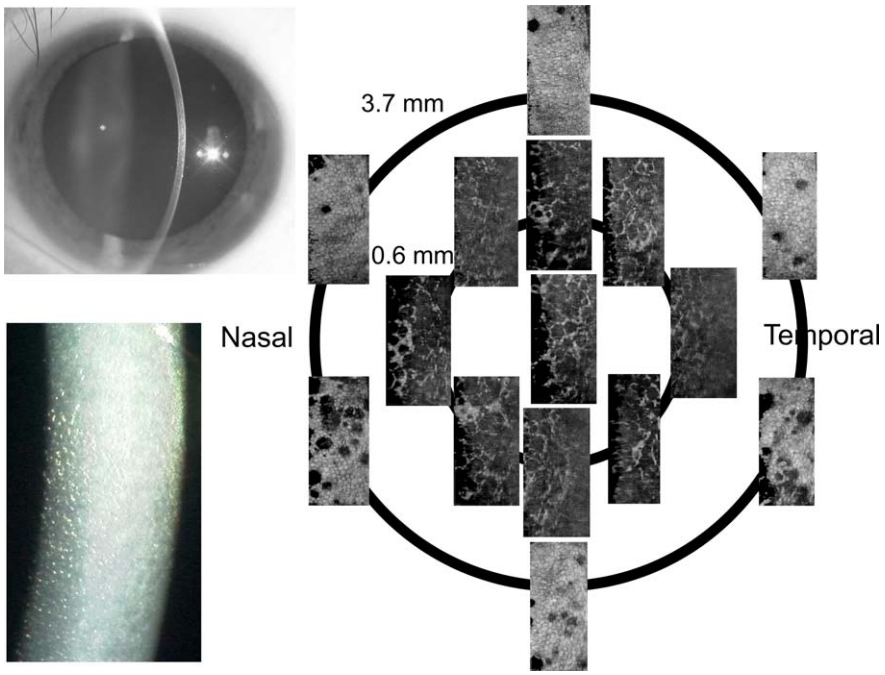

FIGURE 3. Representative image of the left eye of a 30-year-old man with Fuchs' endothelial corneal dystrophy. A slit-lamp photograph (*upper left* and *lower left*), and the specular microscopy images (*right*) are presented. The abnormal areas in nine sites in the central and paracentral areas (center and 0.6 mm from the center) represent 88% ± 6% of the total area, whereas those in the six peripheral areas (3.7 mm) represent 10% ± 8% of the total area. The results differ significantly ($P = 2.1 \times 10^{-11}$, Kolmogorov-Smirnov test).

Correlation Between the Percentages of Abnormal Areas and the Corneal Thickness

Finally, we evaluated the relationship between the percentages of the abnormal areas in the corneal endothelium and corneal thickness at the examined points. In the central and 0.6-mm paracentral zones, the corneal thicknesses were correlated positively with the percentages of the abnormal areas ($P = 1.4 \times 10^{-7}$, $R^2 = 0.090$, generalized estimating equation models, $n = 300$) (Fig. 8). The points at which the corneal thicknesses were not defined by specular microscopy were omitted. However, no significant correlations were observed between the corneal thicknesses and the percentages of the abnormal areas in the 3.7-mm peripheral points ($P = 0.65$, $R^2 = 0.001$, generalized estimating equation models, $n = 206$).

DISCUSSION

In Fuchs' endothelial corneal dystrophy, endothelial cells are damaged more severely in the central and paracentral zones. This is known qualitatively and empirically.[1,4,6,7,22] The disease begins in the central cornea and spreads peripherally, whereas the peripheral endothelium is damaged initially in pseudoexfo-

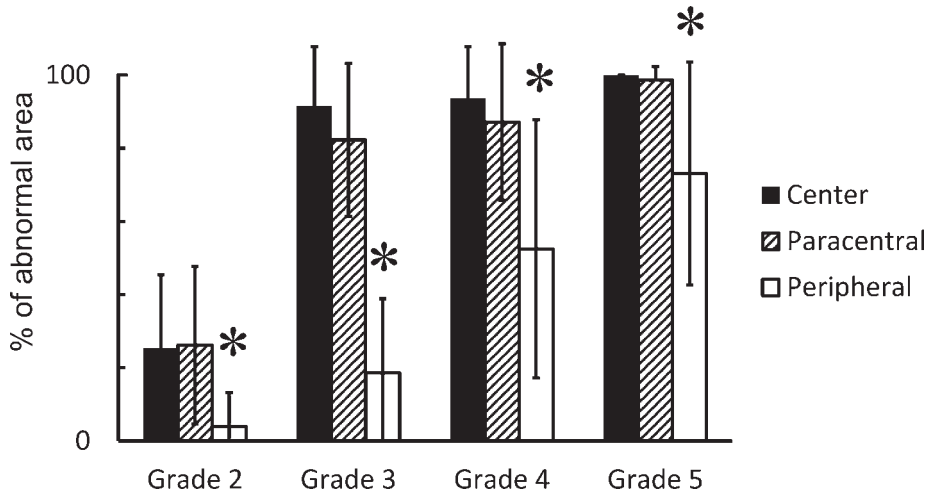

FIGURE 4. The percentages of the abnormal areas in the central, paracentral, and peripheral zones by the Krachmer grading scale. The percentages of the abnormal area in grades 2, 3, 4, and 5 Fuchs' endothelial corneal dystrophy are presented. In each grade, the percentages of the abnormal areas are classified into central, paracentral (0.6 mm from the center), and peripheral (3.7 mm from the center) zones. In grades 2 to 5, the percentages of the abnormal areas in the peripheral zone are significantly ($P < 0.001$, generalized estimating equation models, indicated by *) smaller than those in the central and paracentral zones. The data are expressed as the means ± SD.

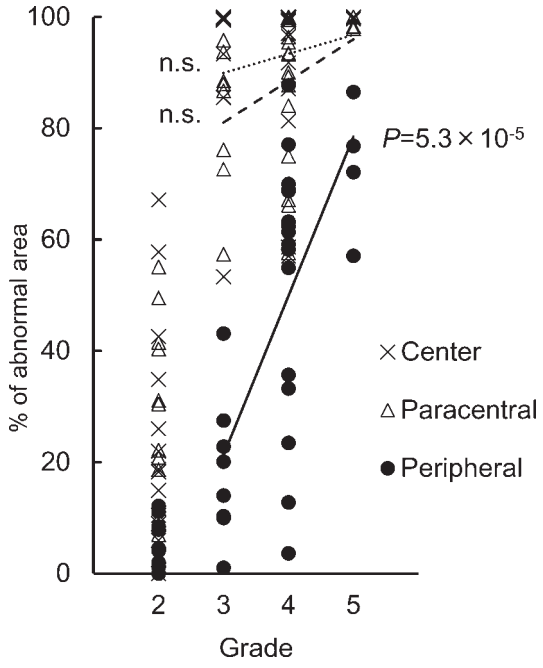

FIGURE 5. The scatterplot shows the relationship between the percentages of the abnormal areas and clinical grade of Fuchs' endothelial corneal dystrophy. The percentages of the abnormal areas in the peripheral zones are correlated significantly with the disease grade (*solid line*), whereas those in the central (*dotted line*) and paracentral zones (*dashed line*) are not in grades 3 to 5. The percentages of the abnormal areas in eight paracentral and six peripheral zones in one eye are averaged and analyzed. n.s., not significant.

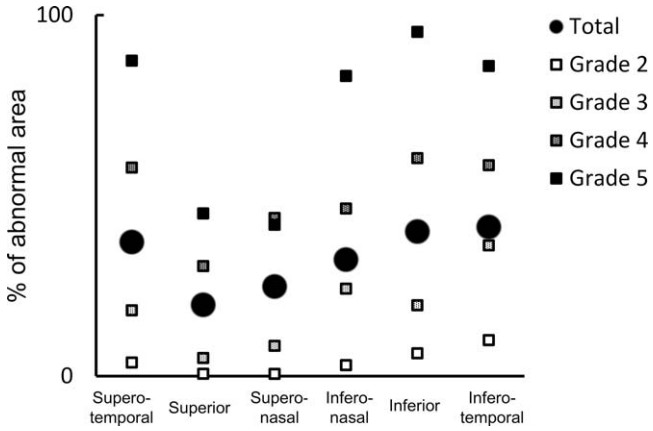

FIGURE 6. The directionally classified percentages of the abnormal areas in the peripheral zones. The percentages of the abnormal area in the peripheral zones are presented based on location. Values are classified by the Krachmer grading scale. The values in all eyes are presented. In every grade, the values in the inferior zones are larger than in the superior zones.

liation syndrome.[23] Hassall-Henle warts are also in the differential diagnosis of Fuchs' endothelial corneal dystrophy, but they also are found earlier in the disease stage in the periphery.[24] This progression of Fuchs' endothelial corneal dystrophy may reflect a pathological property of this disease. In Fuchs' endothelial corneal dystrophy, the central cornea swells more than the peripheral cornea in grade 6 of the Krachmer grading scale.[8] Giasson et al.[22] reported that in subjects with known corneal guttae, the areas of guttae were significantly larger in images of the central cornea compared with the paracentral regions. Moreover, a recent study found that the quotient of the central corneal thickness and 4-mm peripheral corneal thickness is a sophisticated objective clinical grade indicator.[25] In the current study, the endothelial cells were damaged more severely in the central and paracentral zones than in the peripheral zone.

Fuchs[1] reported this disease in 1910 when he mentioned that the corneal opacity is strongest in the pupillary area and develops downward, whereas the upper edge of the cornea remains relatively clear. Graves[26] reported that the disease was confined to the center in the less-pronounced cases, and the abnormalities start near the center of the cornea then take years to spread peripherally. Friedenwald and Friedenwald[27] confirmed that the diffuse corneal opacity in this disease was most intense in the central region and gradually evolved to a clear zone near the margin. Our results confirmed these historical findings[28] quantitatively. Maurice[29] introduced microscopy to examine the corneal endothelium in enucleated eyes at high magnifications. The instruments were developed as clinical noncontact specular microscopes using slit-lamp techniques.[30] Laing et al.[31] divided progression of corneal guttae in Fuchs' endothelial corneal dystrophy into five stages based on specular microscopy. Increased guttae is a characteristic specular microscopy finding in this disease.[32,33] Very few guttata resolved and few new guttata appeared during a 2-year observation of patients with Fuchs' endothelial corneal dystrophy.[34]

One reason for central degeneration of corneal endothelial cells may be that the peripheral corneal endothelium contains precursor cells that compensate for injuries in the periphery,[35,36] whereas involvement of electromagnetic energy or aqueous flow discussed later also may be reasonable. The density of the corneal endothelial precursor cells was significantly higher in the peripheral cornea and decreased toward the center.[35] Corneal endothelial precursor cells may supply new corneal endothelial cells from the periphery. The difference in the areas of guttae between the central and peripheral zones in the current study may reflect the regenerative activity of the endothelial precursor cells and be correlated with the disease progression or prognosis.

Moreover, 3.7 mm from the center, the current results indicated that the inferotemporal area was damaged relatively more severely. The reason is not apparent; however, some pathological mechanisms may be responsible. The first, electromagnetic energy (e.g., ultraviolet light), adversely affects the inferior and temporal areas because of lack of protection by the eyelid or nose.[4,37] The affected areas generally correspond to those that are visible through the palpebral fissure. Rosenblum et al.[38] found that the guttae tended to spread more horizontally than vertically. The current results showed significant differences between the superior and inferior areas and the nasotemporal areas. This implies a subtle difference that cannot be ignored around the corneal endothelial cells in the horizontal and vertical axes. However, the possibility remains that in the current study the corneal center was determined as the primary line of sight and shifted nasally compared with the vertex normal so that the nasal area was determined to be less affected. Second, aqueous flow varies between areas depending on aqueous temperature injury to the endothelial cells. Although studies have not found a consistent correlation between endothelial damage and aqueous flow, possibly because of limited ability to measure the aqueous flow,[39,40] aqueous flow may cause the corneal endothelium injury.

Recent surgical advances include new techniques, such as DMEK.[13,14] An increasing number of patients with early-stage Fuchs' endothelial corneal dystrophy undergo surgery. Recent

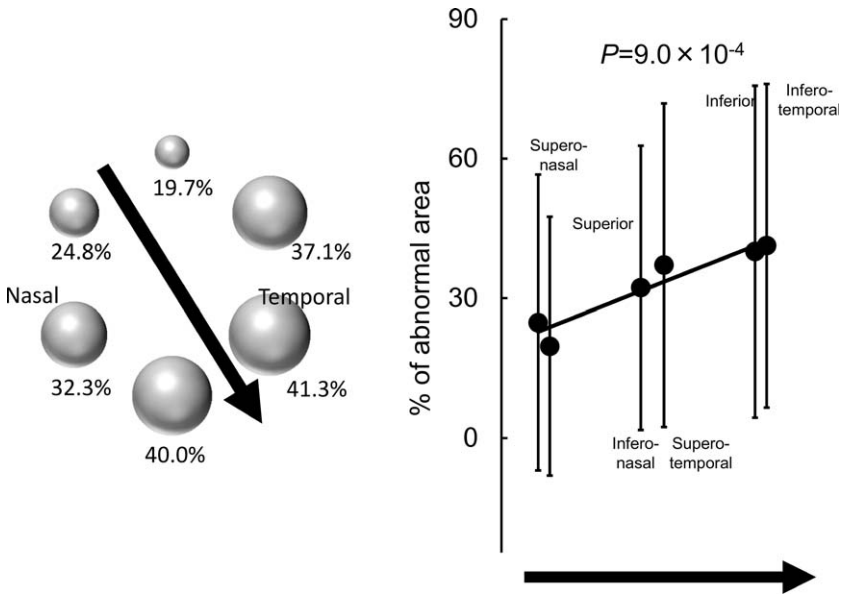

Figure 7. The distribution of the percentages of the abnormal areas in the peripheral zone (*left*). The abnormal areas in the inferotemporal zone are consistently large among the six points studied. From the approximation of the percentages of the abnormal areas by a plane calculated using the least-square method, the abnormal areas are largest inferotemporally (−54.7 degrees in the left eye) (*arrow*) among the six points studied. The values from the left eye and the horizontally reversed values from the right eyes are summed to consider enantiomorphism. The distribution of the percentages of the abnormal areas along the superonasal and inferotemporal axes is shown on the *right*. The percentages of the abnormal areas are projected to the tilting direction of the fitting plane (*arrow*). The results show that the abnormal areas inferotemporally are significantly larger than superonasally ($P = 9.0 \times 10^{-4}$). The data are expressed as the means ± SDs.

studies have shown that guttae evoke forward light scatter and cause visual impairment.[41,42] After DSAEK, forward light scatter remained greater in eyes with Fuchs' endothelial corneal dystrophy than in normal eyes and was correlated with recipient visual acuity.[41] Eyes with Fuchs' endothelial corneal dystrophy that underwent DSAEK had improved contrast sensitivity compared with other untreated eyes despite no difference in the standard Snellen visual quality.[42]

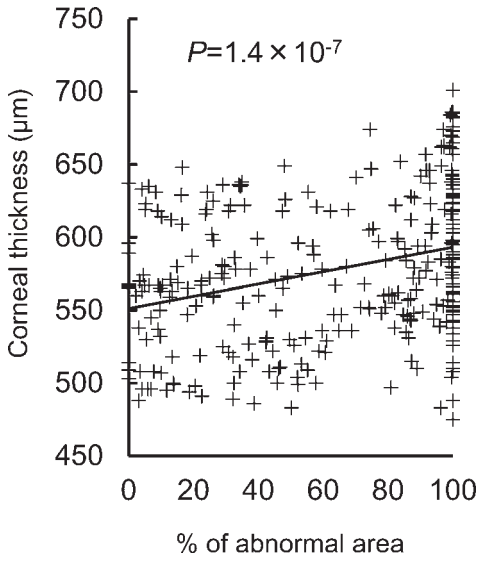

Figure 8. The scatterplot shows the relationship between the corneal thickness and the percentages of the abnormal areas in the center and paracentral zones. They are significantly correlated ($P = 1.4 \times 10^{-7}$, generalized estimating equation models, $n = 300$).

It is important to evaluate visual impairment resulting from guttae without edema. Quantitative measurement of guttae distribution is necessary preoperatively. The current results showed damaged endothelium centrally and in the paracentral zone regardless of the stages of the Krachmer grading scale, although the endothelium remained relatively intact in the peripheral zones especially in the early stages. This implies that guttae in Fuchs' endothelial corneal dystrophy cause visual impairment in the early and advanced stages. In the future, investigation should be undertaken of guttae around the entrance pupil and subtle visual quality changes, such as contrast sensitivity or forward light scatter.

Recent studies have reported a method for objectively grading[25,43] or predicting progression of Fuchs' endothelial corneal dystrophy.[44] The peripheral corneal thickness[25] and the central ratio of guttae[43] are especially useful for objective grading. In advanced cases (grades 3 to 5), the percentages of the abnormal areas differed and were correlated significantly with the grade. However, the central and paracentral endothelia were almost covered completely by guttae, and measuring the abnormal areas in the central and paracentral zones did not distinguish the grades. The current results indicated that the peripheral guttae ratio may be useful for distinguishing grades 3 to 5 and provide another way to objectively grade the disease.

The corneal thickness was correlated significantly with the percentages of the abnormal areas in the center and 0.6-mm paracentral zones but not in the 3.7-mm peripheral zone. In the center and paracentral zones, the ratio of guttae may affect the extent of endothelial pump dysfunction, and the resultant corneal edema may reflect the ratio of guttae. However, no significant correlation was observed in the periphery, a possible reason for which may be that the corneal thickness variations are larger in the peripheral zone than in the central zone. The local change in corneal thickness depending on the

guttae ratio may be smaller than the dispersion of the peripheral thickness.[45]

The current results identified the location of guttae in Fuchs' endothelial corneal dystrophy. However, our examination was limited to 15 corneal points including the peripheral zone but not the entire cornea. An ultra-wide-field specular microscopy study is needed in the future. The current study also was limited in that one patient at a time was examined and not followed chronologically. Further studies are needed to study the comprehensive progression of Fuchs' endothelial corneal dystrophy. Age-related changes in the location of the guttae are important to determine disease prognosis and pathology. In the future, evaluation of damaged endothelial cells, including those in the peripheral zone, might be useful to determine a surgical indication or predict prognosis.

In summary, the corneal endothelium is damaged more severely in the central zone in Fuchs' endothelial corneal dystrophy, and the percentage of the abnormal area in the peripheral zone can be effective for objective grading of the disease in advanced cases. In the peripheral zone, the cells were more damaged inferotemporally than superonasally. These findings might facilitate an understanding of the disease mechanisms.

Acknowledgments

Presented in part at the annual meeting of the Association for Research in Vision and Ophthalmology, Seattle, Washington, United States, May 6, 2013.

Supported in part by Scientific Research from the Japanese Ministry of the Education, Culture, Sports, Science and Technology Grant 25861630 (HF).

Disclosure: **H. Fujimoto**, None; **N. Maeda**, None; **T. Soma**, None; **Y. Oie**, None; **S. Koh**, None; **M. Tsujikawa**, None; **K. Nishida**, None

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