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

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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 53% ± 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, R² = 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...
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 pseudoguttae15,16 and pseudoxfoliation 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. This grading was calculated for every specular microscopy image (percentage of the abnormal area). This percentage of the pixels covered by the abnormal area was calculated using a method to minimize the average of all square errors.20 The original specular microscopy images usually have horizontal inclination, 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.
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 ≥ 0.05, generalized estimating equation models) compared with the center.

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 ≥ 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 × 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.

### Table 1. Clinical Grading of Fuchs’ Endothelial Corneal Dystrophy

<table>
<thead>
<tr>
<th>Grade</th>
<th>Central or Paracentral Guttae</th>
<th>Edema</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>≤12 scattered guttae, nonconfluent</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>&gt;12 scattered guttae, nonconfluent</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>1–2 mm (widest diameter), confluent guttae</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>2–5 mm (widest diameter), confluent guttae</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>&gt;5 mm (widest diameter), confluent guttae</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>&gt;5 mm, confluent guttae</td>
<td>Stromal or epithelial edema</td>
</tr>
</tbody>
</table>

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
Central and Peripheral Zones in Fuchs’ Dystrophy

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

<table>
<thead>
<tr>
<th>Abnormal Area</th>
<th>Central Zone</th>
<th>Paracentral Zone</th>
<th>Periphe ral Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Superior</td>
<td>Superonasal</td>
<td>Nasal</td>
</tr>
<tr>
<td>Position (Clock Hour)</td>
<td>12 (12)</td>
<td>10.5 (7.5)</td>
<td>9 (6)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>71 ± 36</td>
<td>65 ± 35</td>
<td>62 ± 36</td>
</tr>
<tr>
<td>Grade 3</td>
<td>25 ± 22</td>
<td>22 ± 18</td>
<td>22 ± 17</td>
</tr>
<tr>
<td>Grade 4</td>
<td>92 ± 16</td>
<td>77 ± 24</td>
<td>72 ± 26</td>
</tr>
<tr>
<td>Grade 5</td>
<td>94 ± 1</td>
<td>85 ± 18</td>
<td>80 ± 27</td>
</tr>
<tr>
<td>Grade 6</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
</tr>
</tbody>
</table>

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 are also shown.

Circumferential Location of Damage in the Periphe ral Zones

The degrees of abnormality among six points 3.7 mm from the center were examined to address whether the degree of 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. In grades 5 to 6, however, the percentages of the abnormal areas tended to be large at all locations in the peripheral zone. The percentages of the abnormal areas at three temporal points were consistently large among the six points 3.7 mm from the center (Fig. 7). A significant difference was observed among six points in the peripheral zone (Figs. 5). These results indicated that measurement of an abnormal area in grades 5 to 6 was easier to measure the degree of abnormality among six points 3.7 mm from the center. In grades 5 to 6, the percentages of the abnormal areas were significantly larger than at three inferior points. Moreover, the percentages of the abnormal areas at three inferior points were significantly 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 significantly larger than at three inferior points. In addition, projection of the abnormal areas at two nasal points (superonasal and inferonasal) were significantly larger than at two nasal points (inferonasal and inferotemporal). Moreover, the percentages of the abnormal areas at two nasal points (superonasal and inferonasal) were significantly larger than at two nasal points (inferonasal and inferotemporal) (Figs. 6). A significant difference was observed among six points in the peripheral zone (Fig. 7). The percentages of the abnormal areas at six temporal points were significantly larger at three inferior points at the superior site were significantly larger than at the superonasal site (Fig. 5).

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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-
Central and Peripheral Zones in Fuchs’ Dystrophy

**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 in one eye are averaged and analyzed. n.s., not significant.

Recent surgical advances include new techniques, such as DMEK. An increasing number of patients with early-stage Fuchs’ endothelial corneal dystrophy undergo surgery.
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 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 grading25,43 or predicting progression of Fuchs’ endothelial corneal dystrophy.44 The peripheral corneal thickness25 and the central ratio of guttae43 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 paracentral zones, but not in the 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

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**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.

**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, $n = 300$).
guttae ratio may be smaller than the dispersion of the peripheral thickness.\textsuperscript{15}

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.

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**References**


