Progression of Fuchs Corneal Dystrophy in a Family Linked to the FCD1 Locus

Danielle N. Meadows,1,2 Allen O. Egbrari,1,2 S. Amer Riazuddin,3 David G. Emmert,1 Nicholas Katsanis,3,4 and John D. Gottsch1

PURPOSE. Fuchs corneal dystrophy (FCD) is a progressive corneal disease marked by the development of guttata, focal excrescences of Descemet’s membrane. Retroillumination photography is a useful technique for illustrating the presence of guttata and has been used to document progression of disease. This study was undertaken to quantitatively assess disease progression in a cohort of individuals with late-onset FCD linked to chromosome 13.

METHODS. Retroillumination photography was performed on 13 related individuals (26 eyes) with the FCD1 disease haplotype at a 30- to 34-month interval. Individual guttata were counted in each image and the distribution recorded. A polar coordinate system was used to delineate regional differences in development of guttata.

RESULTS. An increase of 29.1% was found in the total number of guttata over approximately 30 months (mean increase of 669 guttata/eye, \( P < 0.001 \)) among 26 eyes. A rapid rate of progression begins at approximately age 50, representing an exponential increase \( (r^2 = 0.60) \) among individuals mildly affected for decades. Individuals with the disease haplotype but with two affected parents demonstrated an earlier disease onset. A significantly greater proportion of guttata were present in the inferotemporal quadrant of the cornea \((P < 0.001)\), an effect that grew in significance over time.

CONCLUSIONS. The study demonstrated quantitative progression of FCD with the use of retroillumination photography in an FCD1-linked pedigree. Comparison of severity versus age suggests a rapid increase in the number of guttata at approximately age 50. Individuals with the FCD1 disease haplotype and a second likely genetic lesion exhibit a markedly increased disease severity suggestive of genetic interaction between FCD loci. (Invest Ophthalmol Vis Sci. 2009;50:5662–5666) DOI: 10.1167/iovs.09-3568

Early a century ago, Ernst Fuchs first described “dystrophia epithelialis cornea,” a condition among 13 patients that included stromal edema, opacity, and loss of corneal sensation.1 Within several years of Fuchs description, “dimples” were identified in the posterior cornea of patients with the disease.2 Vogt described these excrescences as “drop-like,” leading to use of the term corneal guttata.3 Histologic analysis of corneal sections in FCD confirmed the presence of guttata4 and specular microscopy documents guttata distributed across the field.5

FCD typically presents in middle age as a slowly progressive,6,7 bilateral disease with greater severity in females.1,8 A grading system to document progression of FCD was first described by Krachmer et al.9 in 1978. In this scale, grade 1 has a minimum of 12 central, nonconfluent guttata, with grades 2 to 4 representative of increasing area of confluence and grade 5 indicating the presence of stromal or epithelial edema. We have used this scale to document the relationship of increasing age with increased severity of the disease in early- and late-onset FCD.9–11

In the early-onset form of FCD, correlated with an L450W mutation in COL8A2,9 dense, low-elevation guttata have been documented as early as the first decade of life.9,12 In contrast, late-onset phenotypes associated with the FCD1 (13pTel-13q12.13)10 and FCD2 (18q21)11 loci manifest closer to the fifth decade and are associated with coarser, more distinct guttata.

The variability of age and severity in the clinical presentation of FCD produces a challenge in the assessment and documentation of its various phenotypes. A definitive method is needed to delineate the relationship between each phenotype and its corresponding genotype.

Using retroillumination photography, we have demonstrated formation of new guttata in a 30-month time interval.13 These data suggest that measurements of the number and distribution of guttata over time by this technique represent an effective, quantitative profile of disease progression, although it has been difficult to determine whether guttata distribution and rate of appearance can correlate with specific FCD-causative genetic lesions. In beginning to address this question, we used retroillumination photographs taken at 30-month intervals of affected family members in a large pedigree linked to the chromosome 13 FCD1 locus,10 to determine whether quantitative progression of disease could be documented and correlated to FCD1 disease genotype.

METHODS

Recruitment

Previously, 34 individuals of a family with Fuchs dystrophy were genotyped to 13pTel-13q12.13.10 Retroillumination images were acquired from 18 affected family members initially willing to participate; 13 individuals followed up by having sequential photographs taken at a 30- to 34-month interval. Those individuals in the pedigree (Fig. 1) that possess the disease haplotype were photographed at baseline and after a 30- to 34-month interval. Of these, three individuals had inher-

From the 1Center for Corneal Genetics, Cornea and External Disease Service, The Wilmer Eye Institute, the 2Mckusick-Nathans Institute of Genetic Medicine, and the 3Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, Maryland.

2Contributed equally to the work and should therefore be considered equivalent authors.

Supported by National Eye Institute Grant R01EY016835 (JDG), Faller Family LLC, Research Fund, and National Institute of Child Health and Development Grant R01HD04260 (NK).

Submitted for publication February 13, 2009; revised March 11, 2009; accepted August 27, 2009.

Disclosure: D.N. Meadows, None; A.O. Egbrari, None; S.A. Riazuddin, None; D.G. Emmert, None; N. Katsanis, None; J.D. Gottsch, None.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked ‘‘advertisement’’ in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: John D. Gottsch, The Wilmer Eye Institute, Johns Hopkins Hospital, Maumee Building, Room 321, 600 N. Wolfe Street, Baltimore, MD 21287; jgottsch@jhmi.edu.

Copyright © Association for Research in Vision and Ophthalmology
The disease haplotype from one parent, with the other parent also affected but without the disease haplotype.

The study protocol was approved by the Joint Committee on Clinical Investigation at The Johns Hopkins University School of Medicine and was in accordance with the tenets of the Declaration of Helsinki. Written, informed consent was obtained from all study participants after explanation of the nature and possible consequences of the study. The study is in accordance with HIPAA regulations.

**Imaging**

After pupillary dilation of both eyes in each participant, retroillumination images were obtained with a photo slit lamp (Carl Zeiss Meditec, Dublin, CA), as described. In summary, a digital camera (D2xs Nikon Corp., Tokyo, Japan) was used with a 12× magnification changer. Flash power averaged 460 W (range, 240–720), and the lens aperture was set between F32 and F44. A minimum of four photographs were produced of each eye: two with the retroillumination beam from the right, and two with the retroillumination beam from the left.

**Data Analysis**

Images were imported into image analysis software (Photoshop CS4 Extended Package; Adobe Systems Inc., San Jose, CA). Images from individuals without disease or who did not display the disease haplotype were excluded from the analysis.

Characteristic groups of guttae were used to align consecutive images, and a composite representation used corneal images contralateral to the beam. A polar coordinate system (Fig. 2) was developed consisting of eight concentric zones and twelve 30° divisions for a total of 96 sampled sectors. The grid was centered at the central pupil and expanded to include all potentially visible guttae.

Individual gutta in each sector were identified and summed manually on computer (Photoshop Count Analysis; Adobe Systems, Inc., Redmond, WA) for data analysis. Differences in superior versus inferior or nasal versus temporal distribution of guttae were then assessed through statistical comparison of symmetrically opposing quadrants. Coordinates for guttae were determined, to obtain an overall trend of distribution in gutta formation. The average distance and angle of each of the 96 sectors was calculated, and the guttae in each sector summed.

Linear and exponential regression analyses were used to examine the effect of age and density on the cumulative development of guttae (Excel; Microsoft Corp.).

**RESULTS**

Two consecutive sets of images were analyzed from each eye of 12 individuals (9 men, 3 women; average age, 47 years) who were determined to carry the disease haplotype (Fig. 1). We counted the total number of guttae throughout the cornea for all individuals (n = 136,883; Table 1). In total, we found a 29.1% increase in the number of guttae during this interval among all eyes (average increase of 669 guttae); the increase was significant (P < 0.001, paired t-test). Moreover, the total proportion of guttae in the inferior hemisphere of the cornea increased over time from 54.5% to 58.1% of total guttae, a tendency that grew stronger in significance (baseline P < 0.01, post-interval P < 0.0001). Similarly, a comparison between inferotemporal and superonasal quadrants demonstrated an increasingly significant inferotemporal distribution over time (P < 0.0009 - 0.0005). An example of these changes is illus-
trated in Figure 3. We found no significant difference in the rate of formation of guttae between right and left eyes (31.5% and 27.2%, respectively; \(P < 0.84\)).

We confirmed this inferotemporal trend through averaging of coordinates for each gutta and development of a summary vector (Fig. 2). A total of 96 sectors in each of 13 images from each eye were averaged at baseline and repeated postinterval. Guttae continued to develop in an inferotemporal distribution during this interval, centered at an increasingly inferior point.

We found density to be consistently highest in the center and increased throughout each concentric zone during this interval (\(P < 0.0001\), paired \(t\)-test). At both time points, distance from the center was strongly correlated with a decrease in density (\(r^2 < 0.96\)). As the guttae were increasingly distributed throughout the cornea over time, density increased at the greatest rate in the periphery, at a rate approximately 1.8 times that in the central zone. Advanced cases, representing highest density, widespread confluence and complete coverage of the cornea, experienced a relatively lower total percentage of formation of new guttae, as demonstrated in Figure 4.

We also calculated the total increase in guttae in each eye (Fig. 4). A rapid rate of increase in total number of guttae was present at approximately age 50. Cumulative assessment of individuals with the disease haplotype and a single affected parent was consistent with an exponential rate (\(r^2 = 0.60\)). In a notable finding, three individuals with the disease haplotype who represent offspring of two affected parents, as indicated in the pedigree, experienced early onset of disease.

**DISCUSSION**

Using retroillumination photographic analysis, we demonstrate specific patterns of progression that correlate to the previously described FCD1-linked pedigree.\(^{10}\)

All individuals had a quantitative increase in severity over time, as defined by the total number of guttae in each eye. This number increased rapidly among individuals at approximately 50 years of age, consistent with our previous findings among patients with late-onset FCD according to the Krachmer grading scale.\(^9\) The assessment of two individuals with the disease haplotype who are mildly affected as defined by the Krachmer grading scale in the fourth to fifth decade of life suggests that rates of increase in guttae may represent an exponential pattern of progression, with earliest signs present decades before clinical presentation. Our data predict that prospective individ-

**TABLE 1.** Total Number and Proportion of Guttae in Each Quadrant of the Cornea at Baseline and after Interval of Approximately 30 Months, across 26 Images at Each Time Point.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>30 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Guttae (n)</td>
<td>Percentage of Total</td>
</tr>
<tr>
<td>Inferotemporal</td>
<td>17,106</td>
<td>29</td>
</tr>
<tr>
<td>Superonasal</td>
<td>12,823</td>
<td>21</td>
</tr>
<tr>
<td>Superotemporal</td>
<td>14,349</td>
<td>24</td>
</tr>
<tr>
<td>Inferonasal</td>
<td>15,466</td>
<td>26</td>
</tr>
</tbody>
</table>

\* Significant difference in mean number of guttae through comparison of individual quadrant versus inferotemporal quadrant among 26 eyes by paired \(t\)-test.

The total number of guttae increased throughout the cornea, with greatest proportion in the inferotemporal quadrant.

**FIGURE 3.** Comparison of retroillumination photographs before (left) and after (right) 30-month interval in right eye of individual III6, carrier of the FCD1 disease haplotype associated with the 13pTel-13q12.13 locus.\(^{10}\) Top: guttae were distributed inferiorly and temporally. Bottom: the image within the rectangle. Identical sampling regions illustrate progression after 30 months, demonstrated by the greater relative prominence of previously identified guttae (circled, white) and appearance of newly discernible guttae (circled, green).
FIGURE 4. Total number of guttae by age, with (A) linear and (B) logarithmic axes. Guttae developed at an exponential rate, with an increase at approximately age 50. Early onset of disease was manifested in individuals IV1, IV2, and IV4, who possessed the disease haplotype but represented offspring of two affected parents. Individual III4 demonstrated a limited rate of development of guttae, which may be due to environmental factors. Exclusion of this individual results in a factor of 0.12 and an $r^2 = 0.89$. (C) Percentage increase in total guttae during the interval based on the initial count. In advanced cases, density and confluence of guttae increase throughout the cornea, resulting in a decreased percentage of increase in guttae.
uals with the haplotype between the ages of 20 and 30 may exhibit less than 10 corneal guttae and as such not receive a clinical diagnosis of FCD by the Krachmer scale.

In our clinical assessment of family members, we encountered three individuals with the disease haplotype who demonstrated an earlier onset and progression of disease. We have hypothesized previously that in individuals who have inherited FCD-causing alleles from both parents, disease manifestation is accelerated by two to three decades.\(^\text{10}\) In our present findings, we confirm disease progression in these individuals at an early age. Similar to previous findings among a family with an early-onset \(\text{COL8A2} \ L450W\) phenotype,\(^\text{9}\) the disease, once initiated, appears to progress at a rate similar to cases of later onset. In addition, we noted that one individual in the seventh decade of life experienced markedly less severe FCD than others in the family of the same age and with the same disease haplotype. This patient as an outlier may have been exposed to environmental or genetic protective effect(s).

In this family, the majority of guttae in each eye were distributed in the inferior half of the cornea, consistent with our previous clinical observations in late-onset FCD.\(^\text{13}\) However, quantitative measurement of the rate of development of guttae now shows that this is not only a static phenomenon, but a dynamic one that increases in significance over time. We also demonstrate a similar inferotemporal pattern of guttae formation. This distribution may be related to external environmental factors such as light exposure or related to internal aqueous dynamics.

In summary, we used retroillumination photography to quantify and document the progression of gutta formation in a pedigree genotyped to 13pTel-13q12.13. This photographic technique may be useful in quantitatively determining the phenotypic trait of gutta formation in other genotypes identified with Fuchs dystrophy including \(\text{FCD2} (18q21.2-q21.32)\)\(^\text{11}\) and early-onset Fuchs dystrophy (L450W, \(\text{COL8A2}\)).\(^\text{9}\) Differences in progression may represent a distinguishing phenotypic trait among the various genotypes of FCD. Changes in rates of progression for a given phenotype would be an essential parameter to monitor in any therapeutic clinical trial.

Acknowledgments

The authors are grateful to all family members for their enthusiastic participation in this study. The proband was initially identified by Irene Maumenee.

References