Analysis and Documentation of Progression of Fuchs Corneal Dystrophy With Retroillumination Photography

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Purpose: Fuchs corneal dystrophy (FCD) is a degenerative disorder of the cornea that is characterized by the progressive accumulation of guttae, which are small excrescences of Descemet's membrane. We describe a method for documenting the location and number of guttae, and ask whether disease progression can be observed during relatively short periods.

Methods: Patients with FCD were imaged by standard retroillumination photography with a slit lamp. Scanned photographs were analyzed by using NIH ImageJ software to determine the number of individual guttae and areas of confluence.

Results: In 4 FCD patients, photographs taken 23 to 30 months apart revealed that, once formed, individual guttae and their relative positions persisted during this period. Very few guttae disappeared, and the emergence of many new guttae was observed. Determination of the area with confluent guttae was used to quantify disease stage.

Conclusions: Computer-assisted analysis of retroillumination photographs is proposed as an effective way to document the number and distribution of individual guttae. Although the disease typically progresses slowly during decades, we have been able to detect the formation of new guttae within only 2 years. This rapid assessment of disease progression could be used to measure phenotypic differences between genetic subtypes of FCD. It also could provide important baseline information and methodology for clinical trials of therapeutic options, should these become available.

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In Fuchs'[1] initial description of the dystrophy that today bears his name, he noted a progressive disease of mostly elderly women with roughened epithelial changes. Vogt[2] described the characteristic early feature of the disease as posterior dew-drop-like excrescences, which he later termed guttae.[3] Graves[4] used slit-lamp observation of guttate to describe early accumulation of guttae in the central cornea, peripheral spread in advanced disease, and involvement of the endothelium. He also noted the benefit of retroillumination to demonstrate the extent of posterior corneal involvement. Graves observed 22 cases of Fuchs corneal dystrophy (FCD) during 2½ and found that none of these progressed from early to late stages. Goar[5] followed 1 patient during a 4-year period and observed increases in clouding of the posterior cornea with a decrease in vision from 20/40 to 20/70. Krachmer et al[6] studied 64 families with FCD and was able to document that disease severity correlated with increased age of the patient.

We have recently obtained similar results for 63 families with late-onset FCD. By using Krachmer's grading scale, we found a distinct age-severity progression for a single, large family with inherited FCD caused by a mutation in the *COL8A2* gene. Two members of this family were evaluated originally with early-stage disease in the 1970s and were found to have late-stage disease when re-examined 29 years later.

Krachmer defined 5 grades of disease severity, based on the presence of >12 guttae for the first, with more advanced stages defined by the width of a central region of densely packed, confluent guttae, and distinguished from each other by the width, in millimeters, of this central region. Grade 4 was defined by a region of confluent guttae wider than 5 mm, and grade 5 was defined by further progression to stromal or epithelial edema. This scale has proven to be useful for demonstrating correlations between patient age and the stage of disease in relatively large groups,[6,7] with approximately 5 to 8 years between stages. However, the resolution of this method is not adequate to detect progression of FCD individual patients during short periods, such as 1 or 2 years. In this report, we use retroillumination photography and computer-assisted analysis to follow the persistence and formation of individual guttae. During a span as short as 17 months, we have detected disease progression in single patients.

MATERIALS AND METHODS

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. Patients were recruited for study after initial evaluations of patients with FCD who presented to the Cornea Service at The Wilmer Ophthalmological Institute. Patient recruitment and participation followed institutional review board-approved procedures.

Photographic Documentation

After pupillary dilation, patients were positioned for examination of the cornea with reflected light from the

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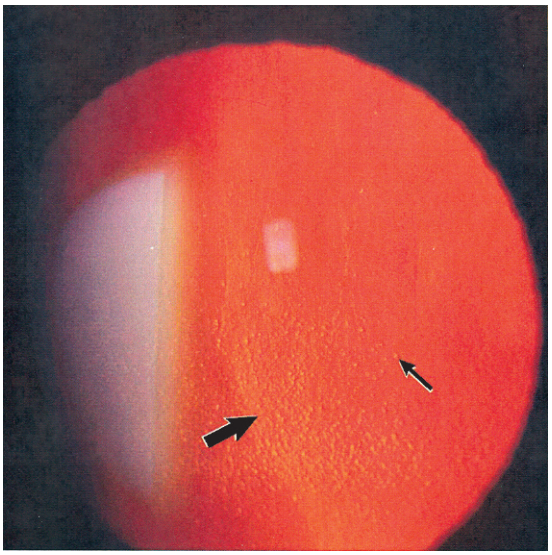

FIGURE 1. Retroillumination photograph. Thin arrow: individual guttae. Thick arrow: confluent guttae. Micrometer calibration gives widest (vertical) dimension of disk as 7.88 mm.

fundus, in which the angle between the biomicroscope and the illumination is small. The slit beam is removed as far as possible from the field by means of an adjustment on the slit lamp.[8] Guttae at the level of the endothelium scatter light and are readily visible (narrow arrow) as well as confluent guttae (thick arrow). Photographic documentation of both eyes was obtained with retroillumination using a Zeiss photo slitlamp camera at 1.6× magnification. For each eye, 6 photographs were taken: 3 with the illumination beam from the right, and 3 with the retroillumination beam from the left. Camera settings included a magnification changer 12× with a 1.6 tele-extender in front of the camera back. Flash power was 720 watt per second and camera aperture was set at f44. Ektachrome 200 film was used and was processed normally.

Computer-assisted Manual Photographic Analysis

Analysis of photographs was performed by scanning the images with 12 to 16 bits per color with a full picture memory size of 35 megabytes per picture with 2× line averaging. The scanned illumination photograph were archived as a JPEG image and analyzed with ImageJ software, a public domain Java image processing program by *NIH image* (http://rsb.info.nih.gov/ij). Using ImageJ, the raw JPEG image is color split. The illumination reflection is mostly in the blue and green splits and can be reduced.

RESULTS

Retroillumination Photography

Retroillumination photography readily demonstrates 2 main pathologic features: individual, isolated guttae, and areas of coalescence of guttae or confluence (Fig. 1). Confluence is defined as the joining of 2 or more guttae into a single structure, often with the appearance of a ridge or plateau. Early confluent areas are characterized by extensive ridges, which may be interspersed with individual guttae. By late-stage disease, the central confluent area has a fine grained, pock-marked quality, in which no individual, isolated guttae are visible.

ImageJ Analysis of Guttae and Confluent Areas in Photographs

Figure 2 shows how a retroillumination image was imported into ImageJ, where it was processed to select the red component, which decreased effects of the central reflection. The grayscale image was imported into ImageJ, and using the crosshair tool, excrescences were marked from the periphery to centrally. This was continued until all guttae were counted, or until those remaining were confluent, could no longer be individually counted, and a boundary was established. The area within these boundary guttae was outlined by tracing a freehand curve. The number of individual guttae, their x and y coordinates, and the circumscribed area were recorded (units in square pixels).

ImageJ calculated an area of 25,982 square pixels within the yellow curve, corresponding to 6.99 mm^2 (Fig. 3D). The conversion of image pixels to millimeters in the cornea was deduced from photographs of a 1-mm stage micrometer, which spanned 61 pixels; 3,721 square pixels in the image

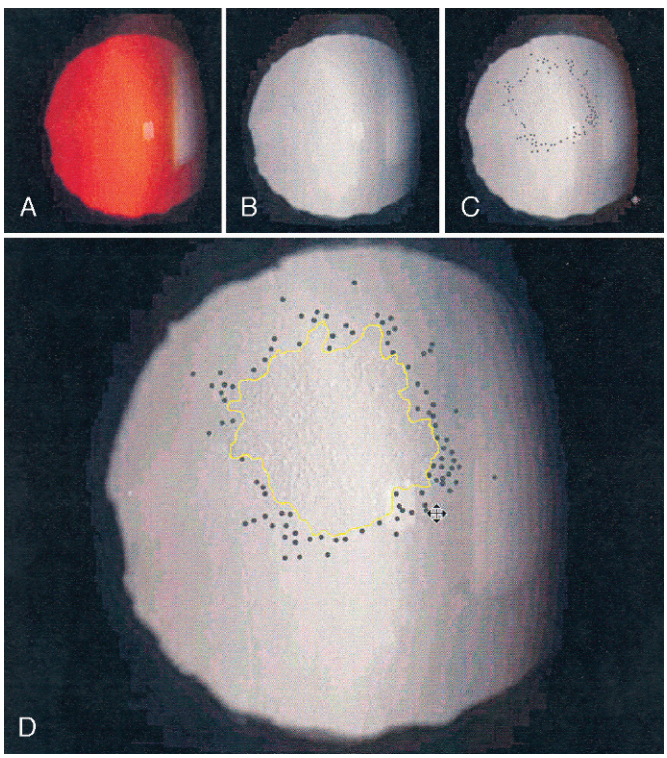

FIGURE 2. Example of computer-assisted analysis. A, Retroillumination photograph B, Color split to red, rendered in grayscale. C, Individually marked guttae with crosshair tool. D, Area of confluent guttae are outlined with yellow curve using the tracing tool.

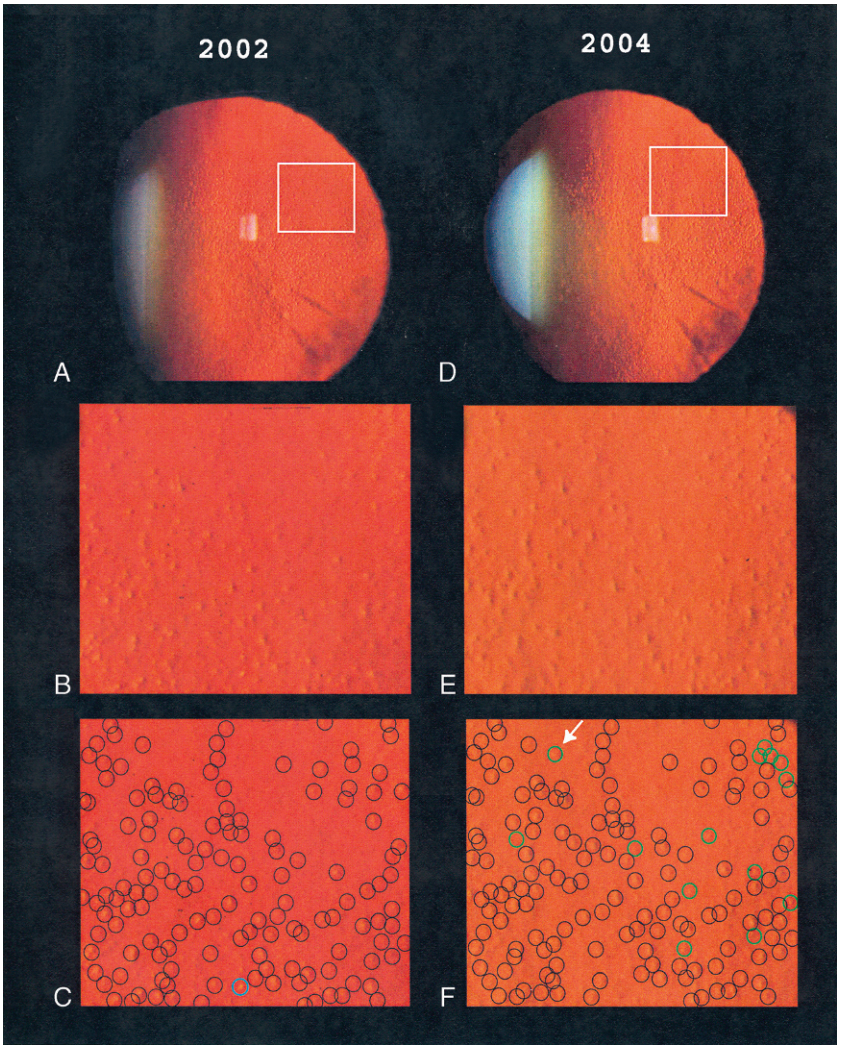

FIGURE 3. Constant and variable features of guttae. FCD patient 1 at age 73 and at age 75. Retroillumination photographs taken 29 months apart in 2002 and 2004. Anterior cortical spokes are noted in both photographs and serve as landmarks. Progression of guttae are noted at arrows. The blue circle in C may represent a gutta that has regressed, cell debris, or some other feature.

corresponded to 1 mm^2. Once the region of confluence has been identified, this provides a rapid and objective means of quantifying progression of the disease, which seems associated with defective endothelial cell function, which leads to corneal edema in late-stage disease.

Changes in Guttae Over Time

Five eyes of 4 patients with the pathology of FCD were photographed in 2002 and 2004. Individual guttae were marked on retroillumination photographs and compared (Fig. 4). In each pair of photographs, all guttata were found at the same position at both the beginning and end of the period. With rare exceptions, there was no clear evidence to indicate that guttae were lost during this period or that they shifted position relative to their neighbors. This excludes regions of lower photographic quality, in which no conclusions could be drawn. The appearance of new guttae could be clearly demonstrated during the 2-year period in 3 of 4 patients.

Figure 3A, D represents a pair of photographs taken 29 months apart. Distinctive radial spokes from cataracts are visible in the lower right quadrant. A rectangular region was selected in the 2002 photograph using patterns of guttae at the corners and along the edges as landmarks. These same landmarks were identified in the 2004 photograph and allowed us to redraw the rectangle over the same territory. There was approximately 2% distortion of the vertical and horizontal dimensions, possibly an optical effect caused by slightly different position of the eye. Great persistence of the pattern of guttae allowed precise placement of the second rectangle in the 2004 photograph (Fig. 3B, E). Marks indicating individual guttae were replaced with black circles to indicate guttae that were observed in the corresponding photographs (Fig. 3C, F). Most of these guttae appeared to be similar in size. Many seem to be larger at the later date, although cannot be readily quantified.

Of special interest are the cases in which new guttae appear in the 2004 photograph (green circles, Fig. 3F). In the analysis of this region, there are 141 persistent or growing guttae and 14 new guttae. Some of these new guttae are quite prominent (arrow Fig. 3F). In most cases, new guttae appear within the existing matrix of individual guttae and ridge-like

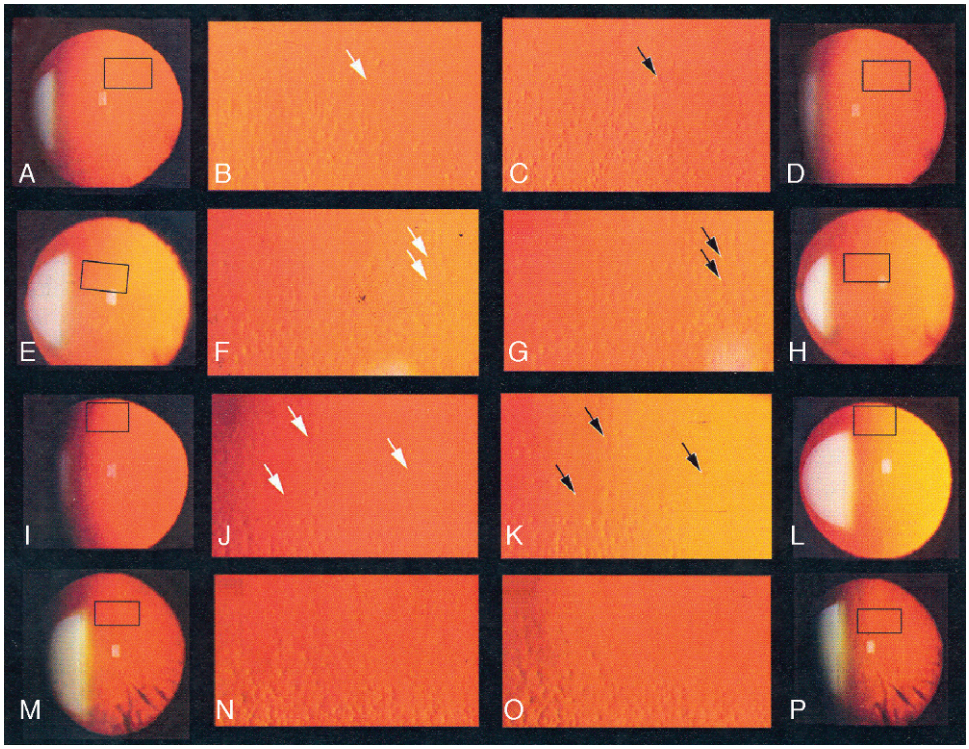

FIGURE 4. New guttae in FCD patients. A–D, Other eye of patient 1 in Figure 3, age 75 years, 29-month interval. E–H, Patient 2, age 69 years, 30-month interval. I–L, Patient 3, age 53 years, 30-month interval. M–P, Patient 4, age 75 years, 23-month interval. First 2 columns: 2002 images. Last 2 columns: 2004 images. Region analyzed is marked with a black rectangle. Black arrows indicate new guttae. White arrows indicate earlier sites corresponding to these new guttae. In patient 4, no new guttae were observed.

extensions of early confluent guttae. This makes sense in terms of ultimate progression of FCD pathology in the posterior cornea toward large regions of densely packed guttae. For this particular region, we observe an approximately 10% increase in the number of guttae during 29 months.

DISCUSSION

As Graves noted more than 75 years ago,[4] retroillumination slit-lamp observation can provide an excellent view of the extent of guttate changes in a cornea. We demonstrate that photographs of the posterior cornea, which show individual and confluent guttae, can be readily archived. Later these photographs can be analyzed in different ways to observe the progression of FCD pathology. Computer-assisted analysis of retroillumination photographs of FCD may be of benefit to readily determine quantitative progression when successive photographs are taken of the same cornea. The ability to follow increases in the number of guttae and the area of confluence should lead to better understanding of the natural history of the disease.

Currently, we have an incomplete understanding of the age of onset and rate of progression of this disease. There are good reasons to believe that yearly examinations will provide insight into a number of features of disease progression, some of which may vary for different genes and mutations. Our preliminary studies of the age-severity distribution of patients with adult-onset FCD suggests an average rate of progression of approximately 10 years from early disease to severe mid-stage disease.[7] It is important to have a detailed and objective record of phenotype to reliably identify effects of mutations that are distinctive to particular genes and to identify phenotypic variations associated with different mutations in the same gene. For example, we already have evidence that a *COL8A2* mutation has guttae with a distinctly different phenotype from that of adult-onset disease.[7] Such basic phenotypic information represents the downstream effect of each gene mutation in the disease. It is important to capture and record this information as part of initial genetic studies, although the significance of these details may not be fully realized until much later.

It is possible that the conception of a steady progression of FCD is incorrect, and that guttae increase in an episodic fashion. In this case, guttae might be expected to show little or no change during several years and increase suddenly, perhaps in response to environmental factors or aqueous substrate levels. On the basis of our observations, neither of these models can be excluded. A generally held concept is that guttae are indelible pathologic features that form and remain static or increase in size during the years. Our

evidence from all 4 patients strongly supports this preconception. However, it is important to realize that this needs to be evaluated further in long-term studies. In the small region examined in Figure 3, all except for 1 of 141 guttae persisted, and 14 new guttae appeared after 29 months. Although this is consistent with a gradual progression, it is not known whether the rate will be more rapid or slower during an extended history of this region. The fact that we could not find evidence of progression in patient 4 (Fig. 4M–P) opens the possibility that the number of guttae can remain in apparent stasis for almost 2 years. A difficulty with advanced cases and highly confluent regions of the cornea is that detecting new guttae, if they occur, is difficult. Earlier stages of the disease should prove to be more amenable to the documentation of individual new guttae.

Finally, in all 5 eyes, we observed a tendency of the superior cornea to be relatively free of guttae, whereas the inferior cornea is almost completely filled with confluent guttae. Although the numbers are too small to draw general conclusion, the observation is striking and may be documented further using these methods. If it can be shown that the superior cornea is more resistant to FCD pathology, investigation of environmental factors, such as light exposure, may be warranted.

A practical rationale for beginning a prospective study and for developing more quantitative methods for following disease progression is that there are currently no adequate data to counsel patients on how quickly FCD is likely to progress and how likely a transplant will be needed in the future if the disease is left to its natural course. A study involving photographic documentation will enable the exploration of some basic issues of disease progression and disease prognosis. The natural progression of FCD would be important baseline information if therapeutic options become available and efficacy is to be determined.

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