

## Corneal guttata associated with the corneal dystrophy resulting from a $\beta$ ig-h3 R124H mutation

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### Abstract

**Aims**—To investigate the frequency of corneal guttata in patients with a corneal dystrophy resulting from an Arg124His (R124H) mutation of  $\beta$ ig-h3 gene.

**Methods**—Slit lamp examination was performed on 30 eyes with corneal dystrophy from a genetically confirmed  $\beta$ ig-h3 R124H mutation and on 50 age matched control eyes. The stage of the corneal dystrophy was classified as stage 0, I, or II and the degree of guttata was classified as none, mild, or severe. Specular microscopic examinations were performed to evaluate the morphology of the corneal endothelium.

**Results**—Slit lamp examination disclosed the presence of corneal guttata in 21 eyes (70%) of the 30 eyes with the corneal dystrophy, but in only one (2%) of the 50 eyes in the age matched control group ( $p < 0.001$ ,  $\chi^2$  with Yates's correction). Of the 12 eyes with stage I  $\beta$ ig-h3 R124H corneal dystrophy, seven had no corneal guttata and five had a mild degree of guttata. Of the 18 eyes with stage II, the degree of guttata was none in two, mild in nine, and severe in seven. The degree of corneal guttata was significantly related to the stage of the corneal dystrophy ( $p < 0.0001$ , Kruskal-Wallis test ANOVA on ranks). There was no significant differences between eyes with  $\beta$ ig-h3 R124H corneal dystrophy and normal eyes in cell density, coefficient of variation, and cell hexagonality of corneal endothelium.

**Conclusion**—Corneal guttata are one of the characteristics of the corneal dystrophy resulting from  $\beta$ ig-h3 R124H mutation.

(Br J Ophthalmol 2000;84:67-71)

Avellino dystrophy is diagnosed by both the clinical manifestation of granular deposition in the cornea and the pathological evidence of amyloid deposition.<sup>1,2</sup> When Avellino dystrophy is first manifested, the corneal granular deposits are small, discrete, and sharply demarcated. As it progresses, the discrete grey-white granular deposits in the anterior stroma of the cornea increase in number and size, and lattice lesions appear in the middle to posterior stroma of the cornea. In addition, an anterior stromal haze is observed between the granular deposits. Histologically, besides the granular deposits, the amyloid deposition is found in the

corneal stroma.<sup>3</sup> The histological changes in Descemet's membrane and the endothelium in the cornea, on the other hand, have not been reported in either Avellino dystrophy or in granular dystrophy.

Recently, Munier *et al* reported that missense mutations in the  $\beta$ ig-h3 gene were detected in patients with four types of corneal dystrophies—granular dystrophy, Reis-Bücklers dystrophy, lattice dystrophy, and Avellino dystrophy.<sup>4</sup> They performed genetic analysis on patients diagnosed clinically with Avellino dystrophy and reported that the  $\beta$ ig-h3 Arg124His (R124H) mutation was present in all of them. However, the amyloid deposition has not been reported in the corneas of those patients. Avellino dystrophy has been diagnosed based on both the histopathological evidence of amyloid deposition and the clinical manifestation of granular deposition in the cornea.<sup>1,2</sup> Therefore, corneal granular deposition is definitely observed in the corneas of the patients with R124H mutation in the  $\beta$ ig-h3 gene. However, it has not been determined whether the cornea of patients with the  $\beta$ ig-h3 R124H mutation completely correspond with the cornea of Avellino dystrophy in term of the histological changes and clinical manifestations.

In this study, we investigated the corneas of patients with the R124H mutation in the  $\beta$ ig-h3 gene confirmed by genetic analysis. We shall refer to these corneas of patients with this mutation as corneal dystrophy and not Avellino dystrophy.

During the observation of the corneas of the patients with the  $\beta$ ig-h3 R124H mutation, corneal guttata were detected after phototherapeutic keratectomy (PTK). Interestingly, we also found corneal guttata that had not had surgery. These observations stimulated us to investigate the incidence of corneal guttata in patients with  $\beta$ ig-h3 R124H corneal dystrophy.

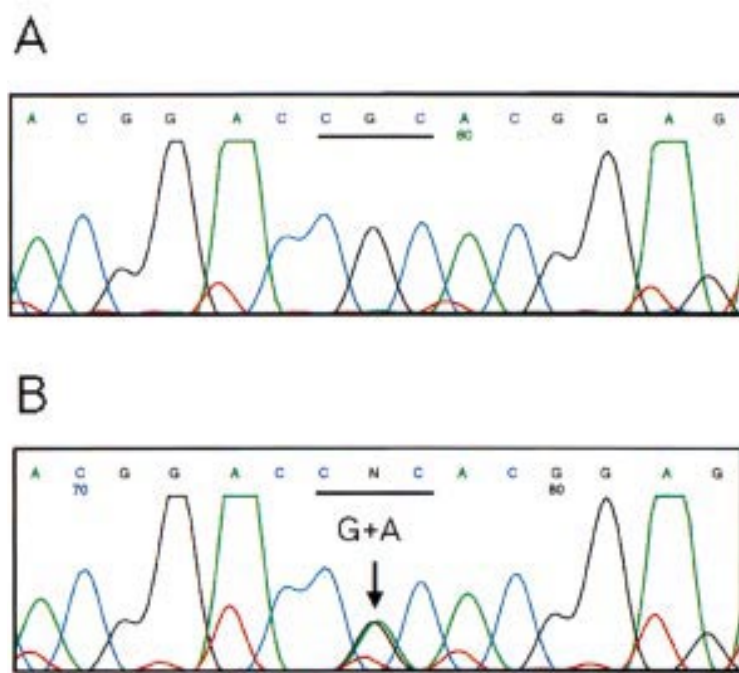
Corneal guttata are droplet-like accumulations of non-banded collagen on the posterior surface of Descemet's membrane. Corneal guttata are often observed in elderly people and are known to be associated with Fuchs' corneal endothelial dystrophy.<sup>5</sup> Guttata have also been found in association with a variety of conditions leading to early corneal damage such as trauma, congenital glaucoma, and macular dystrophy.<sup>5</sup> However, no report has been published to show that corneal guttata are associated with the  $\beta$ ig-h3 R124H corneal dystrophy or Avellino corneal dystrophy.

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Accepted for publication  
31 August 1999



**Figure 1** Molecular analysis of patients with corneal dystrophy from a heterozygous R124H mutation of  $\beta$ ig-h3 gene. The sequence reveals unaffected patients (A) and affected patients (B). G to A transition (CGC to CAC) was found at position 418 in the affected patients.

In this study, we focused on the patients with the R124H mutation of the  $\beta$ ig-h3 gene as confirmed by genetic analysis and investigated whether corneal guttata might be one of the clinical manifestations in these patients.

## Methods

### PATIENT SELECTION

This study was conducted to conform to the tenet of the World Medical Association of Helsinki regarding research involving human subjects. Written informed consent was obtained from all subjects participating in this study.

Molecular analysis was performed on the patients with corneal dystrophy followed by the methods previously reported.<sup>6</sup> We analysed the  $\beta$ ig-h3 gene of each patient with corneal dystrophy by sequencing exon 4 and exon 12 of the  $\beta$ ig-h3 gene which are mutational hot spots of the gene. We collected 20 ml of venous blood from each participant and extracted genomic DNA. Samples of genomic DNA was amplified by the polymerase chain reaction (PCR) with 500 nM of each forward and

reverse primer in an amplification mixture containing 10 mM TRIS-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.2 mM dNTP, and 0.5 unit of Taq polymerase (AmpliTaq Gold, Perkin Elmer, Branchville, NJ, USA). The primer pairs used to amplify exon 4 and exon 12 were based on published sequences. Amplified DNA was purified with a PCR purification kit (Qiagen, Hilden, Germany) and sequenced with an automatic fluorescent DNA sequencer (ABI Prism 377, Applied Biosystems, Foster City, CA, USA) and a dye terminator cycle sequencing kit (Perkin Elmer).

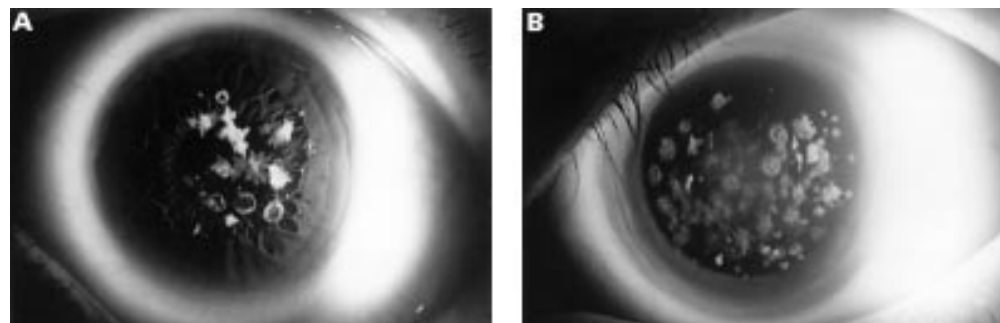
### PATIENT INFORMATION

Fifteen patients who were heterozygous for the R124H mutations in the  $\beta$ ig-h3 gene were enrolled in this study (Fig 1). No patients with a homozygous R124H mutation in  $\beta$ ig-h3 gene were enrolled. None of the patients had a history of intraocular surgery, keratitis, iritis, or corneal endothelial disorder from other causes. In addition, none had been a contact lens wearer. The 15 patients included two men and 13 women and ranged in age from 60 to 69 years (mean 66.8 (SD 7.7)). As control, 25 individuals (50 eyes) of similar age with no history of corneal disease who had visited the department of ophthalmology, Osaka University Medical School were enrolled. The 25 patients in the control group ranged in age from 60 to 69 years (mean 63.7 (3.1)).

### STAGING OF THE CORNEAL DYSTROPHY AND GUTTATA FORMATIONS

Slit lamp examination and non-contact specular microscopy were performed on the 30 eyes with the  $\beta$ ig-h3 R124H corneal dystrophy and the 50 eyes in the control group. Three specialists in corneal disorders classified the stage of corneal dystrophy from a slit lamp examination as: stage 0, no deposition is found; stage I, sharply demarcated granular deposits are few in number and isolated, and the areas between the deposits are clear; and stage II, sharply demarcated granular deposits are abundant with haze between the deposits (Fig 2).

A second group of three corneal specialists, masked to the results of classification on the stage of the corneal dystrophy, evaluated eyes for the presence of corneal guttata. The degree of guttata was classified as none, mild (few guttata), and severe (abundant guttata) as shown in Figure 3.



**Figure 2** Staging of corneal dystrophy. (A) Stage I of the dystrophy is characterised by isolated deposits in an otherwise clear cornea. (B) Stage II is characterised by moderate to abundant deposits and corneal haze.

**Table 1** Relation between stage of corneal dystrophy in patients with a  $\beta$ ig-h3 R124H mutation and degree of corneal guttata

Corneal dystrophy ( $\beta$ ig-h3 R124H mutation)	Corneal guttata			
	None	Presence	Mild	Severe
Stage 0* (normal; n=50)	49	1	1/1	0/1
Stage (I + II)* (n=30)	9	21	14/21	7/21
Stage I† (n=12)	7	5	5/5	0/5
Stage II† (n=18)	2	16	9/16	7/16

\*H=52.7,  $p<0.0001$ , Kruskal–Wallis ANOVA on ranks. Statistical analysis demonstrated that there was a significant relation between degree of corneal guttata and stage of the corneal dystrophy between degree of corneal guttata and stage of the corneal dystrophy.

† $p<0.05$ , Mann–Whitney U test. The degree of corneal guttata has a tendency to increase as the stage of the corneal dystrophy with R124H mutation of  $\beta$ ig-h3 gene increases.

The corneal endothelium and Descemet's membrane could be examined despite the deposits in 17 of the 30 eyes with the corneal dystrophy. In the other 13 eyes, the deposits obscured the corneal endothelium and Descemet's membrane, and caused visual disturbances. The stage of the corneal dystrophy was evaluated and then PTK was performed in these 13 eyes. The degree of corneal guttata was evaluated 1, 3, and 6 months after PTK because the corneal endothelium and Descemet's membrane could not be examined before surgery.

To determine the morphological characteristics of the corneal endothelium, specular microscopy was performed with a non-contact specular microscope (SP1000, Topcon, Tokyo, Japan). The resulting photographs were printed at  $\times 130$  magnification and the endothelial cells in each eye were traced and analysed. The characteristics of the corneal endothelium (cell density, coefficient of variation of cell area, and proportion of hexagonal cells) were evaluated using a corneal endothelial analyser (CA-100, Sun contact lens, Kyoto, Japan).

### Results

All 30 eyes of the patients with the R124H mutation of the  $\beta$ ig-h3 gene had granular-shaped deposits in the subepithelial layer of the cornea. In addition, some dense fusiform-shaped deposits were seen in the middle stroma in all the eyes.

The three cornea specialists agreed on the stage of the corneal dystrophy in 24 of 30 eyes. In the other six eyes, the stage of the corneal dystrophy assigned by one of the specialists

differed by one stage from that selected by the other two specialists. For these six eyes, the stage of selected by the two corneal specialists was adopted.

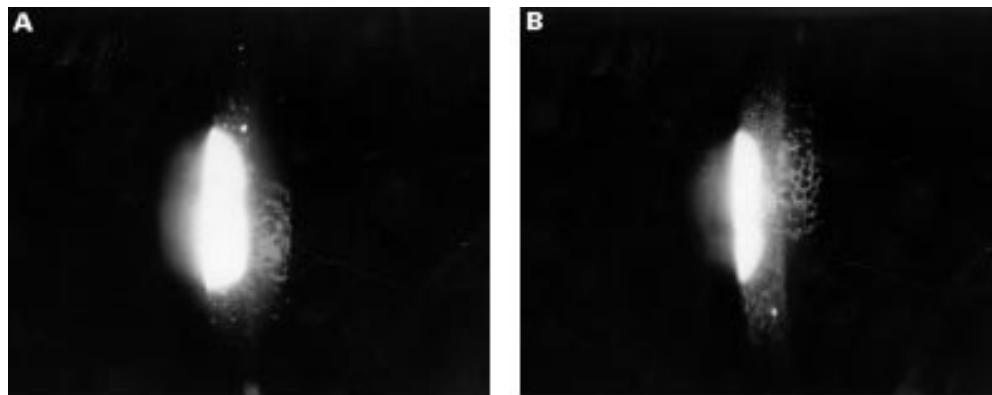
The second group of three cornea specialists agreed in their ratings on the degree of corneal guttata in all 30 eyes with corneal dystrophy and the 50 control eyes. In the 13 eyes with PTK, the evaluation of corneal guttata at 1 month had the same score at 3 and 6 months after the PTK.

Slit lamp examination disclosed the presence of corneal guttata in 21 (70%) of the 30 eyes of patients with the  $\beta$ ig-h3 R124H corneal dystrophy but in only one (2%) of the 50 eyes in the control group ( $p<0.0001$ ,  $\chi^2$  with Yates's correction). The relation between the stage of the corneal dystrophy and degree of corneal guttata is shown in Table 1. There was a significant correlation between the stage of the corneal dystrophy and the degree of corneal guttata (H= 52.7,  $p<0.0001$ , Kruskal–Wallis ANOVA on ranks). The degree of corneal guttata had a tendency to increase as the stage of the corneal dystrophy increased; the differences were significant between stage 0 and I ( $p<0.05$ , Mann–Whitney U test), between stage 0 and II ( $p<0.05$ , Mann–Whitney U test), and between stage I and II ( $p<0.05$ , Mann–Whitney U test). These results indicate that the degree of corneal guttata increases with the progression of corneal dystrophy in these patients with the  $\beta$ ig-h3 R124H mutation. The corneal guttata in eyes with corneal dystrophy were localised in the central cornea and none appeared in the peripheral cornea.

Specular microscopic observation also showed the presence of the corneal guttata in the patients with corneal dystrophy (Fig 4). Analysis of the morphological characteristics of the endothelium (Table 2) showed that the cell density of corneal endothelial cells, the coefficient of variation in cell area, and the proportion of cell hexagonality were not significantly different in patients with or without the corneal dystrophy and with or without corneal guttata.

### Discussion

The results of this study have shown that corneal guttata occurred at a significantly higher frequency in the patients with  $\beta$ ig-h3 R124H corneal dystrophy than in the age



**Figure 3** Grading of corneal guttata. (A) Mild corneal guttata are characterised by a small number of guttata formations. (B) Severe corneal guttata are characterised by abundant guttata formations.

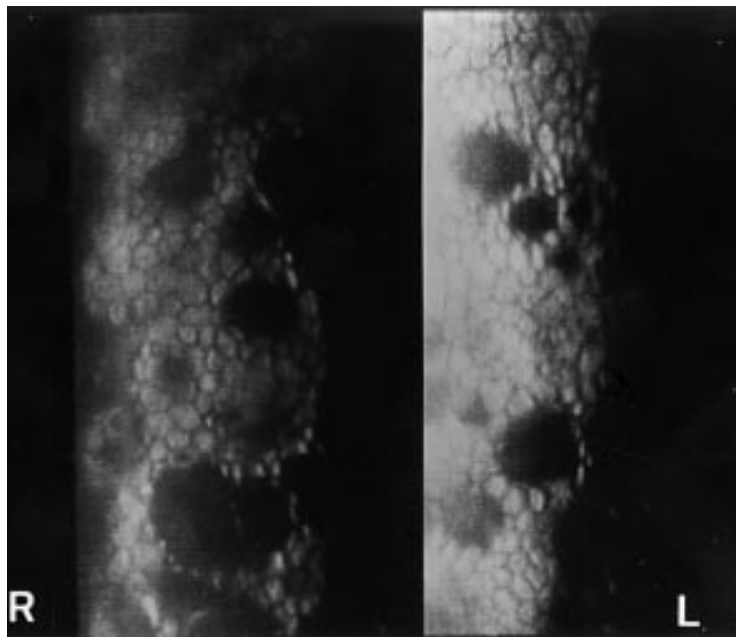


Figure 4 Specular microscopic photograph. Severe corneal guttata were observed in the right eye (R) and mild guttata in the left eye (L) of the same 64 year old patient with  $\beta$ ig-h3 R124H mutation.

Table 2 Characteristics of corneal endothelium in elderly patients with or without the corneal dystrophy from a  $\beta$ ig-h3 R124H mutation

	Cell density ( $\text{mm}^2$ )	Coefficient of variation in cell area	Proportion of hexagonal cells (%)
Control	3271 (363)	0.32 (0.04)	61.2 (7.4)
Corneal dystrophy*	3157 (518)	0.31 (0.05)	60.9 (4.7)
guttata (+)†	3138 (548)	0.31 (0.05)	60.7 (5.0)
guttata (-)†	3200 (465)	0.32 (0.03)	61.5 (3.8)

\*Corneal dystrophy with  $\beta$ ig-h3 R124H mutation.

†The corneal endothelium in the patients of corneal dystrophy with  $\beta$ ig-h3 R124H mutation with and without corneal guttata.

matched control group. It was also shown that the frequency of corneal guttata paralleled the severity of corneal dystrophy although corneal guttata were observed even in eyes in the early stage of corneal dystrophy. These results suggest that corneal guttata are one of the characteristics of  $\beta$ ig-h3 R124H corneal dystrophy. As far as we know, this is the first report of the presence of corneal guttata in corneal dystrophy resulting from a  $\beta$ ig-h3 R124H mutation.

Corneal guttata have been noted in older and younger adults without corneal dystrophy. Goar reported that 9.6% of people older than 40 years and 3.3% of those between 20 and 40 years old had corneal guttata without oedema.<sup>7</sup> However, corneal guttata are seen less often in Japanese people than in the population of the USA.<sup>8</sup> Ohara *et al* found that about 1% of Japanese persons older than 40 years have corneal guttata.<sup>9</sup> Therefore, a high percentage (70%) of corneal guttata in patients with corneal dystrophy from a  $\beta$ ig-h3 R124H mutation supports our conclusion that guttata are one of the characteristics of  $\beta$ ig-h3 R124H corneal dystrophy.

It is unclear why other observers have not reported corneal guttata in patients with corneal dystrophy. Many cornea specialists in our clinic have examined the corneas of patients with corneal dystrophy but none had

noticed this relation. In fact, we did not see corneal guttata until we observed the deep cornea clearly after PTK in patients with corneal dystrophy. We suggest that corneal guttata were overlooked as the corneal specialists were more concerned with the more obvious deposits in the cornea.

It has been long known that the endothelium of corneas affected by interstitial keratitis, especially when the inflammation involves the deeper corneal stroma, are stimulated to induce focal or diffuse thickening of Descemet's membrane. These changes may appear as linear ridges or as excrescences. However, the corneal guttata in the  $\beta$ ig-h3 R124H corneal dystrophy, on the other hand, are different from those seen in interstitial keratitis. Moreover, no patient enrolled in this study had a history of interstitial keratitis.

Although corneal guttata have not been reported as a complication of excimer laser surgery, previous studies have found that excimer laser treatment of the cornea can affect the corneal endothelium leading to the secretion of a substance with high electron density into Descemet's membrane.<sup>10</sup> It may thus be conjectured that corneal guttata may be a secondary reaction to excimer laser treatment to the cornea. In this study, among 30 eyes with the corneal dystrophy, 13 eyes had laser treatment and 17 eyes did not. Corneal guttata were found in nine (69.2%) of 13 eyes with PTK and 12 (70.6%) of 17 eyes without PTK. Thus, there is no difference in the frequency of corneal guttata between those with and without excimer laser treatment. These findings demonstrate that the exposure to excimer laser energy does not induce corneal guttata in corneas with corneal dystrophy.

It may also be suspected that the shadows of the deposits in the cornea were misidentified as corneal guttata. However, after PTK, the corneal guttata were still observed through a clear cornea.

For more than 16 months of follow up of our patients with  $\beta$ ig-h3 R124H corneal dystrophy we have noted that corneal guttata showed no remarkable change in number and shape. Therefore, it is evident that corneal guttata in  $\beta$ ig-h3 R124H corneal dystrophy are not temporary as may be the case with pseudoguttata, but represents a non-reversible change.

Yokoi *et al* noticed that guttata-like formations were present in patients with advanced granular dystrophy who had undergone PTK.<sup>11</sup> However, their diagnosis was based only on the clinical manifestations in the cornea and a genetic analysis was not performed. It is of interest to note that Konishi *et al* reported that most of the patients who were diagnosed clinically with granular dystrophy in Japan have the R124H mutation of the  $\beta$ ig-h3 gene.<sup>12</sup> Our unpublished data have also given similar results. Therefore, it is highly likely that the patients studied by Yokoi *et al* have, in fact, corneal dystrophy with  $\beta$ ig-h3 R124H mutation and not granular dystrophy. If so, their data also support our conclusion that corneal guttata are one of the characteristics of  $\beta$ ig-h3 R124H corneal dystrophy.

The corneal guttata reported in elderly people are round and located in the central cornea. The corneal guttata we report in association with  $\beta$ ig-h3 R124H corneal dystrophy were also located in the central cornea. It would be valuable to know whether there is a histological difference between the corneal guttata in eyes with  $\beta$ ig-h3 R124H corneal dystrophy and the corneal guttata seen in aging eyes and eyes with Fuchs' corneal dystrophy. To determine these characteristics, histological examination is important. However, the corneal deposits in  $\beta$ ig-h3 R124H corneal dystrophy are localised in the superficial and the middle stroma. Thus, lamellar keratoplasty has been preferred in our clinic as the treatment for the corneal dystrophy because it is sufficient to remove the superficial and middle layer of the cornea with the opacity. Moreover, lamellar keratoplasty does not induce endothelial rejection. For these reasons, we were not able to obtain specimens for histological studies of the corneal guttata. Future histological examination will resolve this question as well as provide evidence for the mechanism of corneal guttata in corneal dystrophy in patients with the  $\beta$ ig-h3 R124H mutation.

Supported in part by a grant in aid No 09671800 for scientific research from the Japanese Ministry of Education, Science,

Sports and Culture, by a health science grant from the Japanese Ministry of Health and Welfare and by a Osaka Eye Bank Foundation grant (Dr Watanabe).

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*Br J Ophthalmol* 2000 84: 67-71

doi: 10.1136/bjo.84.1.67

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