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Recurrence of Corneal Dystrophy Resulting from an R124H Big-h3 Mutation After Phototherapeutic Keratectomy

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Purpose. The purpose of the study was to investigate the recurrence-free interval after phototherapeutic keratectomy (PTK) in patients with corneal dystrophies resulting from an Arg124His (R124H) mutation of the Big-h3 gene. **Methods.** Patients with corneal dystrophy resulting from a genetically confirmed Big-h3 R124H mutation were examined with a slit lamp. The patients were divided into two groups on the basis of the mutation genotype, and the recurrence-free interval was analyzed. **Results.** In the 4 eyes of 3 homozygous patients, the mean (± standard deviation [SD]) recurrence-free interval was 9.5 ± 3.1 months, whereas in the 7 eyes of 4 heterozygous patients it was 38.4 ± 6.2 months. The former interval was statistically shorter than the latter (Kaplan–Meier survival analysis with log-rank test, $p = 0.004$). **Conclusions.** These results strongly suggest that the mutation genotype of Big-h3 gene determined the recurrence-free interval as well as the clinical picture after PTK. Therefore, PTK should be considered for patients with Big-h3 R124H corneal dystrophy, on the basis of the expected recurrence-free interval deduced from molecular analysis of the zygosity of the Big-h3 R124H mutation.
Key Words: Big-h3 gene—Corneal dystrophy—R124H mutation PTK—Recurrence.

Avellino corneal dystrophy[1–4] is defined as a disease that exhibits clinical and histologic features of both granular- and lattice-like changes. Avellino dystrophy is inherited as an autosomal dominant trait, and when it is first manifested, the corneal granular deposits are small, discrete, and sharply demarcated. As it progresses, the discrete gray–white granular deposits in the anterior stroma of the cornea gradually enlarge, increase in number, aggregate, and spread into the deeper and more peripheral stroma. In addition, an anterior stromal haze is observed between the granular deposits. Most patients with Avellino dystrophy maintain their vision for many years, and it may give rise to significant visual disabilities only late in life. If necessary, these patients undergo keratoplasty or phototherapeutic keratectomy (PTK) only in the sixth or seventh decade of life.

Recent molecular analyses of Avellino dystrophy, granular corneal dystrophy,[5,6] lattice corneal dystrophy type I,[7] and Reis–Bücklers dystrophy[8,9] have revealed that these corneal dystrophies are linked to the 5q31 gene.[10–12] Munier et al.[13] reported that missense mutations in the Big-h3 gene were detected in patients with these four autosomal dominant corneal dystrophies and that the patients with clinically diagnosed Avellino dystrophy have an R124H (Arg124His) point mutation in the Big-h3 gene.

Avellino dystrophy has been diagnosed by the presence of granular deposits and histologic evidence of amyloid deposits in the affected corneas. Therefore, patients with a corneal dystrophy resulting from Big-h3 R124H mutations definitely have granular deposits; however, they do not always have amyloid deposition. The corneal dystrophies resulting from Big-h3 R124H mutations do not completely correspond with the Avellino dystrophy. In this study report, we refer to the corneal disease resulting from a Big-h3 R124H mutation as corneal dystrophy rather than Avellino dystrophy.

For the treatment of Big-h3–associated corneal dystrophy such as granular, Avellino, lattice, and Reis–Bücklers dystrophy, lamellar keratoplasty (LKP) and PTK have been used, depending on the depth of the opacities in the cornea and the availability of a graft.[1,14] Recurrences of clinically diagnosed granular corneal dystrophy after these procedures have been reported.[1,15]

However, clinically diagnosed granular corneal dystrophy have been reported to result from both Big-h3 R124H and R555W mutation.[16] Moreover, we recently reported a different pattern of recurrence after PTK of the cornea in patients with either a homozygous or a heterozygous R124H mutation.[17] The clinical appearance of the cornea after recurrence in patients with heterozygous R124H mutation was mild; the granular opacities occurred as spot lesions in the central cornea. In contrast, in the patient with a homozygous mutation there was a more severe pattern: the recurrent lesions were diffuse and occurred in the interface between the

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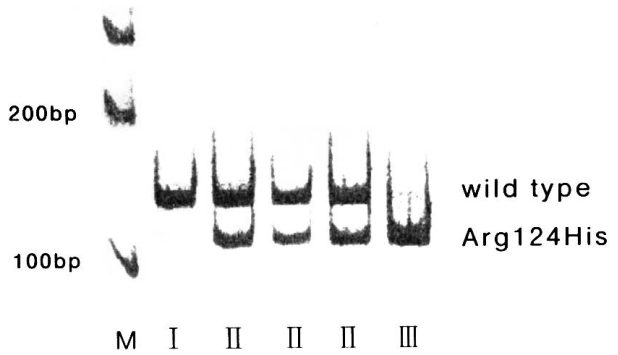

FIG. 1. Restriction enzyme digestion analysis specific to the R124H Big-h3 mutation. Upper bands (140 bp) represent the wild type. Lower bands (approximately 120 bp) represent the R124H mutation. Severely affected patients were homozygous, whereas the clinically mild patients were heterozygous for the Big-h3 R124H mutation. The unaffected person was homozygous for the wild-type sequence.

corneal epithelium and laser-ablated stroma. In addition, the recurrence-free interval after PTK in patients with homozygous R124H mutation was shorter than that with heterozygous mutation.[17]

In this study we performed a statistical analysis of recurrences in the corneas caused by either homozygous or heterozygous R124H mutation. The recurrence-free intervals after PTK in these two groups were compared.

PATIENTS AND METHODS

Patient Selection

This study was conducted according to the tenets of the Declaration of Helsinki and was approved by our institutional human experimentation committees. Informed consent was obtained from all participants in this study.

Molecular analysis was performed by a previously reported established method (Fig. 1).[12,18] We analyzed the Big-h3 gene of each patient with corneal dystrophy by sequencing exons 4 and 12 of the Big-h3 gene. On the basis of these results, 7 eyes from 4 heterozygous patients (age, mean ± standard deviation [SD]: 66.0 ± 6.3 years) and 4 eyes from 3 homozygous patients (age, 40.3 ± 17.0 years) with a Big-h3 R124H mutation were enrolled (Table 1). There were 5 women and 2 men, whose ages ranged from 30 to 74 (54.9 ± 17.4) years. None of the eyes had undergone surgical treatment such as keratoplasty, PTK, or keratectomy, and none had any other ocular disease.

All homozygous patients had severe corneal opacities that were confluent, round, and white in the superficial stromal layer. All of the heterozygous patients had mildly affected corneas with granular-shaped deposits in the subepithelial layer of the cornea and some dense, fusiform deposits in the anterior to middle stroma. These findings are consistent with the clinical features of Avellino dystrophy as previously described.[1–4,18] Thus, the Big-h3 gene genotype determined the clinical appearance of this corneal dystrophy.

In all of these patients with either homozygous or heterozygous mutations, the main opacities that impaired their visual acuity were localized in the superficial and anterior layer of the corneal stroma. Therefore, excimer PTK was used first for all of these patients. The clinical profiles of these patients are shown in Table 1.

PTK and Detection of Recurrence

For PTK treatment, an excimer laser system (ArF; VISX Twenty/Twenty, Sunnyvale, CA) was used. The wavelength of this laser is 193 nm, with a fluence of 160 mJ/cm^2 and a pulse rate of 6 Hz. The laser beam covered a 6-mm-diameter circle centered on the entrance pupil. The depth of the ablation was determined by the observation of the extent of the opacity and deepness in the affected cornea. After the transepithelial ablation (30 μm), the stromal ablation was performed until corneal deposits disappeared within permissible range through the microscope. In this study, peripheral antihyperopic ablation was not performed.

The patients were examined 1 day, 1 week, and 1, 3, and 6 months after surgery. Thereafter, they were examined every 6 months for >3 years. The mean follow-up period for the 4 eyes of the 3 homozygous patients was 36.3 ± 10.7 months, whereas that for the 7 eyes of the 4 heterozygous patients was 55.0 ± 6.5 months.

The cornea was photographed and the image recorded with a camera attached to the slit-lamp biomicroscope. Recurrence was defined as the appearance of opacities that could be observed in the ablation region with slit-lamp biomicroscopy by three corneal specialists (H.W., N.M., and Y.I.). In addition, the photographs were used to confirm recurrence.

We investigated the recurrence-free intervals after PTK in the heterozygous and homozygous patients by statistical analysis with an unpaired *t* test and Kaplan–Meier survival analysis, as well as the Mantel–Cox log-rank test.

TABLE 1. *Clinical characteristics of patients*

Patient no.	Sex	Age (y)	Diagnosis	Eye	Recurrence-free interval (mo)	Preoperative CVA	Postoperative CVA
1	F	30	R124H homo	L	13	20/200	20/33
2	M	31	R124H homo	R	11	20/333	20/33
				L	12	20/333	20/40
3	M	60	R124H homo	R	5	20/2000	20/40
4	F	64	R124H hetero	R	38	20/200	20/40
5	F	59	R124H hetero	R	44	20/200	20/22
				L	49	20/200	20/20
6	F	67	R124H hetero	R	37	20/200	20/50
				L	30	20/100	20/25
7	F	74	R124H hetero	R	35	20/100	20/67
				L	36	20/133	20/50

CVA, corrected visual acuity; F, female; hetero, heterozygous; homo, homozygous; L, left; M, male; R, right.

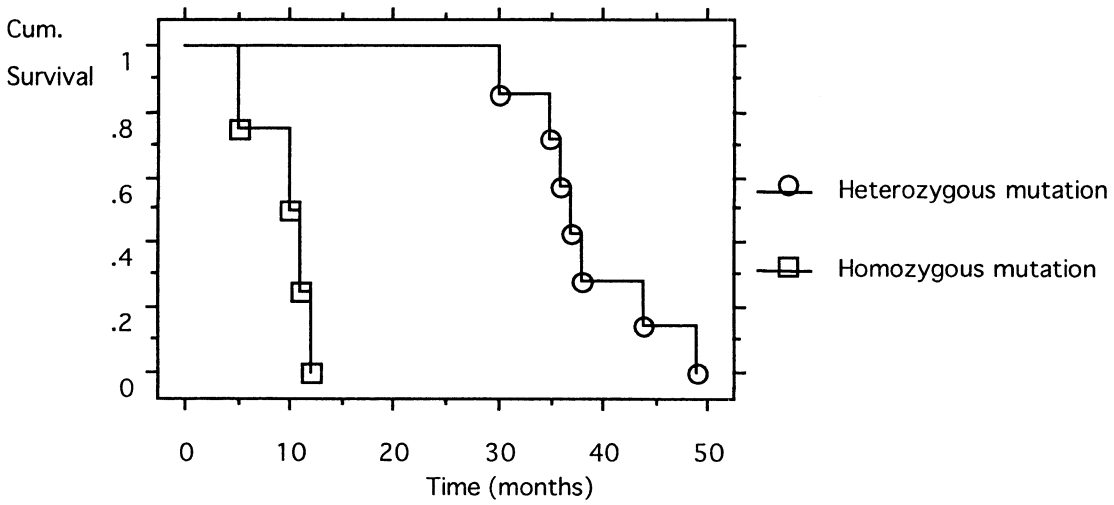

FIG. 2. Kaplan–Meier analysis: the cumulative success probability after PTK (recurrent lesions were classified as failures), analyzed by the Kaplan–Meier method. The success rate in heterozygous mutations was significantly higher than in homozygous cases ($p = 0.004$; log-rank test).

RESULTS

The mean recurrence-free interval for the 4 eyes of the 3 homozygous patients with the R124H mutation was 9.5 ± 3.1 months, whereas that for the 7 eyes of 4 heterozygous patients was 38.4 ± 6.2 months. The interval for the homozygous cases was significantly shorter than the interval for the heterozygous ones (unpaired t test; $p < 0.001$). Furthermore, the cumulative success probabilities in cases of heterozygous mutations were significantly higher than in those of homozygous mutations ($p = 0.004$, Kaplan–Meier survival analysis with log-rank test; Fig. 2).

The mean depth of the ablation in the homozygous cases (90.0 ± 20.0 μm; range, 60.0–100.0 μm) was not significantly different from the mean depth in the heterozygous cases (92.9 ± 7.6 μm; 80.0–100.0 μm) (unpaired t test, $p = 0.736$). Thus, the difference in recurrence-free periods between the two groups was not attributable to the depth of the PTK.

The clinical appearance of recurrent opacities differed between the homozygous and heterozygous mutation groups. The recurrent deposits in homozygous patients started diffusely from the peripheral cornea and showed a rapid centripetal spread. They were localized in the interface between the corneal epithelium and the laser-ablated stroma (Fig. 3A).

In the heterozygous patients, the recurrent lesion reappeared slowly in the superficial central cornea as granular deposits that were small, discrete, and sharply demarcated (Fig. 3B). These features were commonly found in all of the subjects in each group. In addition, the pattern of recurrence did not correlate with visual function in this study.

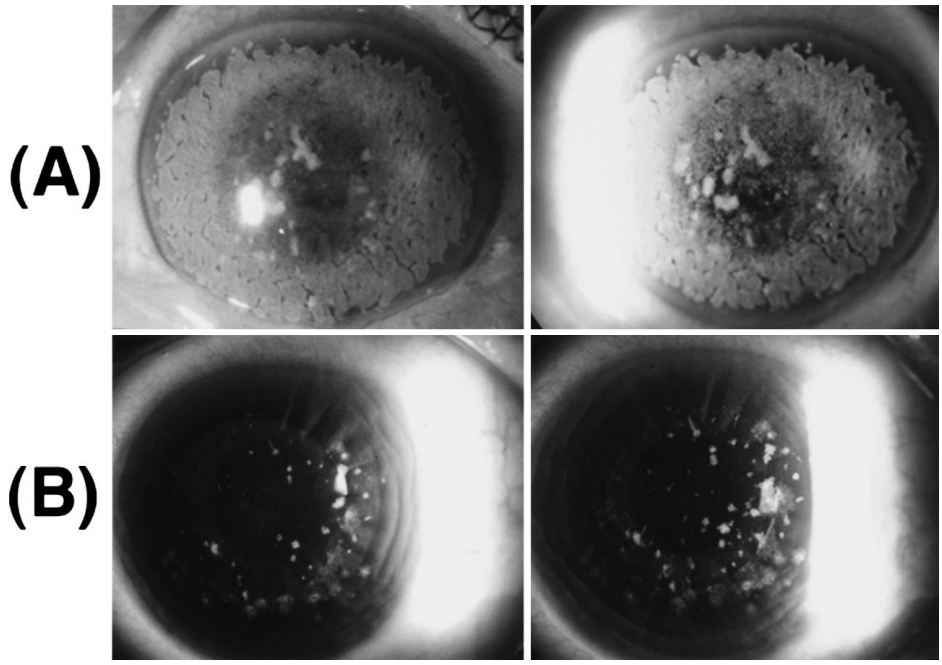

FIG. 3. Slit-lamp photographs of the recurrence after PTK in two patients with corneal dystrophy resulting from a Big-h3 R124H mutation. **A:** Recurrence in a patient with a homozygous Big-h3 R124H mutation, 4 months (left) and 1 year (right) after PTK. The recurrent lesions can be seen as diffuse lesions in the peripheral cornea and are localized in the interface layer between the corneal epithelium and the laser-ablated stroma. **B:** Recurrence in a patient with a heterozygous Big-h3 R124H mutation, 1 year (left) and 4 years (right) after PTK. The recurrent lesions can be seen as granular deposits in the superficial cornea in the central area.

DISCUSSION

In the corneal dystrophy resulting from an R124H mutation of the Big-h3 gene, the recurrence-free interval and clinical features were different and depended on whether the patient was homozygous or heterozygous for the R124H mutation. These results strongly suggest that the mutation genotype determines the recurrence-free period after PTK. Thus, determination of the genotype enables the surgeon to inform the patient about the recurrence pattern after PTK, and PTK should be performed on patients with a corneal dystrophy resulting from a Big-h3 R124H mutation with an expected recurrence-free interval dependent on a molecular analysis of Big-h3 gene.

A severe form of juvenile granular corneal dystrophy has been reported mainly in Japan,[18–20] and the clinical picture of the affected cornea resembled that of the cornea in our patients with homozygous R124H corneal dystrophy. Homozygous patients with severe clinical signs are almost nonexistent, except in rare cases in Japan, where consanguineous marriages were arranged a few generations ago. Therefore, several cases have been reported in Japan in which patients had corneal dystrophy resulting from a homozygous R124H mutation of the Big-h3 gene.[18,20] As far as we know, this is the first report of statistical differences in the recurrence-free period of patients with homozygous and heterozygous mutations of the R124H corneal dystrophy confirmed by genetic analysis.

One may claim that the difference in recurrence-free interval was due to the ablation depth of PTK. However, our PTK was performed with a similar depth and site of ablation. Therefore, variations in the recurrence pattern between the two groups are not related to the total amount of ablated cornea but are associated with the genotype of the patients.

It may also be suspected that recurrent lesions of this disease were misidentified as haze that occurs following PRK. However, haze comprises thin, round opacities like the mesh of a net and appears approximately 1 month after use of an excimer laser and gradually diminishes at approximately 3 months or more after surgery. Thus, haze can be distinguished from the recurrent lesions of this disease by both its clinical appearance and its onset period.

It is still unclear why the recurrence pattern after PTK differs according to the mutation genotype of the Big-h3 gene. The differences in onset and severity of the recurrent deposits may depend on a dose effect of the abnormal Big-h3 mutant allele. To clarify these problems, further investigation and observations will be needed.

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