• Basic Research •

Corneal histomorphology and electron microscopic observation of R124L mutated corneal dystrophy in a relapsed pedigree

Meng-Jun Fu[1,2,3], Jing Zhao[4,5,6], Shan Duan[7], Hao-Run Zhang[1], Jing-Jing Zhao[1], Li Zeng[4,5,6], Rui Wang[1], Xing-Tao Zhou[4,5,6]

[1]Department of Refractive Surgery Center, Weifang Eye Hospital, Weifang 261000, Shandong Province, China
[2]State Key Laboratory of Ophthalmology, Optometry and Visual Science, Eye Hospital of Wenzhou Medical University, Wenzhou 325000, Zhejiang Province, China
[3]National Clinical Research Center for Ocular Diseases, Eye Hospital of Wenzhou Medical University, Wenzhou 325000, Zhejiang Province, China
[4]Eye Institute and Department of Ophthalmology, Eye & ENT Hospital, Fudan University, Shanghai 200031, China
[5]NHC Key Laboratory of Myopia (Fudan University); Key Laboratory of Myopia, Chinese Academy of Medical Sciences, Shanghai 200031, China
[6]Shanghai Research Center of Ophthalmology and Optometry, Shanghai 200000, China
[7]Laboratory of Molecular Medicine, Institute of Maternal and Child Medicine, Shenzhen Maternity and Child Healthcare Hospital, Southern Medical University, Shenzhen 518040, Guangdong Province, China
Co-first authors: Meng-Jun Fu and Jing Zhao
Correspondence to: Rui Wang. Department of Refractive Surgery Center, Weifang Eye Hospital, No.139, Xingfu Road, Kuiwen District, Weifang 261000, Shandong Province, China. 15866565239@163.com; Xing-Tao Zhou. Department of Ophthalmology, Eye and ENT Hospital of Fudan University, No.83, Fenyang Road, Xuhui District, Shanghai 200031, China. doctzhouxingtao@163.com
Received: 2022-01-21 Accepted: 2022-07-21

Abstract

● **AIM:** To investigate the histological characteristics and ultrastructure of recurrent Chinese R124L mutated corneal dystrophy after keratoplasty.

● **METHODS:** The subjects were enrolled from a Chinese family of corneal dystrophy with R124L heterozygous gene mutation and with a history of consanguineous marriage. Normal corneal samples were used as controls.

● **RESULTS:** In this family, 2 patients (3 eyes) underwent penetrating keratoplasty (PKP) and 2 patients (4 eyes) underwent lamellar keratoplasty (LKP). They had recurrence at 33.5±3.0 (range 30-36)mo after keratoplasty. Among them, 1 patient (1 eye) underwent PKP again and 1 patient (2 eyes) underwent LKP again. In the R124L mutated recurrent corneal dystrophy, the corneal turbidity was mainly distributed from the upper corneal cortex to the anterior stroma; the corneal epithelium surface was rougher and more uneven; and, the corneal erosions were larger. Hematoxylin-eosin staining showed that the thickness of the corneal epithelium was uneven; the arrangement of the epithelial cells was disordered; and, some corneal epithelial cells were swollen. The results of Congo red staining, Masson's trichrome staining and Periodic acid-Schiff staining were positive, while that of Alcian blue staining was negative. Under a transmission electron microscope, deposition of high electron density substances between epithelial and basal cells, and, apoptosis of basal cells were observed. Many high electron density depositions were observed in the sub-epithelial and anterior corneal matrix.

● **CONCLUSION:** In the Chinese family of recurrent corneal dystrophy with R124L gene mutation, the corneal epithelia of the recurrent cases are rougher, and the corneal depositions are extracellular amyloid fibrin.

● **KEYWORDS:** corneal dystrophy; R124L mutation; electron microscope observation; pathology

DOI:10.18240/ijo.2022.09.02

Citation: Fu MJ, Zhao J, Duan S, Zhang HR, Zhao JJ, Zeng L, Wang R, Zhou XT. Corneal histomorphology and electron microscopic observation of R124L mutated corneal dystrophy in a relapsed pedigree. *Int J Ophthalmol* 2022;15(9):1416-1422

INTRODUCTION

Corneal dystrophy (CD) with R124L mutation is a familial, primary, binocular autosomal dominant genetic disease[1-2]. According to the 2015 International Classification of Corneal Dystrophies (IC3D) and based on the affected

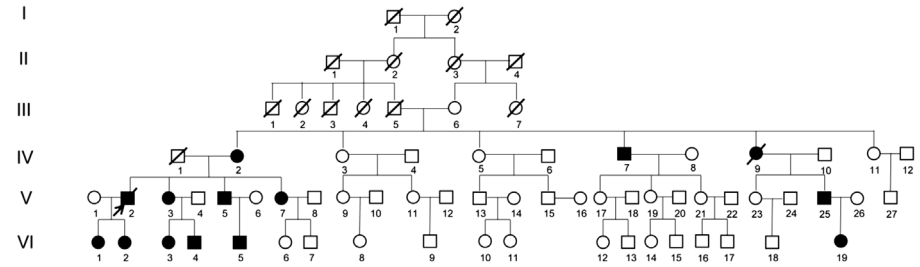

Figure 1 The pedigree diagram of the CD family with R124L mutation III5 was closely related to III6. IV7 underwent binocular penetrating keratoplasty (PKP). There was relapse in both eyes at 36mo after PKP and PKP was performed again on the right eye after recurrence. IV9 underwent binocular lamellar keratoplasty (LKP). Binocular LKP was performed for V2. The patient had CD recurrence at 33mo after the operation and LKP was performed again on both eyes after the recurrence. V25 was treated with PKP for the right eye, and CD recurred at 30mo after surgery.

anatomic site, CD is sub-classified: epithelial/sub-epithelial, epithelial-stromal, stromal, and endothelial dystrophy[3]. CD with R124L mutation belongs to epithelial-stromal CD[3]. The gene mutation sites of CD have been confirmed to be in the transforming growth factor B-induced (*TGFBI* or *BIGH3*) gene on chromosome 5q31[4-7]. The diagnostic basis of CD mainly includes clinical observation of lesion location and morphology, histopathological changes, and genetic testing and typing results[8-10].

Current treatments for CD include phototherapeutic keratectomy (PTK) and keratoplasty[11-14]. After surgery, the symptoms could be improved or relieved and vision could be improved. Some patients may inevitably suffer from aggravation or recurrence of corneal opacity[15]. The pathological changes of R124L mutant CD are reported to be mainly caused by accumulation of abnormal substances in the anterior stroma[1]. However, the corneal pathological changes of recurrent cases have not been reported. In this study, a Chinese CD family with R124L mutation who had a history of consanguineous marriage was enrolled. The pathological changes of cornea tissues with recurrence after keratoplasty were observed by electron microscopy and histopathological analysis. The sites of gene mutations were detected. Our findings may provide evidence for the pathological changes of recurrent CD caused by R124L.

SUBJECTS AND METHODS

Ethical Approval The study was carried out in accordance with the principles of the Declaration of Helsinki and was approved by the Weifang Eye Hospital Ethics Committee. Each patient provided the signed informed consent.

Subjects Fourteen patients with CD from a family with a history of consanguineous marriage in China were enrolled. Among them, the ratio of male to female was 6:8. The youngest was 1 year old, and the oldest was 69 years old. The 14 patients underwent routine ophthalmological examination and peripheral blood collection. The diseased corneal tissues were collected from patients of IV7 and V2 (Figure 1) who had recurrence after keratoplasty and underwent keratoplasty again. For control, 3 pieces of normal corneal tissues donated by Weifang Eye Hospital were collected.

Routine Ophthalmological Examination Routine ophthalmological examinations, including visual acuity examination, slit lamp microscope examination, and, intraocular pressure and fundus examination were performed.

***TGFBI* Mutation Detection** Peripheral blood (10 mL each) was collected from 14 patients with CD. The blood was anticoagulated with EDTA. DNA was extracted from peripheral blood with DNA extraction kit (Qiagen, Santa Clara, CA, USA). Gene sequencing was performed with BigDye terminator V3.1 cycle sequencing kit (Qiagen, Santa Clara, CA, USA) on an ABI 31300XL analyzer (ABI, Foster City, CA). Sequence Scanner V1.0 software was used for analyzing sequencing data, with reference to NCBI GeneBank (NM_000358 for *TGFBI*).

Histopathological Analysis The corneal tissues were fixed with 0.4 g/L paraformaldehyde, dehydrated with graded ethanol, transparent with xylene, embedded with paraffin, and cut into 5 μm sections. Hematoxylin-eosin (HE) staining, Alcian blue staining, Periodic acid-Schiff (PAS) staining, Congo red staining, and Masson's trichrome staining were carried out respectively according to routine procedures. The sections were observed and photographed under a light microscope.

Ultrastructure Observation with Transmission Electron Microscope The corneal specimens were fixed with 2% paraformaldehyde glutaraldehyde, rinsed with phosphoric acid buffer, fixed with 4% osperium tetroxide, dehydrated with ethanol step by step, fixed with epoxy resin, made into ultrathin sections, stained with lead uranium acetate and lead citrate, and were observed under transmission electron microscope (TEM).

RESULTS

Basic Information of Study Subjects The pedigree diagram of the family with a history of consanguineous marriage is shown in Figure 1. There were 59 members (14 patients with

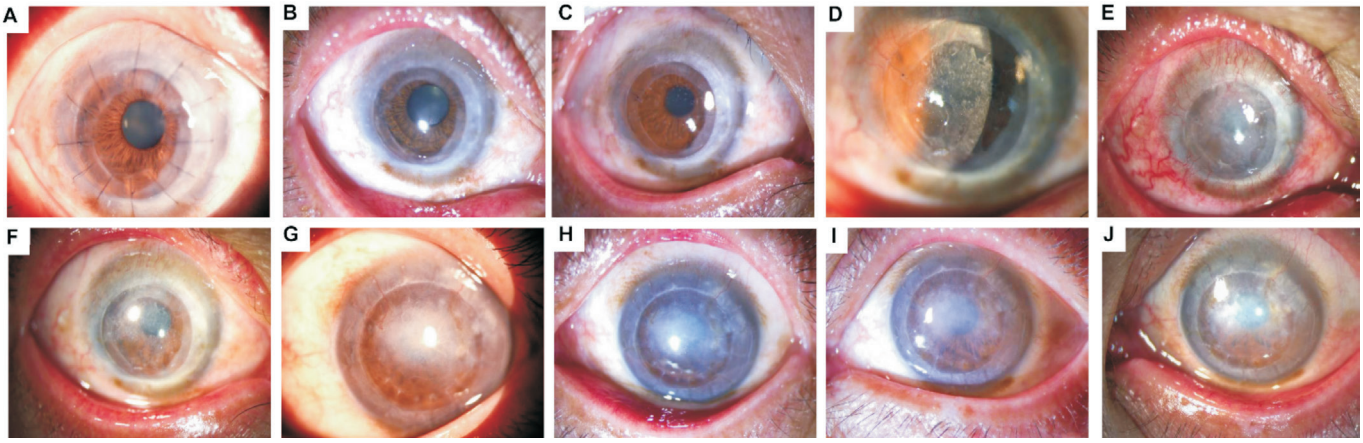

Figure 2 Anterior segment photography A-F: Images of the right eye of IV7 in the family. IV7 had relapse 3y after PKP, and underwent PKP again 27y after PKP. He was followed up 6y after the second PKP. A: Image at 1d after secondary PKP (2013-03). The corneal graft was transparent. The suture was present. B: Corneal graft transparency at 2y and 1mo after secondary PKP (2015-04). C: Image at 3y after secondary PKP (2016-03). Spotty grayish white opacity was observed in the cornea graft. D: Image at 5y and 10mo after secondary PKP (2019-01). Corneal epithelium was rough and eroded into clumps. The cornea was uneven. The deposition was coarse and thick. E: Image at 5y and 11mo after secondary PKP (2019-02). The picture showed conjunctival hyperemia, corneal edema and erosion. F: Image at 6y after secondary PKP (2019-03). The gray and white deposition was fused to flake on the surface of the cornea graft. G-J: Images of the left eye of IV7 in the family. G: Gray and white substance deposition in the center of the corneal graft at 9y after PKP (2013). H: Image at 11y after PKP (2015). I: Image at 12y after PKP (2016). J: Image at 15y after PKP (2019). The image showed that the gray and white deposition in the central cornea gradually increased and thickened with time and the corneal surface was rough. PKP: Penetrating keratoplasty.

CD and 45 healthy people) of 4 generations in this family, all of whom were Han Chinese. Among the 14 patients with CD, 1 patient (2 eyes) underwent penetrating keratoplasty (PKP) and CD recurred 36mo after the operation. The right eye of this patient underwent PKP again because of recurrence. One patient (1 eye) underwent PKP and CD recurred 30mo after the operation. One patient (2 eyes) underwent lamellar keratoplasty (LKP) and CD recurred 33mo after surgery. After recurrence, binocular LKP was performed again.

The 14 patients had recurrent eye pain, eye abrasion and tearing, accompanied by redness and progressive vision loss since childhood. After keratoplasty, the patients IV7, IV9, and V2 (Figure 1) had CD relapse at 33.5±3.0mo. For the corneal morphology of recurrent cases, it was observed that the corneal epithelium was rougher and the corneal roughness was more obvious. The corneal epithelium was eroded into clumps and the deposition was thicker and closer to the corneal surface (Figure 2). The clinical manifestations of the pedigree patients are shown in Table 1. All patients were found to have R124L mutation in the *TGFBI* gene (heterozygous mutation in exon 4 c.418 G>T; Figure 3).

Observation of Corneal Histopathology and Ultrastructure HE staining showed that there were 5 to 6 layers of epithelial cells in normal corneal tissue with regular arrangement and uniform thickness of the pre-elastic layer (Figure 4A). Masson's trichrome staining showed regular arrangement of matrix collagen fiber bundles (Figure 4B). However, in

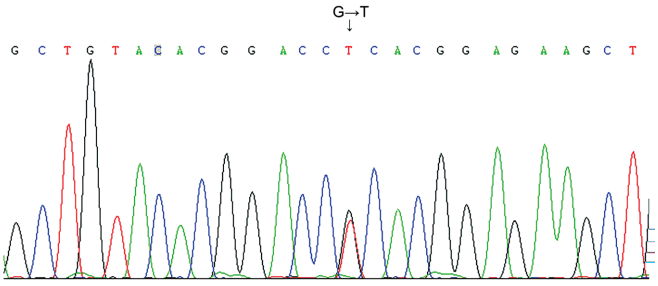

Figure 3 Direct sequencing analysis of exon 4 of *TGFBI* gene The mutation c.418 G>T resulted in Arg124Leu (R124L; indicated by the arrow).

the corneal tissue of recurrent cases, the thickness of corneal epithelium was uneven and the arrangement of epithelial cells was disordered (Figure 4C, 4D). The normal columnar structure of basal cells and the structure of the anterior elastic layer disappeared. Instead, a large amount of red staining substances were observed. Congo red staining was positive (Figure 4E). Orange red was observed in the sub-epithelial and anterior stroma. Masson's trichrome staining was positive (Figure 4F). The sub-epithelial and anterior stroma was stained red and the stromal collagen fibers were stained blue. PAS staining was positive (Figure 4G). Red substance was seen in the sub-epithelial and anterior stroma of the cornea. Alcian blue staining was negative and no specific staining was observed.

Observation of Corneal Ultrastructure Under TEM, it was observed that the epithelium microvilli of recurrent corneal

Table 1 Clinical manifestations of patients in CD family with R124L mutation

Family number	Sex/age (y)	The onset age (y)	Age of visual impairment (y)	Corneal opacity under slit lamp[a]	Corneal epithelial erosion[b]	Recurrent corneal epithelial defects	Treatment
IV7	Male/59	7	12	+++	+	Yes	PKP was performed twice in the right eye and once in the left eye
IV9	Female/56	5	8	+++	+	Yes	PKP was performed once in both eyes
V2	Male/44	7	10	+++	++	Yes	LKP was performed twice in both eyes
V25	Male/31	5	8	++	++	Yes	PKP was performed in the right eye and PTK in the left eye

CD: Corneal dystrophy; PKP: Penetrating keratoplasty; LKP: Lamellar keratoplasty; PTK: Phototherapeutic keratectomy. [a]Corneal turbidity degree under slit lamp: +: Uneven turbidity of the anterior elastic layer of the cornea, turbidity did not reach the limbus of the cornea, anterior chamber and pupil could be seen; ++: Uneven turbidity of the anterior elastic layer of the cornea, turbidity reached the limbus of the cornea, anterior chamber and pupil could still be seen; +++: Uneven turbidity of the anterior elastic layer of the cornea, turbidity reached the limbus, the anterior chamber and pupil were not clear. [b]Degree of corneal epithelial erosion: +: Occasionally; ++: Occasionally, 2-3 times per year; +++: Frequently, more than 4 times a year.

specimens reduced or disappeared. The shape of basal cells varied (Figure 4I). There was swelling of cell mitochondria (Figure 4J). There were vacuoles in the cytoplasm (Figure 4K). The nuclei were hyperchromatic, enriched, and crescent-shaped, or became fragmented, distorted, contracted, or even disappeared. Typical apoptotic features were observed (Figure 4L). Desmosomal junctions between some cells were reduced or disappeared. The boundaries between the anterior elastic layer and basal cells were blurred or disappeared (Figure 4M). A large number of high-density abnormal substances were dispersed and accumulated, and no obvious fibrous structure was observed in the depositions (Figure 4N).

DISCUSSION

The gene mutation of CD and the pathological changes of cornea have been reported previously[2,16]. This paper is the first to report the pathological changes and TEM ultrastructure of the corneal graft with recurrent CD. In the CD classification in 2015, R124L mutation is classified as epithelial-basal CD. This paper was also one of the few families with R124L mutations reported with a history of consanguineous marriage.

The characteristics of the CD family in this study were as follows. First, it has been reported that the earliest onset age was generally around 7 years old[17]. In this study, the youngest age of onset in this family was 1 year old. To our knowledge, this is the youngest reported age of onset. Second, this family was with a history of consanguineous marriage.

In this study, gene sequencing found heterozygous gene mutation at the R124L locus in all patients, which was consistent with previous findings[18]. The R124L mutation is also a common mutation type. In other atypical cases, R125H, R555Q, R555W and a few mutations at the R124C, G623D, H572R and H626P sites have also been reported[19-27]. Our study also found that the CD of this pedigree had high incidence, early onset age, severe condition and high recurrence rate. A possible explanation is the genetic features of consanguineous marriage. The mutation from the polar hydrophilic arginine to the non-polar hydrophobic leucine may change the polarity and hydrophilicity of amino acids, thus changing the three-dimensional structure of protein and contributing to the severity of the disease.

Some studies have reported that the R124L mutation of CD has an early onset age and often has recurrent corneal epithelial erosion from childhood, with obvious symptoms and rapid progress[4,28]. Later, there may be corneal punctuated or map-like turbidity, which may gradually expand and merge, leading to vision loss and blindness[29]. Surgical treatments such as PTK or keratoplasty can be performed. Nevertheless, the disease often relapses within several years after the operation. When there is CD recurrence, PTK or keratoplasty can be performed again[12,29]. Therefore, the patients with CD recurrence in this study underwent PKP or LKP again.

In this study, HE staining clearly showed the scope and location of recurrent lesions, including uneven thickness of corneal epithelial cells, disordered cell arrangement, swelling of some corneal epithelial cells and disappearance of the normal cylindrical structure of basal cells. It is reported that PAS staining could be positive in CD with R124L mutations[8]. In this study, corneal tissue sections with recurrent lesions were stained with Congo red, showing lamellar orange color in the sub-epithelial and anterior stroma of the cornea, which confirmed that the extracellular substance was amyloidosis. Masson's trichrome staining of the corneal epithelium and anterior stroma showed red staining, indicating that the extracellular depositions were acidic and microfibrous. Therefore, in this relapsed CD family with R124L mutation, the corneal depositions were extracellular amyloid fibrin. This was consistent with previous studies[1,30-31].

Histopathological examination serves as the gold standard for diagnosis[32]. HE staining can clarify the scope, size and shape of the lesion. Specific staining can reveal specific components

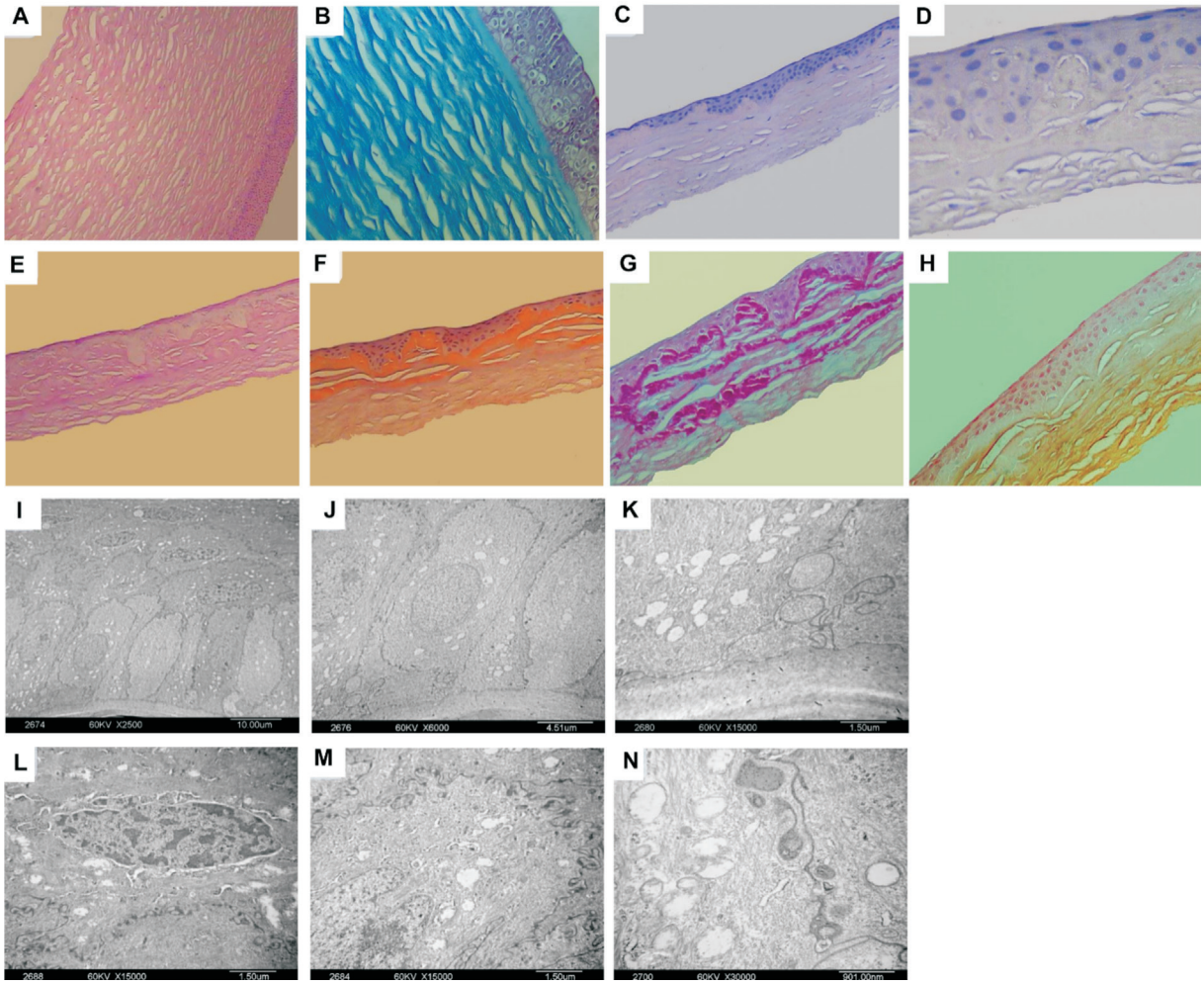

Figure 4 Staining of corneal tissue A, B: Normal corneal tissue sections. A: HE staining of normal corneal tissue showed that there were 5-6 layers of corneal epithelial cells with regular arrangement and the thickness of the pre-elastic layer was uniform (HE staining ×40). B: Masson's trichrome staining of normal cornea showed that the matrix collagen fiber bundles were orderly arranged (Masson's trichrome staining ×200). C-H: Histological staining of the corneal tissue of the recurrent cases. C, D: HE staining of the corneal tissue of the recurrent cases, showing uneven thickness of the corneal epithelium, disordered cell arrangement, and, disappearance of the normal cylindrical structure of the basal cells and the pre-elastic layer structure. A large number of abnormal substances were observed (C, HE staining ×100. D, HE staining ×400). E: PAS staining. Red substance was observed in the sub-epithelial and anterior stroma of the cornea (PAS staining ×100). F: The corneal tissue was stained with Congo red, and the sub-epithelial and anterior corneal stroma were flecked orange (Congo red staining ×100). G: Masson's trichrome staining. The sub-epithelial and anterior corneal stroma were red and the matrix collagen fibers were blue (Masson's trichrome staining ×100). H: Alcian blue staining. No specific staining was observed (Alcian blue staining ×100). I-N: Transmission electron microscopy (TEM) of the recurrent corneal epithelial cells. I, J: It was found that the microvilli of the recurrent corneal epithelial cells were reduced or disappeared. The basal cells were different in morphology and irregular in arrangement, and a few of them were columnar (4J, ×2500, 4K, ×6000). K: The cytoplasmic mitochondria were swelled and vacuolated (×15 000), the nuclei were hyperchromatic, and the chromatin in the nucleus was rough and concentrated. L: The typical apoptotic feature was observed (×15 000). M: Desmosomal junctions between some cells were reduced or disappeared (×15 000). N: The boundary between the anterior elastic layer and basal cells was blurred or disappeared. No obvious fibrous structure was observed in the depositions (×30 000). HE: Hematoxylin-eosin; PAS: Periodic acid-Schiff.

(intrinsic and extrinsic) of the lesion and can give a rough idea of the nature of its depositions. TEM can reveal the pathological changes at the cellular/ultrastructural level. In this study, TEM examination displayed that there were reduced or disappeared epithelium microvilli of recurrent corneal specimens. The basal cell morphology in the epithelium was different. Mitochondria in cells were swelling. Vacuoles were observed in the cytoplasm. Corneal histopathological and TEM ultrastructural changes of the recurrent cases further confirmed that the recurrent CD caused by R124L mutation had characteristic structural changes, which might be related to clinical symptoms and gene mutations[1,3].

Limitations of this study: First, this study only observed the corneal tissues of recurrent cases and did not compare them

with the corneal tissues after the first operation. Second, only one family with a history of consanguineous marriage was studied. The sample size should be expanded in future study.

In this Chinese recurrent CD family with R124L gene mutation, the corneal epithelium of the recurrent cases was rougher. The recurrent specimens suggested extracellular amyloid fibrin in the cornea. Early diagnosis of CD is based on slit lamp examination and clinical symptoms. The advanced cases of CD are classified and diagnosed according to corneal involvement, anatomical site, histopathological features, genetic pattern and genetic testing results. Although consanguineous marriage is now relatively rare in China, it still exists in relatively remote and economically underdeveloped parts of the country. Thus, earlier screening of patients and their families of consanguineous marriage should be performed.

ACKNOWLEDGEMENTS

Foundations: Supported by the National Natural Science Foundation of China (No.81770955); the Major Clinical Research Project of Shanghai Shenkang Hospital Development Center (No.SHDC2020CR1043B); the Project of Shanghai Xuhui District Science and Technology (No.2020-015); the Project of Weifang Science and Technology Bureau (No.2021RKX160).

Conflicts of Interest: Fu MJ, None; **Zhao J,** None; **Duan S,** None; **Zhang HR,** None; **Zhao JJ,** None; **Zeng L,** None; **Wang R,** None; **Zhou XT,** None.

REFERENCES

1 Atia R, Jouve L, Georgon C, Laroche L, Borderie V, Bouheraoua N. Imaging of reis-Bückler corneal dystrophy. *J Fr Ophtalmol* 2019;42(1):105-107.

2 Jun I, Ji YW, Choi SI, Lee BR, Min JS, Kim EK. Compound heterozygous mutations in TGFBI cause a severe phenotype of granular corneal dystrophy type 2. *Sci Rep* 2021;11:6986.

3 Moshirfar M, Bennett P, Ronquillo Y. Corneal Dystrophy. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing; 2022.

4 Cai JQ, Zhu LR, Zha Y, Kang QY. TGFBI gene mutation analysis in Chinese families with corneal dystrophies. *Genet Test Mol Biomarkers* 2016;20(7):388-392.

5 Zhang YL, Ying JL, Zhou WP, Zhu LR, Cai JQ. Analyses of TGFBI gene mutation spectrum in four Chinese families with corneal dystrophy. *Zhonghua Yi Xue Za Zhi* 2015;95(2):116-119.

6 Chao-Shern C, DeDionisio LA, Jang JH, Chan CC, Thompson V, Christie K, Nesbit MA, McMullen CBT. Evaluation of TGFBI corneal dystrophy and molecular diagnostic testing. *Eye (Lond)* 2019;33(6):874-881.

7 Nagano C, Nozu K, Yamamura T, *et al*. TGFBI-associated corneal dystrophy and nephropathy: a novel syndrome? *CEN Case Rep* 2019;8(1):14-17.

8 Weiss JS, Møller HU, Lisch W, *et al*. The IC3D classification of the corneal dystrophies. *Cornea* 2008;27(Suppl 2):S1-S83.

9 Lisch W, Weiss JS. Clinical and genetic update of corneal dystrophies. *Exp Eye Res* 2019;186:107715.

10 Matthaei M, Hribek A, Clahsen T, Bachmann B, Cursiefen C, Jun AS. Fuchs endothelial corneal dystrophy: clinical, genetic, pathophysiologic, and therapeutic aspects. *Annu Rev Vis Sci* 2019;5(4):151-175.

11 Lang SJ, Böhringer D, Reinhard T. Penetrating limbokeratoplasty for gelatinous corneal dystrophy. *Klin Monbl Augenheilkd* 2019;236(2):169-172.

12 Vinciguerra P, Vinciguerra R, Randleman JB, Torres I, Morenghi E, Camesasca FI. Sequential customized therapeutic keratectomy for reis-Bücklers' corneal dystrophy: long-term follow-up. *J Refract Surg* 2018;34(10):682-688.

13 Sridhar U, Tripathy K, Bansal Y. Repeated phototherapeutic keratectomy (PTK) followed by PTK with photorefractive keratectomy for anterior granular corneal dystrophy. *Indian J Ophthalmol* 2020;68(12):3038.

14 Zeng L, Zhao J, Chen YJ, Shang JM, Aruma A, Zhou XT. Multiple phototherapeutic keratectomy treatments in a Chinese pedigree with corneal dystrophy and an R124L mutation: a 20-year observational study. *BMC Ophthalmol* 2019;19(1):191.

15 Chiou AG, Florakis GJ, Copeland RL, Williams VA, McCormick SA, Chiesa R. Recurrent meesmann's corneal epithelial dystrophy after penetrating keratoplasty. *Cornea* 1998;17(5):566-570.

16 Zhao F, Liu Y, Guan T. Analysis of *TGFBI* gene mutations in three Chinese families with corneal dystrophy. *J Ophthalmol* 2019;2019:6769013.

17 Liang QF, Sun XG, Jin XY. *TGFBI* gene mutation in a Chinese pedigree with Reis-Bücklers corneal dystrophy. *Ophthalmic Physiol Opt* 2012;32(1):74-80.

18 Tanhehco TY, Eifrig DE Jr, Schwab IR, Rapuano CJ, Klintworth GK. Two cases of Reis-Bücklers corneal dystrophy (granular corneal dystrophy type III) caused by spontaneous mutations in the *TGFBI* gene. *Arch Ophthalmol* 2006;124(4):589-593.

19 Piao MZ, Zhou XT, Wu LC, Chu RY. Arg555Gln mutation of *TGFBI* gene in geographical-type Reis-Bücklers corneal dystrophy in a Chinese family. *J Int Med Res* 2012;40(3):1149-1155.

20 Yang QN, Zhao YW, Guo LH, Yan NH, Liu XY, Cai SP. Arg124Cys mutation of the *TGFBI* gene in a Chinese pedigree of Reis-Bücklers corneal dystrophy. *Int J Ophthalmol* 2011;4(3):235-238.

21 Ma K, Liu G, Yang Y, Yu M, Sui RF, Yu WH, Chen XM, Deng YP, Yan NH, Cao GQ, Liu XY. *TGFBI* gene mutation analysis in a Chinese pedigree of Reis-Bücklers corneal dystrophy. *Mol Vis* 2010;16:556-561.

22 Li DD, Qi YH, Wang L, Lin H, Zhou N, Zhao LM. An atypical phenotype of Reis-Bücklers corneal dystrophy caused by the G623D mutation in TGFBI. *Mol Vis* 2008;14:1298-1302.

23 Wheeldon CE, de Karolyi BH, Patel DV, Sherwin T, McGhee CNJ, Vincent AL. A novel phenotype-genotype relationship with a TGFBI exon 14 mutation in a pedigree with a unique corneal dystrophy of Bowman's layer. *Mol Vis* 2008;14:1503-1512.

24 Venkatraman A, Hochart G, Bonnel D, Stauber J, Shimmura S, Rajamani L, Pervushin K, Mehta JS. Matrix-assisted laser desorption ionization mass spectrometry imaging of key proteins in corneal samples from lattice dystrophy patients with TGFBI-H626R and TGFBI-R124C mutations. *Proteomics Clin Appl* 2019;13(1):e1800053.

25 Trufanov SV, Tekeeva LY, Salovarova EP, Bag RZ, Sukhanova EV. Corneal dystrophies. *Vestn Oftalmol* 2018;134(5):118-125.

26 de Sousa Peixoto R, Mutch S, Eason J, Jaakson K, Haamer E, Maharajan VS. Association of unilateral lattice corneal dystrophy on slit lamp and bilateral confocal microscopy features with H572R mutation in the TGFBI gene. *Eye (Lond)* 2019;33(12):1973-1975.

27 Wang LY, Zhao CC, Zheng T, Zhang Y, Liu HR, Wang X, Tang XL, Zhao BW, Liu P. Torin 1 alleviates impairment of TFEB-mediated lysosomal biogenesis and autophagy in TGFBI (p.G623_H626del)-linked thiel-behnke corneal dystrophy. *Autophagy* 2022;18(4):765-782.

28 Li DD, Qi YH, Han Q, Lin H, Zhao LM, Zhang CM. Analysis of TGFBI gene mutation in a Chinese family with atypical Reis-Buckler corneal dystrophy. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 2009;26(3):245-248.

29 Qiu WY, Zheng LB, Pan F, Wang BB, Yao YF. New histopathologic and ultrastructural findings in Reis-Bücklers corneal dystrophy caused by the Arg124Leu mutation of TGFBI gene. *BMC Ophthalmol* 2016;16(1):158.

30 Nishino T, Kobayashi A, Mori N, Yokogawa H, Sugiyama K. *In vivo* imaging of reis-Bücklers and thiel-behnke corneal dystrophies using anterior segment optical coherence tomography. *Clin Ophthalmol* 2020;14:2601-2607.

31 Liang QF, Pan ZQ, Sun XG, Baudouin C, Labbé A. Reis-Bücklers corneal dystrophy: a reappraisal using *in vivo* and *ex vivo* imaging techniques. *Ophthalmic Res* 2014;51(4):187-195.

32 Reda AM, Saad El-Din SA. Rare stromal corneal dystrophic diseases in Oman: a clinical and histopathological analysis for accurate diagnosis. *Oman J Ophthalmol* 2020;13(2):70-75.