Macular Corneal Dystrophy and Posterior Corneal Abnormalities

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Purpose: This study reports the presentation of 2 families with macular corneal dystrophy (MCD). The aim of this study was to show whether ultrasound biomicroscopy (UBM) can, based on posterior changes of the cornea in MCD, assist in the choice of surgery, either anterior lamellar keratoplasty (DALK) or penetrating keratoplasty (PK), compared with optical coherence tomography (OCT) and Scheimpflug.

Methods: Six patients with MCD were examined for their best-corrected visual acuity, slit-lamp, OCT, UBM, and Scheimpflug findings. Blood samples for DNA and exons of the *CHST6* gene were screened for mutations.

Results: All 6 patients showed typical MCD signs at the slit lamp. Corneal transplantation was required in 2 patients in both eyes. Recurrence of MCD was observed in 2 eyes after the DALK procedure (patient A5, age 48 years, right eye and B1, 51 years, left eye), whereas the 2 eyes after PK (patient A5, age 48 years, left eye and patient B1, 51 years, right eye) remained clear (for 10 years of follow-up in patient A5 and 4 years in patient B1). In 2 patients (A1 and A3), corneal thinning could be evaluated by OCT. In 3 patients (A2, 3, and 4), UBM disclosed deeper pathologies including opacities, loss of continuity, and focal protrusions of the posterior cornea, which were not evident by other devices. In family A, a novel mutation was identified.

Conclusions: Our UBM examination of MCD shows alterations of the cornea's posterior layer and confirms the known clinical and histological findings of MCD that PK represents the therapy of choice, contrary to DALK. The novel *CHST6* mutation shows the heterogeneity of MCD.

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Corneal dystrophies are a group of hereditary diseases involving opaqueness at different corneal layers manifesting as progressive discomfort and impairment of vision. Macular corneal dystrophy (MCD) is a rare autosomal recessive dystrophy due to mutations in the carbohydrate sulfotransferase 6 gene (*CHST6*) on chromosome 16q22, which encodes N-acetylglucosamine 6-O-sulfotransferase.[1] This enzyme has a key role in keratan sulfate biogenesis, and because of failure of keratan sulfation, glycosaminoglycans (GAGs) accumulate in different corneal structures.[2–4]

Initially, starting in childhood, central superficial irregular whitish fleck-like opacities develop, which give the condition the name and unlike granular corneal dystrophy type-1, these flecks involve the limbus and deep stroma down to Descemet membrane. Simultaneously, a progressive diffuse haze develops, throughout the whole stroma with corneal thinning.[1,5] Severe visual impairment occurs between 10 and 30 years of age. As a result of these changes, constant discomfort, photophobia, tearing, and progressive impairment of vision occur, which ultimately necessitate corneal transplantation. Traditionally, penetrating keratoplasty (PK) has been considered as the best treatment option in stromal dystrophies.[6] However, the recent introduction of deep anterior lamellar keratoplasty (DALK) with the advantage of retaining the host endothelium placed this procedure as an attractive alternative.[7] Disease recurrence emerged as a significant problem after lamellar graft procedures,[8] with estimates that recurrence risk may climb more than 5 times higher after these types of surgeries.[9] Consequently, most surgeons prefer PK whenever deep lamellar involvement is evident,[10,11] highlighting the important focus on examining this layer. On histology, abnormal GAGs are seen abundantly —underneath the epithelium, between stromal lamellae, and within keratocytes and endothelial cells.[12,13] However, their preoperative detection is often difficult. On slit-lamp biomicroscopy, the most prominent findings are observed at the center of the cornea just under the epithelium, whereas deep and peripheral deposits are obscure and harder to identify. Recent studies have used optical coherence tomography (OCT)[14] and the Scheimpflug camera[15] to exemplify in vivo changes in MCD, including diffuse corneal thinning

and superficial hyperreflective depositions; however, these devices were less successful in demonstrating deep and peripheral changes, probably because of light attenuation.

In this study, we have screened *CHST6* for mutations in 2 unrelated families and identified a yet unrecognized null mutation and a recently described missense mutation. We also describe their related phenotypes before and after corneal transplantation. Our in vivo studies included for the first time ultrasound biomicroscopy (UBM), which clearly illustrated posterior corneal changes.

MATERIALS AND METHODS

Clinical Assessment and Genomic DNA Extraction

Patients

Six patients from 2 families suspected to have MCD were recruited. A detailed medical history was obtained from each family by conducting personal interviews and screening available medical records (Table 1). In 1 family (family A), consanguinity was reported, whereas in the other (family B), the parents who shared the same ethnic background were not related (Fig. 1). Ophthalmic examinations took place at the Ophthalmo-Genetic Eye Clinic, and the study protocol has been approved by the Institutional Review Board of the Assaf Harofeh Medical Center, Zerifin, Israel. Written informed consent was obtained from all participants. Family members underwent a detailed ophthalmologic examination that included slit-lamp biomicroscopy and in vivo corneal imaging using the following: anterior segment spectral domain OCT (Heidelberg Engineering GmbH, Heidelberg, Germany), Scheimpflug camera (Pentacam system; Oculus Optikgeräte GmbH, Wetzlar, Germany), and UBM (Aviso A/B mode Ultrasound Driven PC System; Quantel Medical, Cedex, France).

Mutation Screening

Blood samples were drawn, and genomic DNA was extracted using a commercial kit (Gentra System Inc, Minneapolis, MN). Molecular analysis was performed at the Matlow's Ophthalmo-Genetic Laboratory, Assaf Harofeh Medical Center, Israel.

The 5′ untranslated region (UTR) of the *CHST6* gene and all exons and their boundaries were amplified and sequenced using specific primer pairs as previously described.[16] Briefly, polymerase chain reaction (PCR) amplification was performed using 3 sets of the following primers: for exon 1 part 1 and 5′UTR region: Fw. 5′-GTTATGTCTTAAGGCCACATTTCC-3′; Rw. 5′-GAAGAAGCGCACCTCCTTG-3′, for exon 1 part 2: Fw. 5′-GCCGCAACCTGTCCGACCTC-3′; Rw. 5′-GAGACGTTGAGCGCATTCCTGG-3′, for exon 1 part 3 and 3′UTR: Fw. 5′-CAGAAATCCGTGCGCTCTAC-3′; Rw.5′-GTGATTTCAGACAAGTCCTTTCAC-3′. The reaction took place in a 25-μL volume containing 50 ng of DNA, 13.4 ng of each primer, 1.5 mM dNTPs, in 1.5 mM $MgCl_2$, PCR buffer, with 1.2 U of Taq polymerase (Bio-Line, London). After initial denaturation for 5 minutes at 95 degrees, 30 cycles were performed (94 degrees for 2 minutes, 57 degrees for 3 minutes, and 72 degrees for 1 minute), followed by final extension of 7 minutes at 72 degrees. PCR amplicons were sequenced in both directions using a commercial sequencing service (Hylabs Ltd, Park Tamar, Rehovot, Israel).

Validation studies of the variants included: segregation analysis of mutations within the families and verification of their rarity in our in-house exome database composed of 300 exomes, of them 25 are of Ashkenazy Jews and 20 are of Arab ancestry.

RESULTS

Clinical Data

Family A was a consanguineous Arab family with 5 affected individuals, and family B consisted of a single Ashkenazy Jewish patient. We recorded that the ages when symptoms began differed between members of the 2 families. All patients of family A recalled symptoms of tearing, photophobia, and visual disturbances starting at childhood, which contradicts the late onset of symptoms in the sporadic patient of family B who could not recall any symptoms before

TABLE 1. Clinical Findings and Identified Mutations

Patient	Ethnicity	Age	Age at First Symptoms	RE Vision	LE Vision	RE Surgery	LE Surgery	Mutation
A1	Arab Muslim	18	Before age 10	6/12+ PH 6/7.5P	6/6P PH NC	—	—	p.E364Gfs8
A2	Arab Muslim	13	Before age 10	6/12− PH 6/9	6/12− PH 6/7.5	—	—	p.E364Gfs8
A3	Arab Muslim	23	Before age 10	6/9+ PH NC	6/12 PH 6/9	—	—	p.E364Gfs8
A4	Arab Muslim	22	Before age 10	6/30 PH 6/12	6/7.5 PH NC	—	—	p.E364Gfs8
A5	Arab Muslim	48	21	6/30	6/24	DALK 2008, recurrence under the epithelium	PKP 2005	p.E364Gfs8
B1	Ashkenazy Jewish	51	35	CC 6/18++	CC 6/18	2 PK, last one in 2011	DALK 2015, recurrence at the surgical interface	p.P64L

CC, with correction; LE, left eye; NC, no change; PH, pinhole; RE, right eye.

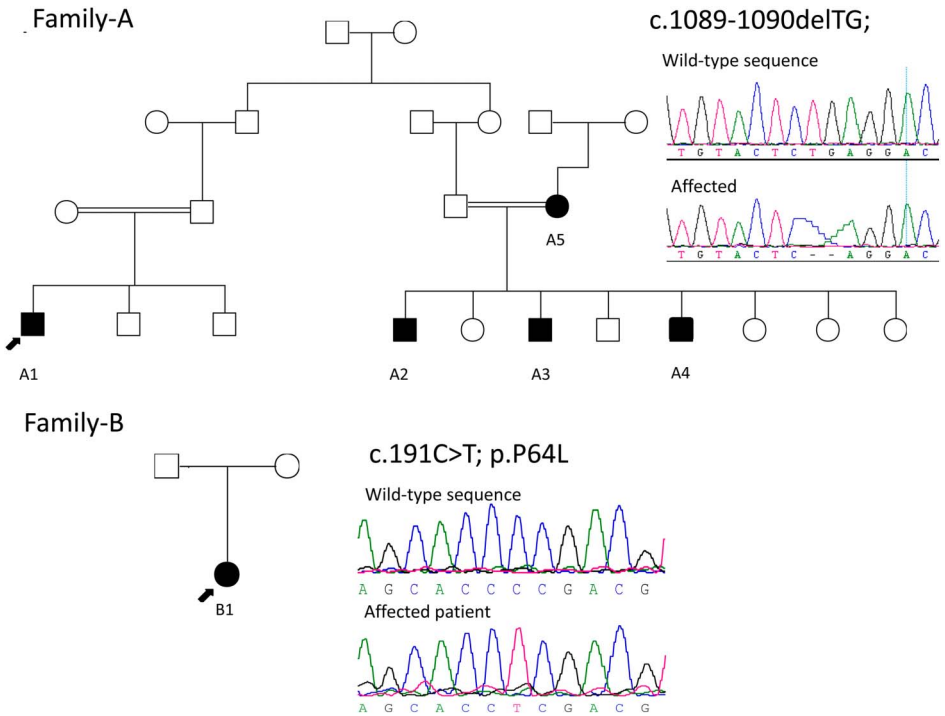

FIGURE 1. Families and identified mutations.

age 35. Clinical characteristics and identified mutations of the 2 families are presented in Table 1.

On slit-lamp biomicroscopy, characteristic grayish opacities at the central anterior stroma were evident in all patients (Fig. 2A). Some cases showed additional opacities at deep peripheral locations (Figs. 2B, C). As previously reported for MCD, OCT illustrated very pronouncedly the central lesions that appeared hyperreflective just under the epithelium and Bowman layer. Larger deposits frequently caused "optical shadow," and pushed the overlying epithelium, which became thinner, anteriorly (Figs. 2D, E).[14,15] However, aggregates at the deep peripheral location were much smaller and harder to identify (Fig. 2D*). Scheimpflug imaging had exemplified thinning of the cornea and anterior deposits (Fig. 2J) but could not demonstrate pathologies of the posterior lamella. Interestingly, some measurements disclosed a yet unrecognized aberration pattern consisting of focal, round, posterior elevations on "back-elevation" maps (Figs. 2J-1). Curiously, inspection of the corresponding locations by Scheimpflug (Figs. 2J-2), slit-lamp, or OCT (data not shown) could not identify any deposits or abnormalities of the posterior corneal curvature at the analogous coordinates. Therefore, we have followed former suggestions to use UBM whenever other imaging modalities could not provide adequate evaluation.[17] Satisfactorily, in addition to superficial (Fig. 2K-1) and mid-stromal deposits (Figs. 2K-3,4), which have already been recognized by the other methods, this utility uncovered hidden interruptions, loss of continuity (Figs. 2K-1–4), and focal protrusions (Figs. 2K-1,2,4) of the posterior corneal surfaces.

In their fourth decade, corneal transplantation was required in 2 patients (Figs. 1, A5 and B1). Each of them had a different procedure in his alternate eye, for example, PK in 1 eye and DALK in the other (4 eyes). Notably, recurrence of MCD was observed only in eyes that underwent DALK (Figs. 2F, H, I), whereas the full-thickness corneal buttons remained clear for 7 years and 1 year of follow-up in our patients A5 and B1, respectively (data not shown). Remarkably, the location of recurrence differed between both patients; abnormal gray deposits were noticed under the epithelium of patient A5 (Figs. 2F, H), whereas in patient B1, depositions appeared at the surgical interface (Fig. 1J). Differences in the location of recurrence have already been described for MCD9.

Molecular Analysis

The presumed diagnosis of MCD was confirmed by direct sequencing of the *CHST6* gene. In family A, a novel homozygous binucleotide deletion (Fig. 1A) was identified; a normally occurring TG (thymine–guanine) sequence at cDNA location 1089 to 1090 was deleted, causing a translation change of glutamic acid at position 364 of the designated *CHST6* protein to glycine, and creation of a stop-codon 8 amino acids downstream (c.1089-1090delTG; p.E364Gfs8*). This mutation demonstrated full segregation within all members of this family.

The Ashkenazi Jewish patient of family B carried a different mutation; a homozygous C→T substitution at position 191 of the cDNA resulted in a change of an evolutionarily conserved proline to leucine at position 64 of the encoded N-acetylglucosamine 6-O-sulfotransferase (c.C191T; p.P64L) (Fig. 1B). This missense mutation has recently been described in the heterozygous state in a Polish patient with MCD.[14]

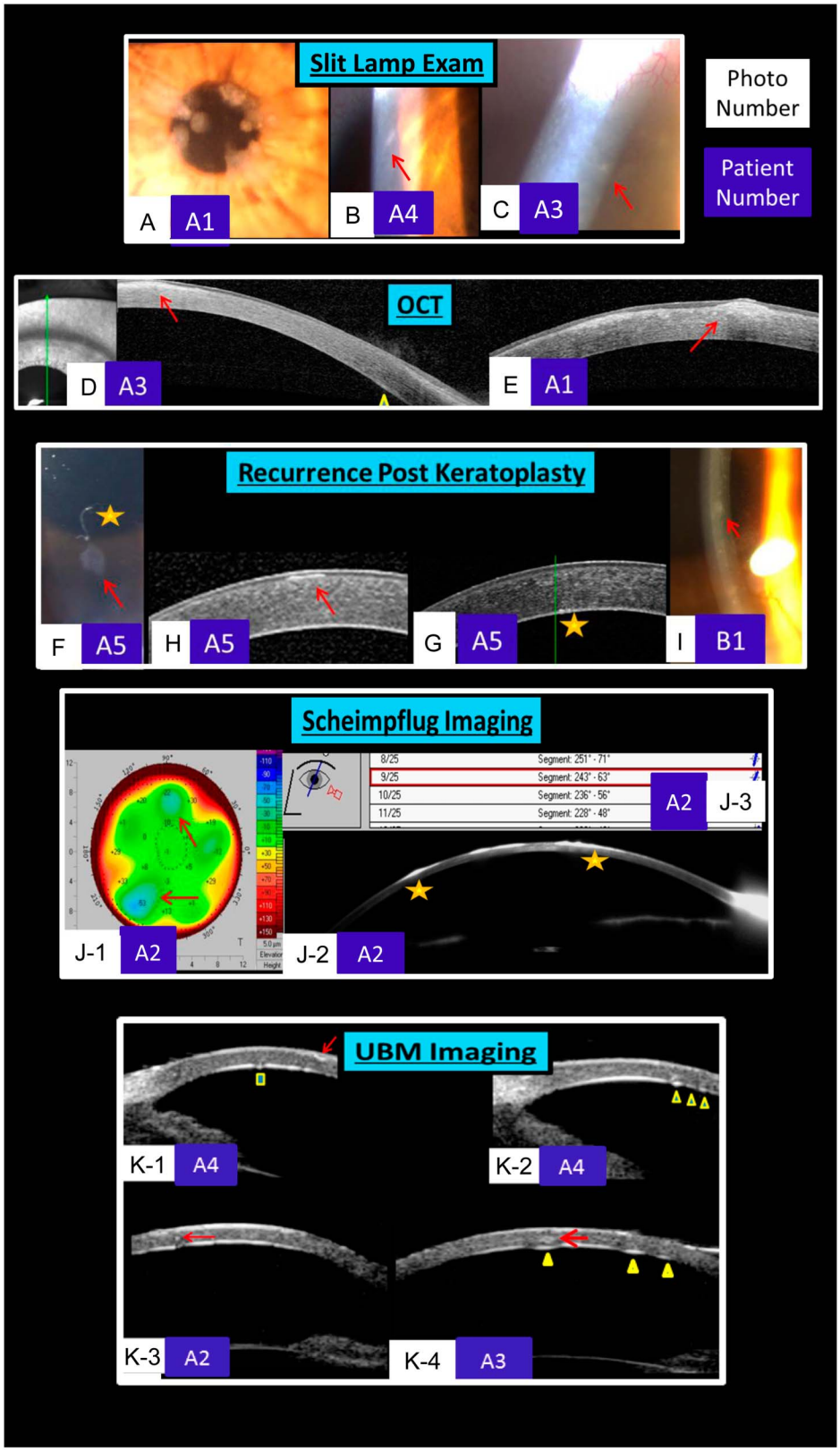

FIGURE 2. Photographs and imaging of representative patients. A, Broad slit lamp displaying prominent fleck-like, grayish opacities in the anterior central stroma. B and C, Smaller slit lamp showing diffuse opacities of the stroma. D and E, OCT imaging illustrating large deposits (arrows), which frequently cause an "optical shadow" and push the overlying epithelium anteriorly. Peripheral sediments hint to posterior alterations (D, arrowhead) (the green line in the upper left insert, indicates the location of measurements across the superior corneal limbus); (F, G, and H) recurrence under the epithelium after DALK in patient A5. An unintentional leftover fiber located at the surgical interface (asterisk) is shown on slit-lamp photograph (F) and OCT (G). Just inferior to the fiber, grayish discrete hyperreflective sediment is observed under the epithelium [F and H (arrows)]. I, Recurrence at the surgical interface after DALK in patient B1 appeared under the transplanted button; (J): Scheimpflug imaging of patient A2. J1 shows a yet unrecognized aberration pattern consistent of focal, round, posterior elevations on "back-elevation" maps (arrows). J2 shows diffuse corneal thinning and hyperreflectivity at the anterior cornea. No pathologies of the posterior lamella are recognized. J3 shows the image at the 63- to 243-degree meridian that corresponds to sites of abnormalities in back-elevation map; (K) UBM imaging of patients A2, A3, and A4 (Fig. 1) exemplifies aberrations at different locations: anterior and midstromal deposits (red arrows), and posterior corneal surface interruptions, loss of continuity, and focal protrusions (yellows arrowheads and triangles).

DISCUSSION

To our knowledge, this study has used for the first time UBM for in vivo evaluation of patients with genetically verified MCD. We confirm previous reports that imaging by OCT and Scheimpflug demonstrates hyperreflective deposits and corneal thinning as integral signs of MCD and offer UBM as an appropriate tool to examine important posterior lamellar changes that should be taken into consideration before corneal graft transplantation.

UBM is a well-established tool for imaging the eye's anterior segment especially when clarity is impaired.[18] It provides high magnification power, with good penetration, which has already been exploited for diagnosis, follow-up, and management of other corneal degenerations and dystrophies.[19–21] The preferred surgical option for managing MCD has not been established yet. Most surgeons would prefer DALK as the procedure of choice in patients with corneal diseases not involving the endothelium, but whenever it does, traditional full-thickness PK is considered as a better alternative. Higher rates of recurrence remain a significant disadvantage after DALK,[9] probably because of the leftover diseased corneal tissue.[8] Unfortunately, our patients have also experienced this undesired complication after DALK. According to medical records, involvement of deep corneal structures was not evident before their operations, although their preoperative assessments did not include UBM. In retrospect, given the acknowledgment by histology of endothelial participation in MCD, it is most likely that such undiagnosed involvement had also occurred in our patients, and as a whole, PKP should be regarded as the procedure of choice in MCD. In the future, prospective studies could determine whether examining transplantation candidates with UBM may help clinicians tailor their surgical plan more appropriately and improve graft survival outcomes. Interestingly, the location of disease recurrence was different between subjects; the deposits in patient A5 (Fig. 2) appeared in the donor cornea under the epithelium, whereas in patient B1 (Fig. 2), they occurred at the surgical interface. One plausible explanation for this finding is that remaining GAGs in the host became significant only after DALK took place. Another explanation for the varying recurrence location could relate to differences in migration of diseased keratocytes and/or GAGs between the 2 subjects. Differences in mutation types or background genes could also influence this. Differences in migration of cells could also relate to nongenetic influences such as local inflammation, and differences in medications. Although MCD has extensively been studied and various *CHST6* mutations were reported, very little information on phenotype–genotype correlations is available. One study reported more severe phenotypes in patients harboring frameshift mutations as compared with missense ones.[22] This brought up the assumption that the mutation type by itself could serve as a criterion in the decision whether to excise the entire cornea in PK or to use lamellar grafts. However, another study did not identify any meaningful genotype–phenotype correlations.[14] Our study consisting of only 6 patients with MCD and 4 operated eyes is too small to draw correlations; however, it does provide intriguing insights on relationships between mutations and their associated phenotype. Two mutations were identified: a novel truncating mutation (c.1089-1090delTG; p.E364Gfs8*) and an already reported missense one (c.C191T; p.P64L). In family A, 5 patients harboring the TG deletion reported a much earlier age of disease onset as compared with the missense mutation carrier. One intuitive conclusion could relate the 20-year difference in disease onset solely to genetic influence, which could be in agreement with the observations of Gruenauer-Kloevekorn et al[22]; however, other factors such as recall bias may also have influenced this determinant. Another risky deduction relates to disease recurrence; our study exemplified that this complication is not characteristic for a specific mutation as it occurred in both mutation types; therefore, whether the underlying mutation is missense or nonsense cannot stand as a criterion in surgical decision making. In our perspective, further larger-scale studies on relations between genotypes and phenotypes are needed before firm correlations could be drawn for this rare disorder.

In summary, we have identified a novel mutation and an already described missense mutation in Israeli patients with MCD. We also used for the first time UBM, which identified yet unrecognized posterior surface changes with possible importance for surgical decision making and graft survival.

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