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Congenital Corneal Endothelial Dystrophies Resulting from Novel *De Novo* Mutations

Khrishen Cunnusamy, PhD¹, Charles B. Bowman, MD², Walter Beebe, MD², Xin Gong, MD¹, R. Nick Hogan, MD, PhD¹, and V. Vinod Mootha, MD^{1,3}

¹University of Texas Southwestern Medical Center, Department of Ophthalmology, Dallas, Texas, USA

²Cornea Associates of Texas, Dallas, Texas, USA

³University of Texas Southwestern Medical Center, McDermott Center for Human Growth and Development/Center for Human Genetics, Dallas, Texas, USA

Abstract

Purpose—To describe two cases of congenital corneal endothelial edema resulting from novel *de novo* mutations.

Methods—**Case A** patient was a 15 months old Caucasian infant and **Case B** patient was a 3 year old Hispanic child presenting with bilateral cloudy corneas since birth. Clinicopathological findings are presented. DNA samples were screened for mutations in candidate genes by Sanger sequencing.

Results—Slit-lamp examination of **Case A** patient revealed stromal edema and haze. Histology of keratoplasty button showed stromal thickening with loss of endothelium and thin Descemet's membrane. Sanger sequencing established the diagnosis of congenital hereditary endothelial dystrophy (CHED) by detection of a compound heterozygous mutation in *SLC4A11*. The proband displayed a novel *de novo* frameshift mutation in one *SLC4A11* allele, p.(Pro817Argfs*32), in conjunction with a maternally inherited missense mutation in *SLC4A11*, p.(Arg869His). **Case B** patient similarly presented with stromal edema and stromal haze. Histopathological analysis revealed a spongy epithelium, focal discontinuities in Bowman's layer, stromal thickening with areas of compacted posterior stroma, variable thickness of Descemet's membrane, and regional multilayered endothelium. Sanger sequencing found a novel *de novo* nonsense mutation in the first exon of *ZEB1*, p.(Cys7*).

Conclusions—To our knowledge, we present the earliest clinical presentation of posterior polymorphous corneal dystrophy resulting from a *de novo* mutation in *ZEB1*. Additionally, we present a CHED case with a thin Descemet's membrane with a novel compound heterozygous *SLC4A11* mutation. In the absence of a family history or consanguinity, *de novo* mutations may result in congenital corneal endothelial dystrophies.

Corresponding Author: V. Vinod Mootha, MD, University of Texas, Southwestern Medical Center, 5329 Harry Hines Blvd, Dallas, TX, 75390 – 9057, Vinod.Mootha@UTSouthwestern.edu.

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Keywords

Congenital hereditary endothelial dystrophy; CHED; posterior polymorphous corneal dystrophy; PPCD; *SLC4A11*; *ZEB1*

INTRODUCTION

Corneal endothelial dystrophies are a subset of bilateral inherited diseases of the cornea that can result in a severe decline in visual acuity. Characterized by diffuse, non-inflammatory corneal edema and opacification arising from severe endothelial cell dysfunction, the disorders encompass several subtypes including congenital hereditary endothelial dystrophy (CHED; MIM #217700), posterior polymorphous corneal dystrophies (PPCD1; MIM #122000, PPCD3; MIM #609141), Fuchs' endothelial corneal dystrophy (FECD), and X-linked endothelial corneal dystrophy (XECD). Despite their clinically unique features, many of the dystrophies still harbor similar histopathological hallmarks including structural aberrations in the individual layers of the cornea, degeneration of corneal endothelial cells, anomalous deposition of collagen on the posterior surface of the Descemet's membrane, and loss of corneal deturgescence.¹⁻³

In the current report, we describe two cases of congenital corneal edema without a family history of inherited corneal disease or consanguinity where candidate gene sequencing assisted in making the diagnosis by the identification of novel *de novo* mutations. We first describe a case of CHED with a *de novo* variant resulting in a compound heterozygous mutation in *SLC4A11* gene. We further report a case initially presumed to have the clinical hallmarks of CHED which was subsequently re-diagnosed to be a case of PPCD3 following identification of a novel, *de novo* mutation in the *ZEB1* gene. In this current era of human genomics with its promise of personalized patient care, the two cases reported here epitomize how genetic testing can complement careful clinical examination, and histopathological review, for the proper diagnosis, management, and counseling of the patients with congenital corneal endothelial dystrophies.

MATERIALS AND METHODS

The study protocol had the approval of the institutional review board of the University of Texas Southwestern Medical Center and was in compliance with the tenets of the Declaration of Helsinki. All study subjects were recruited at UT Southwestern after informed consent. The subjects underwent a complete eye examination including slit-lamp biomicroscopy and/or examination under anesthesia by cornea fellowship-trained ophthalmologists (VVM, CBB, WB).

Penetrating keratoplasty specimens were fixed in formalin and embedded sections were processed for standard hematoxylin and eosin staining as described previously.⁴ Genomic DNA was extracted from leukocytes of peripheral blood using AutoGen FlexiGene by Qiagen (Valencia, CA). Exon coding regions were amplified by PCR with flanking primers and subsequently sequenced by Sanger sequencing. Primers for *ZEB1* were selected using

ExonPrimer and Primer3 (Helmholtz Center Munich, Institute of Human Genetics)⁵ while previously described primers for *SLC4A11* were utilized.⁶

CASE REPORTS

The **Case A** patient was a 15 months old Caucasian toddler who presented to the pediatric ophthalmologist with a chief complaint of limited ambulation. Parents reported that the child “bumped into things” because of poor vision, noting that the onset was gradual and that the condition was worsening. Parents also reported that the child had bilateral cloudy corneas since birth. The family history was absent for eye disease or consanguinity. The patient’s ocular history was also remarkable for strabismus. The pediatric ophthalmologist noted mild stromal edema and stromal haze. A clinical diagnosis of CHED was made by a corneal specialist and future keratoplasty was advised. Pre-operative visual acuity was 20/80 OD and 20/80 OS as measured by Allen cards. Corneal pachymetry results showed thickened corneas at 800 µm OD and 800 µm OS. Intraocular pressures (IOPs) were deemed normal by tactile digital pressure and corneal diameters were also noted to be normal. The anterior and posterior segments of the eye were otherwise normal. At the age of 3 years and 4 months, the patient underwent penetrating keratoplasty (PKP) in both eyes over 2 months. Figure 1A shows 2 months status post PKP to the left eye at which point right eye was operated on. Five days post PKP to the right eye, the patient suffered a rupture of OD corneal transplant wound after a fall. Examination under anesthesia revealed that the dehiscence involved 270 degrees of the wound with lens and vitreous prolapse. The patient underwent emergent lensectomy, anterior vitrectomy, and repair of dehiscent keratoplasty wound. At the most recent follow-up visit (17 months after initial transplant), both corneal grafts were noted to be clear and best corrected visual acuity was 20/60 in each eye.

Histopathological analysis of keratoplasty button showed areas of thickening but also focal loss of Bowman’s layer centrally (Figure 1B), stromal thickening with edema, and sparse endothelial cells with a thin Descemet’s membrane (Figure 1C). The ocular pathologist’s report noted “some features of CHED are present; however, Descemet’s membrane is extremely thin. Only a few endothelial cells remain. Hence, clinical correlation is required.” Both parents were examined and had normal corneal examination by slit-lamp examination. Given the absence of consanguinity and absence of thickening of Descemet’s membrane, PCR amplification and traditional Sanger sequencing of the coding exons of *SLC4A11* were performed on genomic DNA from the proband and parents. The mother was noted to have a pathogenic heterozygous single nucleotide polymorphism predicted to result in a missense mutation [SNP rs121909392: NM_032034.3:c.2606G>A, p.(Arg869His)]. In addition to the p.(Arg869His) mutation from his mother, the proband showed a novel *de novo* mutation in the father’s *SLC4A11* allele (Figure 1D) resulting in a frameshift mutation [NM_032034.3:c.2450del, p.(Pro817Argfs*32)]. Paternity was confirmed by microsatellite loci genotyping using PowerPlex 1.2 System from Promega (Madison, WI).

The **Case B** patient was a Hispanic child who presented with corneal clouding at the age of 3 to a pediatric ophthalmologist. Per history by mother, the corneas were “hazy since birth.” Family history was unremarkable for eye disease and without consanguinity. At 4 years of age, best spectacle corrected visual acuity was 20/70 in the OD and 20/80 OS. Exam under

anesthesia showed evidence of stromal edema and haze with band keratopathy (Figure 2A). Central corneal pachymetry measured 763 μm OD and 734 μm OS using a Corneo-gage Plus ultrasonic pachymeter (Sonomed, Cleveland, OH). IOPs were 15 mmHg OD and 18 mmHg OS. Corneal diameters were normal. No signs of anterior chamber or intra-ocular inflammation were noted. The anterior and posterior segments of the eye were otherwise normal. Treatment of band keratopathy was done by a pediatric ophthalmologist at the time of the examination under anesthesia. A clinical diagnosis of CHED was made and PKP was advised upon referral to a corneal specialist. At the age of 4, patient underwent bilateral PKP - with 3 months separation between procedures. At 8 years and 3 months of age, on the most recent follow-up visit, the patient's visual acuity was 20/25 in each eye and both corneal grafts were noted to be clear.

The preliminary report by a general pathologist remarked on the keratoplasty button findings (Figures 2B, 2C, 2D) "as consistent with an endothelial dystrophy." The patient's parents and siblings were examined and all were noted to have normal corneal examinations by slit-lamp examination. PCR amplification and traditional Sanger sequencing of the coding sequence of *SLC4A11* revealed no pathogenic variants in proband, siblings or parents. Sanger sequencing of the exons of the *ZEB1* gene was performed in the proband, siblings, and parents. A novel *de novo* *ZEB1* mutation was found in the proband [NM_001174093.1:c.21T>A, p.(Cys7*)] (Figure 2E) which was absent in both parents and siblings. Paternity was confirmed genetically. This single nucleotide substitution is predicted to result in a nonsense mutation and a premature stop codon. The pathology slides were subsequently reviewed with an ocular pathologist (RNH). Light microscopy analysis of the excised left cornea showed a spongy epithelium with minimal basal epithelium (Figure 2B). Discontinuities in Bowman's layer were noted. The stroma was thickened but areas of the posterior stroma were compacted. Descemet's membrane displayed regional thinning and thickening and abnormal endothelium (Figure 2C). The ocular pathologist noted that the focal multi-layered endothelium was consistent with posterior polymorphous corneal dystrophy (PPCD) (Figure 2D).

DISCUSSION

The presented cases illustrate the importance of genetic testing in conjunction with meticulous clinical examination and histopathological review for the diagnosis, management, and counseling of patients with congenital corneal edema. Additionally, these cases highlight that *de novo* mutations should be considered in the etiology of congenital corneal endothelial dystrophies in the absence of a family history of corneal disease or presence of consanguinity.

Manifesting at birth or early childhood, CHED (MIM #217700) distinguishes itself by its histological hallmarks which include dystrophic endothelial cells, a typically thickened Descemet's membrane, and diffuse stromal edema that give a distinct "ground glass" appearance to the corneas. The affected gene for CHED has been identified as *SLC4A11*.⁷ The protein product from the *SLC4A11* transcript was originally described as a sodium borate cotransporter 1 (NaBC1).⁸ More recent work investigating the role of the protein specifically in bovine corneal endothelial cells suggests that the protein does not have any

significant affinity for borate and instead appears to function as a sodium-dependent intracellular pH modulator transporting mainly hydroxyl anions.⁹ Additional observations from exogenous expression and knockout of *SLC4A11* in multiple experimental animal models and human corneas suggest that the protein may also promote basolateral entry of water molecules into corneal endothelium.¹⁰ These observations provide a mechanistic basis for the loss of deturgescence and progressive disorganization of the endothelial layer seen in CHED. Numerous homozygous or compound heterozygous *SLC4A11* mutations have been described in CHED individuals.^{1,11–14} To our knowledge, *de novo* mutations in *SLC4A11* have not been previously described in the literature prior to this report.

Case A patient had clinical features of CHED but the pathology showed marked loss of endothelium without a thickened Descemet's membrane precluding a definitive diagnosis. Additionally as an autosomal recessive disorder, CHED occurs primarily in patients with a history of consanguinity. Sanger sequencing of the subject and his parents helped make the diagnosis of CHED by the detection of a novel *de novo* mutation in the paternal *SLC4A11* allele combining with a maternal missense mutation p.(Arg869His) to result in a compound heterozygous mutation in the gene. The maternally inherited missense mutation has been previously implicated in CHED and is specifically thought to interrupt the interaction between a specific domain (the transmembrane surface of helix 12) of the cotransporter encoded by *SLC4A11* transcript and the cytoplasmic membrane.⁶ Interestingly, recent detailed light and electron microscopic studies of the Descemet's membrane of CHED cases have revealed that marked thickening of this layer is not a universal finding of the disorder.^{15,16} Moreover, a majority, of the reported homozygous mutations in exon 18 were associated with only moderate if any thickening of the Descemet's membrane.^{15,16}

Although the **Case B** patient also had presented with clinical features of CHED, the pathological findings and detection of a *de novo* mutation in *ZEB1* helped establish the diagnosis of PPCD3. In contrast to CHED, corneal changes in PPCD usually occur in the second or third decade of life. PPCD also features a more expansive array of clinical manifestations encompassing grouped vesicles, scalloped bands, geographic lesions, and opacities.^{2,3} Although band keratopathy may be a rare association with PPCD,¹⁷ it is a landmark finding of late X-linked endothelial dystrophy presenting in the form of endothelial changes resembling “moon craters”.¹⁸ Mutations in *ZEB1* (a zinc finger transcription factor) (MIM #609141) are found in nearly one third of all PPCD probands.^{2,3} *De novo* mutations in *ZEB1* have been described in several families.^{3,19} By modulating genes responsible both for maintenance of the epithelial phenotype and the epithelial to mesenchymal (EMT) transition, *ZEB1* has been shown to regulate neurodevelopment and tumor metastasis.^{1,20}

Interestingly, the Case B patient was only 3 years old but presented all the hallmarks of PPCD3 including corneal edema and an abnormal multilayered endothelial cell layer. To our knowledge, our study is the first to report such an early onset PPCD3 from a *de novo* mutation. Familial PPCD3 cases of congenital onset¹⁵ and early onset at 3 months of age²¹ have been reported previously.

In this era of genomics and promise of personalized medicine, these cases highlight the important role that gene sequencing may play in the diagnosis of congenital corneal endothelial dystrophies with atypical clinical and/or histopathological presentations. Genetic counseling at the appropriate age is warranted for the case B patient with the *de novo* heterozygous mutation in *ZEB1* is at risk of passing on the deleterious allele to half of his offspring. Meticulous correlation of mutations to clinicopathological findings may improve our clinical understanding of these endothelial disorders and also shed light on their underlying molecular biology.

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Figure 1.

Case A Patient. **A.** Photographs obtained with operating microscope 2 months status post penetrating keratoplasty to the left eye shows a cloudy edematous cornea in the right eye; and a clear and compact PKP graft in the left eye. Histopathological findings of keratoplasty button from **Case A.** Light microscopy showed features of CHED included **B)** focal loss in Bowman's layer centrally (arrowheads), areas of thickened Bowman's layer (arrow); and **C)** thin Descemet's membrane with only a few remaining endothelial cells (arrow). **D)** DNA sequence chromatogram of proband revealing a novel *de novo* mutation (c.2450delC) in the

paternal allele resulting in a frameshift mutation in exon 18 of the *SLC4A11* gene. Co-inheritance of a maternal missense mutation (c.2606G>A) in exon 18 leads to the generation of the compound heterozygous mutation.

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Figure 2.

Case B Patient. **A)** Operating microscope photographs at age 3 prior to any surgical intervention shows stromal edema and haze with associated band keratopathy in the right and left corneas. **B)** Light microscopy demonstrated characteristic features of PPCD including a spongy epithelium (arrow) with minimal basal epithelium, discontinuities in Bowman's layer (arrowhead), **C)** a compacted posterior stroma (*) with regional thinning (open arrowhead) and thickening (closed arrowhead) of the Descemet's membrane, and **D)** a focal multilayered (arrow) and vacuolated endothelium (arrowhead). **E)** DNA sequence

chromatogram of proband revealing a *de novo* nonsense mutation resulting in a premature stop in exon 1 of the *ZEB1* gene.

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