CASE REPORT

Compound heterozygous mutations in the SLC4A11 gene associated with congenital hereditary endothelial dystrophy in a Chinese family

Min Liu, Jia-Li Xia, Hong Yang, and Ling Yu

*Department of Ophthalmology, The Affiliated Hospital of Southwest Medical University, Luzhou, Sichuan Province, China; †Department of Ophthalmology, The People’s Hospital of Wenchang Chengdu, Chengdu, Sichuan Province, China; ‡Department of Ophthalmology, Daping Hospital, Army Medical Center, Army Medical University, Chongqing, Shapingba, China

ABSTRACT

Background: In this case report, we have described congenital inherited endothelial dystrophy (CHED) caused by two heterozygous missense mutations in two patients.

Methods: A Chinese family affected by CHED was recruited to identify potential genetic mutations. The proband developed bilateral corneal opacity after birth, and was diagnosed with CHED based on the clinical manifestations. Her younger sister had the same symptoms. Blood samples were collected from four members of the family, including the two sisters and their parents, and full exon sequencing (WES) was used to identify potential genetic mutations in the proband. To verify the identified mutations, Sanger sequencing was performed on samples from other family members.

Results: Two heterozygous missense variants were found in SLC4A11, a variant NM_032034.4; c.1237 G > A (p.G413R, rs1286683365) in exon 10 and a variant NM_032034.4, c. 698 G > T (p.R233L) in exon 6, and the latter was reported for the first time in this disease. Bioinformatics tools, such as SIFT and PolyPhen, showed that changes in these two amino acids probably affected protein function.

Conclusions: This study reported the typical clinical symptoms of CHED caused by two heterozygous missense variants (c.1237 G > A and c. 698 G > T) in the SLC4A11 gene in a Chinese family.

Introduction

Congenital inherited endothelial dystrophy (CHED) is a rare genetic disorder, which is clinically characterized by corneal turbidity and stromal layer edema caused by corneal endothelial dysfunction (1). The symptoms presenting at birth or within the first few years of life may slowly alleviate over the next few years (2). Subsequently, this disease may lead to nystagmus and significant impairment of vision. Many cases have been reported in consanguineous families, suggesting that CHED may be inherited as an autosomal recessive disease. Patients with CHED have specific corneal histopathologic changes, including diffuse corneal epithelial and stromal edema, loss of Bowman’s membrane, thickening of the retroelastic membrane, and abnormal morphology of the endothelial cells (3). Previously, CHED was divided into autosomal recessive (CHED2) and autosomal dominant (CHED1). In 2015, Weiss et al. developed a new IC3D classification of corneal dystrophies based on new clinical, histopathological, and genetic information. CHED1 mainly manifested as posterior polymorphous corneal dystrophy (PPCD) and was excluded from the diagnosis (4). Genetic linkage analysis showed that CHED was caused by homozygous or compound heterozygous mutations of the solute carrier family 4 member 11(SLC4A11) gene 610206, (5), which encodes a sodium borate cotransporter on chromosome 20p13. The SLC4A11 gene codes for BTR1, which is essential for cellular boron homeostasis, cell growth, and proliferation (6). To date, more than 100 mutations of SLC4A11 have been reported, of which approximately 94 mutations are related to CHED (7).

Here, we have reported two heterozygous missense mutations of the SLC4A11 gene in a Chinese family, one of which has already been described in the literature, whereas the other is a new mutation.

Materials and methods

The study was approved by the Ethics and Medical Research Committee of the Army Medical Center of the PLA in China. Members of a family with CHED who attended the outpatient department of the Army Medical Center of PLA were recruited.

All participants completed a detailed eye examination, including visual acuity, intraocular pressure, and slit lamp examination. The diagnosis of CHED was based on the fact that the two patients developed bilateral diffuse corneal opacity and edema in infancy, which was non-progressive. Participating family members signed an informed consent form and agreed to venous blood samples. Genomic DNA was extracted from 3 mL of peripheral blood samples obtained from the patients and their family members.
Whole-exome capture was performed using the Agilent SureSelect Human All Exon V6, and Illumina NovaSeq 6000 platform (2X150 bp) was used for high-throughput sequencing with an average coverage depth of ~150X. The process of whole exon sequencing included quality control, mapping, variant calling, and variant annotation using Fastqc, BWA, GATK, and ANNOVA, followed by The GATK Best Practices workflow. Then, the detected variants were filtered by 1000 Genome, EXAC, and gnomAD_exome_EAS, and those with allele frequency > 0.01 were discarded. We eliminated non-coding variants and selected functional variants with missense, splicing, stop gain/stop loss, and indel mutations. Sift, POLYPHPhen, MutationTaster, and ClinPred were used to predict whether exon variants were damaging or possibly damaging. For splicing variants, the score was required to be > 0.6 in dbscSNV_ADA and dbscSNV_RF. The pathogenicity of the variants was determined by the American College of Medical Genetics and Genomics (ACMG) classification. After identifying possible genetic mutations, Sanger sequencing was performed on the pathogenic or likely pathogenic compound heterozygous mutations of the two sisters and their parents using the Life Technologies 3730 sequencer.

Case reports

Case 1 was a 6-year-old Chinese girl who presented with severe impairment of eyesight, which affected her studies and life. Parents reported that their children had “white eyes” from birth, but that the “whiteness” lessened as they grew older. Because of her poor eyesight, the patient needed to listen to sounds to find direction, and the parents also received complaints from her teachers. The parents denied any history of eye disease or consanguineous marriage. On performing a detailed eye examination (Figure 1a), we found that the patient had obvious nystagmus and strabismus. Slit-lamp examination revealed severe corneal turbidity and edema. The best corrected visual acuity (BCVA) was 40/200 in the right eye and 40/200 in the left eye. Anterior segment optical coherence tomography (Heidelberg Engineering, Heidelberg, Germany) and Corneal Visualization Scheimpflug Technology (Corvis ST; Oculus, Wetzlar, Germany) were used for anterior segment examination and biometric measurements. The results suggested that the corneal thickening was 1060 μm in the right eye and 1124 μm in the left eye, and the axial length was 27.6 mm in the right eye and 27.67 mm in the left eye. The corneal diameter, anterior chamber depth, and lens were normal, but the optic nerve could not be accurately assessed. Covis and Icare were used to measure the intraocular pressure, and it was 26.1 mmHg in the right eye and 23.6 mmHg in the left eye. Due to severe corneal turbidity, the abnormal structure of the corneal endothelium could not be observed through confocal microscopy, although the loss of Bowman’s membrane and diffuse stromal edema could be observed. A bilateral hearing test was performed, and the results were normal. Based on these examinations, we made a diagnosis of CHED. The proband was diagnosed with glaucoma due to corneal turbidity and high intraocular pressure in another hospital. From the perspective of embryology, CHED and congenital glaucoma may coexist, however, based on the examination results, the evidence for the diagnosis of glaucoma was not sufficient. Abnormal lesions were also found in the patient’s teeth and were diagnosed as caries by the dentist (Figure 1b).

Case 2 was the proband’s 4-year-old younger sister. She also had corneal turbidity, nystagmus, strabismus, and poor vision. BCVA showed CF/50 cm in the right eye and CF/50 cm in the left eye. Corneal thickening measured by Corneal Visualization Scheimpflug Technology was 951 μm in the right eye and 946 μm in the left eye, whereas the axial length was 26.55 mm in the right eye and 25.42 mm in the left eye. Intraocular pressure was 16.1 mmHg in the right eye and 17.0 mmHg in the left eye, as measured by Icare. Her eye signs were similar to the proband’s. Severe dental caries were also diagnosed in the younger sister, and the hearing test results were normal. Both parents underwent a detailed ophthalmic examination and dental examination, and all results were normal.

Two heterozygous missense variants in SLC4A11 were found in the proband and her younger sister; one was the known variant NM_032034.4; c.1237 G > A (p.G413R, rs1286683365) in exon10 that was acquired from the father, whereas the other was a newly discovered variant NM_032034.4; c.698 G > T (p.R233L) in exon 6 that was acquired from the mother. The two variants had MAF < 0.01% in the normal population, based on the databases of 1000 genomes, EXAC and gnomAD_exome_EAS. Bioinformatics tools were used, and these two variants were designated as “probably damaging” by PolyPhen, “disease causing” by MutationTaster, and “damaging” by SIFT. Based
on the ACMG criteria, the variants of G413R and R233L classified as likely pathogenic were PS1 PM1 PM2 PP3 and PS3 PM1 PM2 PP3, respectively. Hence, we speculate that changes in the amino acids from Gly to Arg at 413 position and from Arg to Leu at 233 position affected protein functions (Figure 2).

**Discussion**

Patients with CHED have characteristic manifestations, such as diffuse corneal edema, corneal turbidity or ground-glass appearance, and corneal thickening up to 2–3 times the normal thickness. Corneal endothelial cell count was found to be significantly reduced, and was 10 times less than that in the control group of the same age (8). Corneal transparency depends on a “pump-drain” system of the corneal endothelial cells, which maintains a dynamic balance between the anterior chamber and the corneal stroma in an active and passive manner. Endothelial cell dysfunction causes excess water to enter the stroma, causing the collagen fibrils to rupture and the cornea to become cloudy (9). Mutations in SLC4A11 can affect corneal endothelial function through the mechanisms of oxidative stress, impaired mitochondrial function, endoplasmic reticulum stress, and pump defects (10).

Previous studies have mapped the CHED locus to a unique region of chromosome 20p13, and the SLC4A11 gene has been identified as the main gene responsible for this disease (11). As a protein-coding gene, SLC4A11 plays an important role in sodium-mediated fluid transport in different organs. The gene encodes protein for voltage regulation, electrogenic sodium-conjugated borate co-transporters, and is critical for borate homeostasis, cell growth, and cell proliferation (12). The SLC4A11 gene is highly expressed in the kidney, testis, salivary gland, thyroid gland, and corneal endothelium, and mutations can affect the function of these tissues (10).

In this study, two heterozygous mutations were identified in the SLC4A11 gene screening. According to Sanger sequencing, the parents were found to be heterozygous carriers of the SLC4A11 mutation in the family and showed no abnormal phenotype. The two sisters received two heterozygous mutations from their parents, which exhibited abnormal phenotypes. Mutation analysis further confirmed the involvement of SLC4A11 in the disease pathology. Variant c.1237 G > A (p.G413R) was first reported in a genetic study of corneal dystrophy (CD) in Han Chinese patients in 2019 (13). The variant resulted in replacement of the locally charged environment with positively charged arginine, and introduced interactions with nearby helices and rings, affecting protein function. The second variant c.698 G > T (p.R233L), derived from the mother, is the first to be reported as related to CHED, and is caused by a G-to-T change in exon 6 at position 698, resulting in the positively charged polar amino acid arginine being replaced by the non-polar amino acid leucine, probably affecting the secondary structure of the protein. ClustalW was used for amino acid conserved analysis, which showed that glycine at site 413 and arginine at site 233 remained fully evolutionarily conserved in different species, further supporting the pathogenicity of the mutation (Figure 3). The compound heterozygous mutation in SLC4A11, composed of two missense mutations, led to the occurrence of typical CHED symptoms in the two daughters of the family, which also conforms to the characteristics of autosomal recessive inheritance.

Mutations in the SLC4A11 gene can lead to two other corneal disorders in addition to CHED: autosomal recessive Harboyan syndrome and autosomal dominant late Fuchs endothelial dystrophy (FECD) (14). In the present case, a hearing assessment was performed in both patients, and the results suggested that there was no hearing loss yet. However, many reports have suggested that hearing loss
may occur in adolescents (15). Therefore, it is particularly important to monitor the presence of progressive hearing loss in patients with CHED. In studies of different populations, heterozygous mutations in SLC4A11 have been found in approximately 3%-4% of patients with late-onset FEDC (16). In the present study, the parents of the two girls were heterozygous carriers of the SLC4A11 mutation, and rigorous ocular examination was performed to detect late FEDC. Although no evidence of FEDC was found, long-term follow-up is required.

In addition to ocular symptoms, the two patients had definite dental problems, including complete caries of the anterior crown, residual gingival roots, caries on the adjacent surface of the anterior teeth under the deciduous teeth, and incisor involvement (Figure 1b). The parents reported that the deciduous teeth of the sisters developed normally after birth, and the caries appeared from the age of 3 years with gradual worsening. They denied that the sisters had abnormal eating and living habits. However, it was clear that both sisters showed a strong susceptibility to dental caries. Studies (17,18) have shown that resistance or susceptibility to caries is the result of one or more genotypes, phenotypes, and environmental influences. To date, more than 20 mutations have been found to be associated with susceptibility to caries, including in AMBN, FOKL, and HLA-DR4. However, it is not clear whether the appearance of caries in our patients was related to the mutation reported in this study, or was due to the presence of a new site in the non-coding region of the genome, which requires further study by whole-genome sequencing.

Keratoplasty is currently the only effective treatment for CHED, and penetrating keratoplasty is widely used as the classical method, although it is associated with a risk of infection and rejection. Nowadays, procedures with fewer complications and better outcomes, such as Descemet membrane endothelial keratoplasty and Descemet-stripping automated endothelial keratoplasty, are being successfully used to treat endothelial dysfunction in children (19). In addition, with an in-depth understanding of the mechanism of action of SLC4A11 and other pathogenic genes in CHED, non-steroidal anti-inflammatory drugs and drugs that promote the clearance of mitochondrial reactive oxygen species may be effective for the treatment of the corresponding patient population (20,21).

In the present study, in the absence of a histopathological examination, genetic testing combined with careful clinical examination and typical clinical symptoms led to the correct diagnosis of CHED in two patients with congenital corneal edema. This research adds to the mutation spectrum of CHED and proposes a new phenotype. With the continuous expansion of the mutation spectrum, the etiology of CHED will be more widely understood, laying the foundation for genetic counseling, genetic diagnosis, and guided treatment.

The above study was based on only one family and had some limitations. In order to find more new mutation sites, more CHED families should be concerned. At the same time, although a variety of bioinformatics tools were used to predict mutations, the functional aspects of the newly discovered mutations were not discussed. Therefore, additional experiments, such as in vitro cell experiment, are needed to verify the role of mutation sites in the pathogenesis of CHED.

Figure 3. Conservation analysis of the two novel mutations identified in this study. Homology comparison of SLC4A11 gene from 10 different species with human confirmed the conserved nature of amino acid residues among different species. The arrows indicate the conserved nature of Gly413 (a) and Arg233 (b) mutated residues in SLC4A11 among different species. The number refers to the position of the amino acid residue.

Acknowledgments
We thank all the family members who participated in this study.

Disclosure statement
No potential conflict of interest was reported by the author(s).

Funding
This work was supported by the Affiliated Hospital of Southwest Medical University under Grant number 16004.

ORCID
Ling Yu http://orcid.org/0000-0001-6395-6630
References

1. Sultana A, Garg P, Ramamurthy B, Vemuganti GK, Kannabiran C. Mutational spectrum of the SLC4A11 gene in autosomal recessive congenital hereditary endothelial dystrophy. Mol Vis. 2007 Jul 26;13:1327–32.

2. Choe S, Yoon CH, Kim MK, Hyon JY, Yu YS, Oh JY. 2020. Spontaneous regression of congenital corneal opacity. Graefes Arch Clin Exp Ophthalmol. 258(2):359–66. doi:10.1007/s00417-019-04526-5.

3. Cunnusamy K, Bowman CB, Beebe W, Gong X, Hogan RN, Mootha V, Mootha VV. 2016. Congenital corneal endothelial dystrophies resulting from novel de novo mutations. Cornea. 35(2):281–85. doi:10.1097/ICO.0000000000000670.

4. Weiss JS, Möller HU, Aldave AJ, Seitz B, Bredrup C, Kivelä T, Munier FL, Rapuano CJ, Nischal KK, Kim EK, et al. IC3D classification of corneal dystrophies—edition 2 [published correction appears in Cornea. 2015 Oct;34(10):e32]. Cornea. 2015;34(2):117–59. doi:10.1097/ICO.0000000000000307.

5. Vithana EN, Morgan P, Sundaresan P, Ebenezer ND, Tan DT, Mohamed MD, Anand A, Khine KO, Venkataraman D, Yong VH, et al. Mutations in sodium-borate cotransporter SLC4A11 cause recessive congenital hereditary endothelial dystrophy (CHED2). Nat Genet. 2006;38(7):755–57. doi:10.1038/ng1824.

6. Park M, Li Q, Shcheynikov N, Zeng W, Mualem S. NaBC1 is a ubiquitous electronegative Na(+)-cotransporter essential for cellular boron homeostasis and cell growth and proliferation. Mol Cell. 2004;16:331–41. doi:10.1016/j.molcel.2004.09.030.

7. Zhang W, Frausto R, Chung DD, Griffis CG, Kao L, Chen A, Azimov R, Sampath AP, Kurtz I, Aldave AJ, et al. Energy shortage in human and mouse models of SLC4A11-associated corneal endothelial dystrophies. Invest Ophthalmol Vis Sci. 2020;61(8):39. doi:10.1167/iovs.61.8.39.

8. Bourges JL. 2017. Corneal dystrophies. J Fr Ophtalmol. 40(6):e177–e192. doi:10.1016/j.jfo.2015.05.003.

9. Vilas GL, Loganathan SK, Liu J, Riau AK, Young JD, Mehta JS, Vithana EN, Casey JR, et al. Transmembrane water-flux through SLC4A11: a route defective in genetic corneal diseases. Hum Mol Genet. 2013;22(22):4579–90. doi:10.1093/hmg/ddt307.

10. Malhotra D, Casey JR. Molecular mechanisms of fuchs and congenital hereditary endothelial corneal dystrophies. Rev Physiol Biochem Pharmacol. 2020;178:41–81. doi:10.1007/112_2020_39.

11. Hand CK, Harmon DL, Kennedy SM, FitzSimon JS, Collum LM, Parfrey NA. 1999. Localization of the gene for autosomal recessive congenital hereditary endothelial dystrophy (CHED2) to chromosome 20 by homozygosity mapping. Genomics. 61(1):1–4. doi:10.1006/geno.1999.5920.

12. Paliwal P, Sharma A. Congenital hereditary endothelial dystrophy - mutation analysis of SLC4A11 and genotype-phenotype correlation in a North Indian patient cohort. Mol Vis. 2010 Dec 31;16:2955–63. PMID: 21203343; PMCID: PMC3013067.

13. Zhang J, Wu D, Li Y, Fan Y, Chen H, Hong J, Xu J. Novel mutations associated with various types of corneal dystrophies in a Han Chinese population. Front Genet 2019 Aug 29;10:881. doi:10.3389/fgen.2019.00881.

14. Kodaganur SG, Kapoor S, Veerappa AM, Tontanahal SJ, Sarda A, Yathish S, Prakash DR, Kumar A. Mutation analysis of the SLC4A11 gene in Indian families with congenital hereditary endothelial dystrophy 2 and a review of the literature. Mol Vis. 2013;19:694–706.

15. Siddiqui S, Zenteno JC, Rice A, Chacón-Camacho O, Naylor SG, Rivera-de la Parra D, Spokes DM, James N, Toomes C, Inglehearn CF, et al. Congenital hereditary endothelial dystrophy caused by SLC4A11 mutations progresses to Harboyan syndrome. Cornea. 2014;33(3):247–51. doi:10.1097/ICO.0000000000000241.

16. Hand CK, McGuire M, Parfrey NA, Murphy CC. 2017. Homozygous SLC4A11 mutation in a large Irish CHED2 pedigree. Ophthalmic Genet. 38(2):148–51. doi:10.3109/13816810.2016.1151901.

17. Opal S, Garg S, Jain J, Walia I. 2015. Genetic factors affecting dental caries risk. Aust Dent J. 60(1):2–11. doi:10.1111/adj.12262.

18. Cavallari T, Arima LY, Ferrara A, Moysés SJ, Tetu Moysés S, Hirochi Herai R, Iani Werneck R. Dental caries: genetic and protein interactions. Arch Oral Biol. 2019;108:104522.

19. Pereira NC, Pereira Gomes JÁ, Tonin C, Verardo RS, Felipe RS, Dos Santos Forseto A. 2021. Descemet membrane endothelial keratoplasty in children. Cornea. 40(4):453–57. doi:10.1097/ICO.000000000000254.

20. Alka K, Casey JR. 2018. Ophthalmic nonsteroidal anti-inflammatory drugs as a therapy for corneal dystrophies caused by SLC4A11 mutation. Invest Ophthalmol Vis Sci. 59(10):4258–67. doi:10.1167/iovs.18-24301.

21. Okumura N, Ueno M, Koizumi N, Sakamoto Y, Hirata K, Hamuro J, Kinoshita S. Enhancement on primate corneal endothelial cell survival in vitro by a ROCK inhibitor. Invest Ophthalmol Vis Sci. 2009;50(8):3680–87. doi:10.1167/iovs.08-2634.