Congenital hereditary endothelial dystrophy (CHED) is defined as a rare inheritable disorder of the corneal endothelium characterized by bilateral, symmetric, non-inflammatory corneal clouding (edema) seen at the time of birth or shortly thereafter [1]. The degeneration of the corneal endothelium leads to corneal edema, especially of the stroma, and gives the cornea an appearance resembling ground glass. Two subtypes of CHED are known – autosomal dominant (CHED1; OMIM 121700) and autosomal recessive (CHED2; OMIM 217700) – based on differences in the mode of inheritance [2]. Clinically, both forms have similar features. The former is characterized by bilateral, symmetric, non-inflammatory corneal edema seen at birth or shortly thereafter, while the latter is more severe. Different loci have been mapped to 20p11.2-q11.2 [3] and 20p13, for CHED1 and CHED2, respectively [4].

The Solute Carrier family 4 (sodium borate cotransporter) member 11 (SLC4A11; OMIM 610206) has been identified as the candidate gene for CHED2 [5]. This gene has 19 exons that encode the bicarbonate transporter–related protein 1 (BTR1) of 891 amino acids and 14 transmembrane domains with intracellular amino and carboxyl terminals. Several functions have been defined for BTR1; the important ones are its role in maintenance of boron homeostasis, cell growth and proliferation, and activation of the mitogen-activated protein kinase pathway [6]. Vithana et al. [5] hypothesized that a deregulation of the mitogen-activated protein kinase pathway and a failure of the mutant protein to reach the cell surface to perform its function leads to the symptoms of CHED2 [5]. Mutations in SLC4A11 are described in Harboyan syndrome, also known as Corneal Dystrophy and Perceptive Deafness (CDPD; OMIM 217400), which is characterized as CHED2 with hearing loss [7].

Several studies have been performed to analyze the role of this gene in causation of CHED2, which involves mainly patients from South India [8-10]. There are no published data...
available from the Northern part of India. Therefore, the present study was undertaken to assess the role of SLC4A11 in the pathogenesis of CHED2 in North Indian patients.

METHODS

The study protocol adhered to the tenets of Declaration of Helsinki and the Institutional Ethics Committee provided ethical approval for the study. In total, 25 patients from 20 families diagnosed with CHED2 were included in the study. Corneal dystrophy was diagnosed on clinical examination. Detailed family histories were collected and pedigree charts were constructed for all of the patients; the presence of consanguinity was noted based on marriage histories.

Clinical studies: The clinical examination included routine slit lamp biomicroscopy, confocal microscopy, specular microscopy, ultrasonic pachymetry, orbscan, and ultrasonography (USG) for posterior segment evaluation. Patients who were bilaterally affected and without any other systemic involvement formed the study group. The diagnosis of CHED was made on the basis of the following characteristics: presence of mosaic corneal haze with corneal edema, increased central corneal thickness (>0.7 mm in all cases), normal horizontal corneal diameter (10–11 mm), and no evidence of congenital glaucoma (e.g., no buphthalmos, Haab’s striae, or optic disc cupping).

The control population consisted of 50 healthy, unrelated, population-matched individuals who had no history of any ocular disease in their family.

Histological studies: Histopathology and electron microscopic (EM) studies were performed on 7 corneal buttons obtained from 7 patients after penetrating keratoplasty surgery. Each corneal button was divided in half; one half was used for histopathology (fixed in 10% buffered formalin) and the other half for electron microscopic (EM) studies.

For histopathological studies, the formalin-fixed corneal tissue samples from 7 patients were embedded in paraffin blocks and cut into 4 μM thick sections. Sections were analyzed by light microscopy after staining with hematoxylin and eosin (H&E), periodic acid Schiff, and Congo red dyes.

Ultrastructural analysis: The corneal tissues were collected from five patients at the time of penetrating keratoplasty and immediately fixed in Karnovsky fixative. Two corneal tissue samples could not be processed for transmission electron microscopy as the patients had undergone keratoplasty 8 years previously.

After the dehydration and clearing processes, the tissue samples were embedded in araldite blocks. Sections were cut with an ultramicrotome (UC-6; Leica; Lab India, New Delhi, India) using a glass knife and the electron micrographs were taken with an electron microscope (MORGAGNI 268D; FEI, Eindhoven, The Netherlands).

Genetic analysis: A 5 ml sample of peripheral blood was collected by venipuncture in EDTA from all patients and controls after taking informed consent from all of the participants. Genomic DNA was extracted by the salting out method [11].

The DNA was amplified in a thermocycler (ABI 9700; Applied Biosystems [ABI], Foster City, CA) by PCR using the primers as described previously (Table 1) [5]. The amplification reaction mixture consisted of 10 ng DNA, 1.5 mM MgCl₂, 0.25 mM of each dNTP, 10pM of each primer, and 0.7 units of Taq polymerase (Roche, ABI, Foster City, CA), and Q-sol (Qiagen GmBH, Hilden, Germany) in a total volume of 25 μl. The amplified PCR products were subjected to gel purification using QIAmp gel extraction kits (Qiagen GmBH) and the purified PCR products were screened for sequence changes by bidirectional sequencing. The amplified products were sequenced directly with BigDye Terminator Mix version 3.1 (ABI) according to the manufacturer’s instructions and were then analyzed on an ABI-3100 Genetic Analyzer (ABI). Nucleotide sequences for the coding regions were compared with the nucleotide sequence of the published SLC4A11 human cDNA (NM_032034). The families that did not show SLC4A11 coding region changes were screened for mutations in the putative promoter region using primers described earlier [8].

One family was screened for underlying changes in the carbohydrate (N-acetylglucosamine 6-O) sulphotransferase 6 (CHST6; OMIM 605294) gene as the mother was suspected to have macular corneal dystrophy based on her phenotypic presentation. PCR amplification was done using the primers as described previously (Table 1) [12] in a reaction mixture consisting of 100 ng DNA, 1.5 mM MgCl₂, 0.25 mM of each dNTP, 10pM of each primer, and 1.0 units of Taq polymerase (Roche, ABI), and Q-sol (Qiagen GmBH) in a total volume of 25 μl.

Polyphen 2 and SIFT analysis: The PolyPhen 2 and SIFT (Sorting Intolerant From Tolerant) tools were used for analysis of novel mutations to characterize the pathogenic nature of the identified changes. The SIFT tool generates multiple sequence alignments of a gene over different species and assesses the degree of conservation of the substituted positions over the course of evolution. It gives a value as a score, where a score <0.05 is considered to represent a potentially damaging mutation.

RESULTS

Several clinical parameters were assessed to confirm autosomal recessive CHED2 in the patients forming the study group.

Clinical examinations: Corneal pachymetry revealed increased corneal thickness in all the cases. The age of the patients ranged from 4 to 34 years (Table 2). The intraocular pressure was within normal range (below 21 mmHg) for all patients. Nystagmus was present in 19 patients and 38 eyes. A high degree of consanguinity (9 out of 20 families) was
noted among the CHED2 patients. Corneal haze was reported to have been present since birth or shortly thereafter in all the cases. Clinical examinations (slit lamp biomicroscopy) of parents (thirty parents from seventeen families) revealed unaffected clear corneas. None of the patients in the present study group had associated sensorineural hearing loss.

**Light microscopy analysis:** Histopathological examination for the patients undergoing keratoplasty confirmed the diagnosis of CHED2 in all of the patients. Light microscopy revealed widening of the stroma and marked thickening of Descemet’s membrane. Few atrophic endothelial cells could be identified in two cases, whereas the rest of the corneal tissues had completely atrophied endothelium (Table 2).

**Ultrastructural analysis:** Electron microscopy (EM) revealed degeneration of keratocytes. Fragmentation and disorganization of collagen fibers was seen, leading to separation of collagen lamellae. An overall increase in stromal thickness was observed, with the presence of numerous vacuoles or water clefts in the stroma. The most conspicuous observation was an increase in the total thickness of the Descemet’s membrane, which was due to an increase in the thickness of the non-banded zone (5–8 times thicker than the normal); the banded zone had normal thickness and morphology. The non-banded zone had numerous collagen bundles, but no endothelial cells were observed, indicating almost complete absence and attenuation of the endothelium. These findings were similar for all of the CHED2 patients, in whom the ultrastructural imaging was done, confirming the clinical diagnosis.

**Mutation analysis:** Direct DNA sequence analysis showed 14 CHED2 patients from 9 families had underlying SLC4A1I mutations that co-segregated with the disease phenotype and which were absent in 50 population matched controls.

A novel in-frame homozygous deletion mutation of one of the four leucine residues c2518-c2520 del CTG in exon 18 was seen in two affected siblings of a family (Figure 1A) who also had associated spheroidal degeneration (Figure 1B). The presence of mutation was confirmed on bidirectional sequencing in both the siblings (Figure 1C). Conservation of the amino acid residues involved in the novel deletion mutations is shown in Figure 1D. Ultrastructural studies of the proband carrying the novel mutation revealed the presence of large vacuoles in the stromal keratocytes and a marked increase in the thickness of the Descemet’s membrane (Figure 1E-G).

Other mutations documented in the study included a homozygous missense mutation c.2470G>A in exon 18 in 6 affected patients from 4 families (Figure 2A). A homozygous missense mutation, c.1156T>C, was seen in three affected individuals from 2 families that led to substitution of a cysteine residue at amino acid position 386 with arginine (C386R; Figure 2B). Both of these mutations have been reported in patients from South India. A splice site mutation c.2240+1G>A was seen in a family that showed a variable phenotype (Figure 2C). Apart from these, two homozygous changes in introns 2 and 11 were identified in two patients from two families, respectively, but no coding region changes, either heterozygous or homozygous, were seen in them. No

---

**Table 1. Table showing primer sequences and PCR conditions used for amplification of SLC4A1I and CHST6 genes.**

| SLC4A1I gene | Primer Sequence 5′to 3′ | Annealing temperature (°C) |
|--------------|-------------------------|---------------------------|
| Exons        | Forward                 | Reverse                   |                           |
| 1            | CCTAGCAGATGGGCTAAGCA    | GAGCAAAAGCCACAGGACTCT     | 60                        |
| 2 and 3      | CCCAGGAGGAGCTCAACAG     | CTCCCTTGAAGTGCTCTCTT      | 62                        |
| 4 and 5      | CACGAGGAGCTCAACAG       | CAGCCCTTCTTCCCAAGTT       | 57                        |
| 6            | CCAACAAACTTGGGAGAAGA    | CTTTCAGGCCAGCCAGGACTCT    | 52                        |
| 7 and 8      | AAAACCTGTGCTGCGGATTC    | CTAGGAGATGGGGAGATTT       | 57                        |
| 9 and 10     | ACTGATGGTCTAGGGCTATTCT  | CGTCCATCGTAAAGAGGTG       | 58                        |
| 11 and 12    | TCTACATCAAGGGTGCAGT    | CGTTGAGAGCTGCAACTTGCTT    | 56                        |
| 13 and 14    | AGGCCCTTTCTCCTGGATAG    | GTGCAGGAGGAGGACTGAT       | 61                        |
| 15 and 16    | CCGGAAATTCGAGAGGTATT   | CGTTCTCTCTAGCAGACCTC      | 54                        |
| 17 and 18    | CTGGCCACATGGGAGCATAG    | CTTAGCAGGAGCACCAGACTCC    | 53.5                      |
| 19           | CAGGAGAGGCTCCTGATCTA   | CTGTCATGCTGATTCCACCTT     | 55                        |
| Putative Promoter region 1 | GCCCTACTCAACCAATCTATGC | CTTAGCTCATCCTTCTTCCGAC | 61                        |
| Putative Promoter region 2 | GGAGGAGGAGAAGGACTGTC | GCACACTGCGCAGCCTCAC  | 55                        |

| CHST6 gene | Primer Sequence 5′to 3′ | Annealing temperature (°C) |
|------------|-------------------------|---------------------------|
| Coding region | Forward                 | Reverse                   |                           |
| 1          | GCCCCTAACCGCTGCCTCTCTCTC- | GGCTTCAGACAGGCGCTCGCT     | 57                        |
| 2          | GACGTGTGTTGCTACTGCTCTTG- | CGGCGGCGACAGGTGCACCTC     | 55                        |
| 3          | CTCCCGGGGAGCACAGCAGCACA | CTCCCGGGGCTTAGGCGCT       | 57                        |
## Table 2. Details of Families with Autosomal Recessive Congenital Hereditary Endothelial Dystrophy and SLC4A11 Mutations.

| S. no | Family no. | Age/sex | Nystagmus | CCT RE/LE | Age at PK | BCVA at last F/up | Histopathological findings | Complications | Consanguinity | Exon affected | cDNA position of the change | Amino acid position | Indian state of origin |
|-------|------------|---------|-----------|-----------|-----------|------------------|---------------------------|---------------|---------------|--------------|------------------------|---------------------|----------------------|
| 1     | Q1         | 16/F    | P         | PE/PE     | ND        | CF               | CF                        | -             | -             | P            | c.2240+1G>A            | Inactivation of splice site | India               |
| 2     | Q2         | 18/M    | A         | PE/PE     | 10        | 6/24             | CF                        | ED+DMT        | A             | 18           | c.2470G>A             | Va824Met            | Uttar Pradesh        |
| 3     | Q3         | 22/F    | P         | PE/PE     | 12        | CF               | CF                        | ED+DMT        | -             | A            | c.2470G>A             | Va824Met            | Bihar               |
| 4     | Q4         | 7/M     | A         | PE/921    | ND        | 6/60             | CF                        | Graft rejection | A             | 18           | c.2470G>A             | Va824Met            | Bihar               |
| 5     | Q5         | 17/M    | A         | PE/PE     | ND        | 6/24             | -                         | -             | -             | P            | NMD                  | U.P.                | Uttar Pradesh        |
| 6     | Q6         | 28/F    | P         | PE/770    | 26        | 4/60             | CF                        | ED+DMT        | A             | 18           | (NOVEL) c.2518-2520 delCTG | Leu840del           | Haryana             |
| 7     | Q7         | 24/M    | P         | 101/PE    | ND        | 2/60             | CF                        | -             | -             | P            | NMD                  | U.P.                | Haryana             |
| 8     | Q8         | 11/M    | P         | PE/PE     | ND        | 1/60             | CF                        | -             | -             | P            | NMD                  | U.P.                | Haryana             |
| 9     | Q9         | 14/M    | A         | PE/994    | ND        | 6/60             | CF                        | -             | -             | P            | NMD                  | U.P.                | Haryana             |
| 10    | Q10        | 34/M    | P         | PE/PE     | ND        | 1/60             | 6/60                      | -             | -             | P            | c.1156T>C              | Cys386Arg            | Uttar Pradesh        |
| 11    | Q12        | 4/M     | P         | PE/PE     | ND        | 1/60             | Seeing                    | -             | -             | P            | c.1156T>C              | Cys386Arg            | Haryana             |
| 12    | Q13        | 22/M    | A         | 838/PE    | ND        | 2/60             | CF                        | -             | -             | A            | NMD                  | U.P.                | Jammu & Kashmir      |
| 13    | Q14        | 9/M     | P         | PE/1023   | ND        | 6/24             | 6/60                      | -             | -             | P            | NMD                  | U.P.                | Jammu & Kashmir      |
| 14    | Q15        | 20/F    | P         | PE/PE     | ND        | 6/36             | CF                        | -             | -             | P            | NMD                  | U.P.                | Jammu & Kashmir      |
| 15    | Q16        | 16/F    | P         | PE/920    | ND        | 1/60             | CF                        | -             | -             | P            | c.2470G>A             | Va824Met            | U.P.                |
| 16    | Q17        | 7/F     | P         | PE/1008   | ND        | 3/30             | 3/30                      | -             | -             | A            | 16                   | c.2470G>A             | Punjab              |
| 17    | Q18        | 8/M     | P         | PE/987    | ND        | 1/60             | 1/60                      | -             | -             | P            | NMD                  | U.P.                | Delhi               |
| 18    | Q19        | 21/M    | A         | PE/PE     | 18        | 6/24             | CF                        | ED+DMT        | A             | 16           | c.2470G>A             | Va824Met            | U.P.                |
| 19    | Q20        | 4/M     | P         | PE/PE     | ND        | 4/60             | CF                        | -             | -             | A            | NMD                  | U.P.                | Bihar               |
| 20    | Q21        | 12/F    | P         | PE/PE     | 2/60      | 4/60             | CF                        | NMD           | 18           | c.2470G>A             | Va824Met            | U.P.                |

PK-Penetrating keratoplasty, P- Present, A- Absent, F- Female, M- male, ND-Not Done, ED- endothelial degeneration, DMT- Descemet Membrane Thickening, NMD - No mutation Detected, UP- Uttar Pradesh, J & K- Jammu & Kashmir, IOP-Intraocular pressure, CCT- Central corneal thickness, BCVA-Best corrected visual acuity after keratoplasty, RE-Right eye, LE-Left Eye, F/up period- follow up period, PE-Error on pachymetry.
other coding region variations were identified in 11 patients from 11 families. In addition, screening of the upstream putative promoter region also failed to reveal any change in these 11 individuals.

Details of the family (Q1) with a variable phenotypic presentation and a splice site mutation c.2240+1G>A: The proband, a 20 year old male (Figure 3A), presented with bilateral ground glass cornea with bullae. Corneal cloudiness and nystagmus were reported to have been present right from birth (Figure 3B,C). Family history revealed parental consanguinity and one more affected sibling. Visual acuity of the proband was counting fingers from a 1 m distance in the right eye and counting fingers close to the face in the left eye. Corneal pachymetry revealed increased corneal thickness that was beyond the measurement limits of the specular microscope. Molecular analysis identified a homozygous splice site mutation c.2240+1G>A.

Histopathological analysis identified a markedly thickened Descemet’s membrane and an atrophied endothelium, confirming the diagnosis of CHED2. Additionally, the patient’s cornea also had amyloid deposition and spheroidal degeneration. The presence of amyloid was confirmed based on the presence of apple green birefringence when viewed under a polarizing filter (Figure 3D).

The affected sibling, i.e., a younger sister of the proband, was also examined. She was 15 years old, with cloudy corneas since birth, but no nystagmus. Bilateral diffuse limbus-to-limbus stromal haze with stromal thickening and guttae changes was seen (Figure 3E). Vision in both eyes was limited to counting fingers from 1 m. Corneal pachymetry revealed an increased corneal thickness (right eye- 980, left eye-1010). Molecular analysis identified a homozygous splice site mutation c.2240+1G>A. None of the unaffected siblings showed the mutation in either homozygous or heterozygous state.

The 60-year-old mother was also clinically examined and she reported to have had a history of bilateral lime injury during childhood. Defective vision, photophobia, and
watering had been present for the past 15 years (Figure 3F).
Vision in the right eye was recorded as counting fingers from
1 m distance and the left eye had vision restricted to counting
fingers close to the face. Corneal pachymetry revealed a
normal corneal thickness (right eye-524µm and left
eye-527 µm) but bilateral endothelial opacities and stromal
haze were noted. Additionally, a few epithelial opacities were
seen. The phenotype of the mother resembled macular corneal
dystrophy with epithelial and endothelial involvement.
Mutation analysis identified the heterozygous splice site
mutation c.2240+1G>A. No other changes were noted in the
coding region, overlapping splice sites, and the putative
promoter region of SLC4A11.

In view of the different clinical presentation seen in the
mother, who resembled macular corneal dystrophy, the
affected family members were screened for mutations in the
CHST6 gene, which are known to cause macular corneal
dystrophy. No coding region changes were seen in CHST6,
either in the mother or in any of her affected offspring.

**DISCUSSION**

The SLC4A11 gene codes for BTR1 that functions as a sodium
borate co-transporter (NaBC1) and plays a role in activation
of the mitogen activated protein kinase pathway [6].
Mutations in the SLC4A11 gene lead to nonsense mediated
mRNA decay or formation of a truncated protein that is unable
to reach the surface and perform its function. It is the
subsequent deregulation of the MAPKinase pathway that is thought to cause CHED2.

In the present study we identified a novel c2518-c2520 delCTG mutation and assessed its pathogenic nature using SIFT and PolyPhen 2 tools. The deleted leucine residue lies in the transmembrane domain 11 and in silico analysis showed that the leucine residues at all the positions in the transmembrane domain, ranging from 840 to 843, are conserved over a wide range of species. Deletion of any one of them can disrupt the appropriate assembly or localization of the protein within the membrane. This may consequently have lead to CHED2 in the two siblings, although the exact mechanism remains unknown.

The splice site mutation c.2240+1G>A, identified in family Q1 with CHED2 affects the first nucleotide of the splice donor site and may probably cause mis-splicing of the pre-mRNA transcript. This would lead to either, exon skipping or intron retention, which would consequently result in an altered protein structure. However, it is difficult to determine the exact nature of the mis-splicing and the fate of the mutant transcripts, due to non-expression of SLC4A11 in lymphocytes [13]. Gene expression studies to determine the nature and variable expression of SLC4A11 would require corneal tissue of heterozygous individuals, which was not possible in the present study.

The most interesting feature of family Q1 was the intrafamilial variability with presence of variable phenotypes in the affected siblings who were homozygous for the splice site mutation and a completely different phenotypic presentation in the mother who was a heterozygous carrier. Other family members who were heterozygous carriers of the same mutation were unaffected and had clear corneas. The mutation c.2240+1G>A has been reported as the single heterozygous change leading to CHED2 in a British white (Caucasian) from the UK (UK), while the same change was not observed in 30 South Indian and 50 European controls of the study [13]. Similarly, in the present study, this change was not seen in the 50 controls also screened for SLC4A11 mutations. The reason for the presence of corneal opacities in some heterozygous carriers and not in others is not clear. The most plausible explanation could be that it is an autosomal dominant change with variable penetrance and expressivity. This change could also be described as a rare polymorphism segregating with the disease phenotype in the family. However presence of the same change in heterozygous condition in a CHED2 patient of British origin is suggestive of it being a pathogenic mutation.

Other previously reported mutations that were seen included c.2470G>A in 6 affected patients from 4 families and c.1156T>C in 3 affected individuals from 2 families. Both the
mutations showed interfamilial and/or intrafamilial variability and no genotype-phenotype correlation was seen. Similar studies from South India have also reported absence of evident correlations between clinical and histopathologic findings, and SLC4A11 mutations [9,14]. These reports implicate that phenotype presentation depends not only on the underlying mutation but also involves role of other genes, developmental and/or environmental factors.

In the present study, mutations were seen in 14 affected individuals from 9 families in a cohort of 25 patients. Several reports have been published describing the role of SLC4A11 mutations in the causation of dystrophies. In reports by Sultana et al. [9], Jiao et al. [10], and Hemadevi et al. [8], mutations in SLC4A11 were identified in 35 of 42 (83 percent), 12 of 16 (75 percent), and 11 of 20 (55 percent) CHED2 families screened. These studies involved Indian patients, but most of them were from South India. We found mutations in 14 of 25 (56 percent) patients diagnosed with CHED2 in North India. A decrease in the number of patients with underlying mutations in SLC4A11 in North India can be due to the difference in origin of these two populations and a relatively low rate of consanguinity in the Northern part as compared to South. The disease, being autosomal recessive, is associated with a high rate of consanguinity and thus is seen to occur at a higher frequency in the South Indian population.

Cases of CDPD are reported to have SLC4A11 mutations that result in an alteration of gene expression in the stria vascularis causing impairment in the hearing efficiency of these individuals [7]. However none of the patients in the present study group had associated sensorineural hearing loss.

Heterozygous changes in SLC4A11 have been reported in cases of late onset Fuchs endothelial corneal dystrophy (FECD; OMIM 136800) in which haploinsufficiency and accumulation of aberrantly folded protein is reported to result in FECD pathology [15]. The onset of symptoms for CHED2 and FECD are different, yet they share a common feature of an abnormal non-banded zone of the Descemet’s membrane which points to endothelial dysfunction starting in the late prenatal period [16]. In view of this, the parents of the affected patients, who were identified as heterozygous carriers of the SLC4A11 changes were clinically examined by slit lamp biomicroscopy. No signs and symptoms of FECD or associated guttus changes were seen in any of the parents examined, whose age range varied from 45 to 72 years.

Posterior Polymorphous Corneal Dystrophy (PPCD; OMIM 122000) is another dystrophy which results from primary endothelial dysfunction inherited as a dominant trait. Incomplete penetrance and de novo mutations have been observed in PPCD cases with identified zinc finger E-box binding homeobox 1(ZEB1; OMIM 189909) mutations which can lead to consideration of recessive inheritance. A few PPCD cases have been reported to present with corneal haze at birth [16] which might result in an overlap in phenotypic presentation of CHED and PPCD. Considering this, CHED2 cases with no SLC4A11 coding region changes should be screened for mutations in ZEB1.

The studies on endothelial dystrophies i.e., CHED, FECD, and PPCD suggest that all these forms can actually be allelic variants of the same disease continuum and that genetic interaction between genes that cause corneal dystrophies can modulate the expressivity of the phenotype [17]. Moreover, they all are considered to represent defects of terminal differentiation of the neural crest cells [17] and share common features of disease manifestation like endothelial metaplasia, and secretion of an abnormal Descemet’s membrane [17]. Considering this variability in expression, all cases of endothelial dystrophies should be screened for the presence of SLC4A11 and/or ZEB1 changes for confirmation and categorization.

In conclusion, to the best of our knowledge, this is the first report of mutation screening for the SLC4A11 gene in CHED2 cases from North India. We identified 1 novel and 3 previously reported mutations in 14 individuals from 9 families and documented inter- and intrafamilial variability, apart from the presence of genetic heterogeneity in our cohort of patients.

ACKNOWLEDGMENTS

The study was supported by a financial grant provided by the Department of Biotechnology (DBT), India. Financial assistance from the Department of Science and Technology (DST) to the Electron Microscope Facility, AIIMS and Senior Research Fellowship from Council of Scientific and Industrial research (CSIR), India to Preeti Paliwal is gratefully acknowledged.

REFERENCES

1. Ehlers N, Módis L, Moller-Pedersen T. A morphological and functional study of congenital hereditary endothelial dystrophy. Acta Ophthalmo Scand 1998; 76:314-8. [PMID: 9686844]

2. Weiss JS, Moller HU, Lisch W, Kinoshita S, Aldave AJ, Belin MW, Kivelä T, Busin M, Munier FL, Seitz B, Suphin J, Bredrup C, Mannis MJ, Rapuano CJ, Van Rij G, Kim EK, Klintworth GK. The IC3D classification of the corneal dystrophies. Cornea 2008; 27:S1-83. [PMID: 19337156]

3. Toma NM, Ebenezer ND, Inglehearn CF, Plant C, Ficker LA, Bhattacharya SS. Linkage of congenital hereditary endothelial dystrophy to chromosome 20. Hum Mol Genet 1995; 4:2395-8. [PMID: 8634716]

4. Hand CK, Harmon DL, Kennedy SM, FitzSimon JS, Collum LMT, Parfrey NA. Localization of the gene for autosomal recessive congenital hereditary endothelial dystrophy (CHED2) to chromosome 20 by homozygosity mapping. Genomics 1999; 61:1-4. [PMID: 10512674]

5. Vithana EN, Morgan P, Sundaresan P, Ebenezer ND, Tan DT, Mohamed MD, Anand S, Khine K, Venkataraman D, Yong VH, Salto-Tellez M, Venkataraman A, Guo K, Hemadevi B, Srinivasan M, Prajna V, Khine M, Casey JR, Inglehearn CF,
Aung T. Mutations in sodium-borate co-transporter SLC4A11 cause recessive congenital hereditary endothelial dystrophy, CHED2. Nat Genet 2006; 38:755-7. [PMID: 16767101]

6. Park M, Li Q, Shcheynikov N, Zeng W, Muallem S. NaBC1 is a ubiquitous electrogenic Na+-coupled borate transporter essential for cellular boron homeostasis and cell growth and proliferation. Mol Cell 2004; 16:331-41. [PMID: 15525507]

7. Desir J, Moya G, Reish O, Van Regemorter N, Deconinck H, David KL, Meire FM, Abramowicz MJ. Borate transporter SLC4A11 mutations cause both Harboyan syndrome and non-syndromic corneal endothelial dystrophy. J Med Genet 2007; 44:322-6. [PMID: 17220209]

8. Hemadevi B, Veitia RA, Srinivasan M, Arunkumar J, Prajna NV, Lesaffre C, Sundaesan P. Identification of Mutations in the SLC4A11 Gene in Patients with Recessive Congenital Hereditary Endothelial Dystrophy. Arch Ophthal 2008; 126:700-8. [PMID: 18474783]

9. 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; 13:1327-32. [PMID: 17679935]

10. Jiao X, Sultana A, Garg P, Ramamurthy B, Vemuganti GK, Gangopadhyay N, Hejtmancik JF, Kannabiran C. Autosomal recessive corneal endothelial dystrophy (CHED2) is associated with mutations in SLC4A11. J Med Genet 2007; 44:64-8. [PMID: 16825429]

11. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16:1215. [PMID: 3344216]

12. Akama TO, Nishida K, Nakayama J, Watanabe H, Ozaki K, Nakamura T, Dota A, Kawasaki S, Inoue Y, Maeda N, Yamamoto S, Fujiwara T, Thomar EJ, Shimomura Y, Kinosita H, Tanigami A, Fukuda MN. Macular corneal dystrophy type I and type II are caused by distinct mutations in a new sulphotransferase gene. Nat Genet 2000; 26:237-41. [PMID: 11017086]

13. Ramprasad VL, Ebenezer ND, Aung T, Rajagopal R, Yong VH, Tuft SJ, Viswanathan D, El-Ashty MF, Liskova P, Tan DT, Bhattacharya SS, Kumaranamickavel G, Vithana EN. Novel SLC4A11 mutations in patients with recessive congenital hereditary endothelial dystrophy (CHED2). Mutation in brief #958. Hum Mutat 2007; 28:522-3. [PMID: 17397048]

14. Mehta JS, Hemadevi B, Vithana EN, Arunkumar J, Srinivasan M, Prajna V, Tan DT, Aung T, Sundaresan P. Absence of phenotype-genotype correlation of patients expressing mutations in the SLC4A11 gene. Cornea 2010; 29:302-6. [PMID: 20118786]

15. Vithana EN, Morgan PE, Ramprasad V, Tan DT, Yong VH, Venkataraman D, Venkatraman A, Yam GH, Nagasamy S, Law RW, Rajagopal R, Pang CP, Kumaranamickavel G, Casey JR, Aung T. SLC4A11 mutations in Fuchs endothelial corneal dystrophy. Hum Mol Genet 2008; 17:656-66. [PMID: 18024964]

16. Cibis GW, Krachmer JA, Phelps CD, Weingeist TA. The clinical spectrum of posterior polymorphous dystrophy. Arch Ophthal 1977; 95:1529-37. [PMID: 302697]

17. Riazuddin SA, Zaghloul NA, Al-Saif A, Davey L, Diplas BH, Meadows DN, Eghrari AO, Minear MA, Li YJ, Klintworth GK, Afshari N, Gregory SG, Gottsch JD, Katsanis N. Missense mutations in TCF8 cause late-onset Fuchs corneal dystrophy and interact with FCD4 on chromosome 9p. Am J Hum Genet 2010; 86:45-53. [PMID: 20036349]