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## **Homozygous *SLC4A11* mutation in a large Irish CHED2 pedigree**

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**Running head:** Homozygous *SLC4A11* mutation in CHED2

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## **Abstract**

*Background* Congenital hereditary endothelial dystrophy (CHED) is a genetic disorder of corneal endothelial cells resulting in corneal clouding and visual impairment. Autosomal dominant (CHED1) and autosomal recessive (CHED2) forms have been reported and map to distinct loci on chromosome 20. CHED2 is caused by mutations in the *SLC4A11* gene which encodes a membrane transporter protein.

*Materials and Methods* Members of a large CHED2 family were recruited for clinical and genetic study. Genomic DNA was sequenced for the exons and intron-exon boundaries of the *SLC4A11* gene.

*Results* Twelve family members were recruited, of which 8 were diagnosed with CHED. A homozygous *SLC4A11* mutation (Leu843Pro) was detected in the eight patients; a single copy of the mutation was present in 3 unaffected carriers.

*Conclusions* A missense *SLC4A11* mutation (Leu843Pro) is responsible for CHED2 in this family; this is the first report of this mutation in a homozygous state.

## **Keywords**

Congenital hereditary endothelial dystrophy (CHED)

Corneal dystrophy and perceptive deafness (CDPD)

Fuchs endothelial dystrophy (FECD)

Harboyan Syndrome

*SLC4A11* gene

## Introduction

Congenital hereditary endothelial dystrophy (CHED) is a rare inherited disorder of the cornea which results in bilateral opacification and impaired vision in early life. Both autosomal dominant (CHED1; MIM 121700) and autosomal recessive (CHED2; MIM 217700) forms exist. CHED2 manifests at birth or in the neonatal period and is generally more severe than CHED1 which presents within the first two years <sup>1</sup>. CHED1 has been mapped to a locus on chromosome 20 but no causative gene has yet been described <sup>2</sup>. We previously mapped the CHED2 locus to a distinct region on chromosome 20p13 <sup>3</sup> and subsequently CHED2 –causing mutations have been described in the *SLC4A11* gene (MIM 610206) <sup>4</sup>. Mutations in this gene have also been shown to cause two other corneal disorders - autosomal recessive Harboyan Syndrome or corneal dystrophy and perceptive deafness (CDPD; MIM 217400) and autosomal dominant late onset Fuchs endothelial dystrophy (FECED4; MIM 613268) <sup>5-7</sup>.

Corneal dystrophies can be established on clinical grounds <sup>1</sup> but advances in recent years have led to molecular based classification of disease according to underlying genetic pathogenesis <sup>8</sup>.

Corneal swelling in CHED2 is due to stromal oedema and enlargement of collagen fibrils which result in altered light scattering and the clouding of the cornea. Corneal transplantation to restore vision is the primary treatment option <sup>1</sup>.

The aim of this project was to screen the *SLC4A11* gene for mutations in the large Irish consanguineous pedigree used to map the CHED2 locus.

## **Materials and Methods**

The study was approved by Ethics and Medical Research Committee of the Royal Victoria Eye and Ear Hospital, Dublin. Members of a large Irish consanguineous CHED2 family attending clinics in the Royal Victoria Eye and Ear Hospital were recruited for this study (Table 1). The diagnosis of CHED2 was based on the presence of bilateral corneal stromal clouding involving the whole corneal stroma with onset during infancy. Participating family members gave written informed consent and venous blood samples were obtained.

Genomic DNA was extracted from blood samples using the QIAamp Blood Kit (Qiagen). Mutation screening of the 19 coding exons and flanking splice sites of the *SLC4A11* gene was performed by direct sequencing using previously described primers<sup>9</sup>.

## Results

In total 12 individuals participated; 8 are affected by CHED2, 3 are probable carriers based on the affected status of their children and one individual is unaffected and does not have any children.

Of these participants, seven were part of the original mapping study<sup>3</sup> from which haplotype data is available (Figure 1).

Table 1 describes the demographic and clinical characteristics of the affected individuals. All of the patients had childhood onset nystagmus and amblyopia. All had undergone corneal transplantation in one or both eyes, with poor graft survival in most patients. Four of the patients had developed glaucoma following corneal transplantation and one had congenital glaucoma. One of the patients, (Table 1, patient 6; Figure 1, V:22), had sensorineural hearing loss.

Mutation screening of the *SLC4A11* gene identified a homozygous DNA sequence variant c.2528 T>C (p.Leu843Pro) in exon 18 in all 8 affected individuals (Figure 1, IV:4, IV:21, IV:25, V:9, V:15, V:16, V:21, V:22). The probable carriers (Figure 1, IV:16, IV:17, IV:26) are heterozygous and the unaffected individual (Figure 1, V:24) is homozygous wildtype. This pathogenic change has previously been described in compound heterozygotes with Harboyan syndrome/ CDPD in two distinct families<sup>5</sup>.

## Discussion

We detected a homozygous missense *SLC4A11* mutation in eight CHED-affected individuals in a large consanguineous family. Three heterozygous carriers have been confirmed and one unaffected individual is homozygous wildtype. Mutated *SLC4A11* has been described in patients with CHED2, CDPD and late onset FECD. CDPD patients present with endothelial dystrophy at birth and develop sensorineural hearing loss generally in the second decade. This raises the

question as to whether CHED2 is unrecognised CDPD and several studies recommend monitoring CHED2 patients for progressive hearing loss<sup>5,10,11</sup>. The Leu843Pro mutation was previously reported as one of two distinct mutations detected in two non-consanguineous CDPD families of South American Indian and Dutch background<sup>5</sup>. The finding of this mutation in an Irish pedigree means that this is the most ethnically diverse mutation reported to date; the majority of reports have been from Indian and middle eastern populations. Additionally, studies have demonstrated the high conservation of the L843 residue which is located within the thirteenth transmembrane region<sup>5</sup>. This is the first report of this mutation in a homozygous state. The families in which L843P was identified previously were both very small with only one or two affected individuals in which hearing loss was diagnosed at 4 and 19 years, fitting the picture of deafness by the second decade as generally reported in CDPD<sup>5</sup>.

Within the family presented here, one individual (Figure 1, V:22) suffers from sensorineural hearing loss, although formal audiological evaluation was not performed in her or any of the other participants in this study. None of the other family members have reported hearing difficulties (age range 18-62 years). This suggests that a diagnosis of CHED2 rather than CDPD is appropriate. As corneal clouding is diagnosed at an early age with hearing loss presenting often in the teenage years; the concern of progression from CHED to CDPD may apply only to a small portion of families who have a final CDPD diagnosis.

A number of reported *SLC4A11* mutations cause both CHED2 and CDPD; an 8 bp deletion causing a frameshift at Arg158 has been reported to cause CDPD with hearing loss diagnosed at 5 years<sup>5</sup> and CHED2<sup>12-14</sup> with no reports of hearing loss, though cases ranged from 9 – 13 years, so hearing deficits may develop subsequently. The Ser213P missense mutation causes CDPD in a Shepari Jewish patient, with deafness noted at 17 years, while a Ser213Leu mutation was reported in a CHED patient with no comment on hearing loss at 10 years of age. Val824Met

causes at least CHED2 in a homozygous Indian patient who was too young for deafness to be assessed (2 years).

Therefore, to date all cases of CDPD have early diagnosis of CHED2 with hearing loss detected by the second decade.

*SLC4A11* mutations have also been described in patients with late onset FECD<sup>6,7</sup> though this gene accounts for only approximately 5% of such cases<sup>15</sup>. While several FECD loci have been identified,<sup>6, 16-21</sup> to date no linkage analysis has mapped FECD to the CHED2 locus<sup>22</sup>. If FECD is caused by heterozygous mutations of *SLC4A11*, a high incidence of this disease would be expected in populations where CHED2 is common. However, FECD is a very uncommon indication for corneal transplantation in countries where CHED2 is most common<sup>1,22</sup>. FECD is a common disease of corneal aging, present in about 4% of individuals over 40 years<sup>21</sup>. Therefore, when considering FECD in carriers of CHED2 *SLC4A11* mutations it is important to consider if these cases are subclinical and how many actually present with visual disturbances. FECD has been reported in a heterozygous parent of a CHED2 patient with homozygous *SLC4A11* nonsense mutation at residue Cys386<sup>23</sup>. However, the other carrier parent and a heterozygous sibling did not show signs of FECD. This raises the question of whether the single mutation was causative in this case. A homozygous Cys386Arg mutation has been reported in several CHED2 cases in Indian families<sup>24 13,25</sup>. Another site of both FECD and CHED2 mutations is residue 240. Trp240X homozygous mutation causes CHED2 while a heterozygous Trp240Ser mutation was reported in a late onset FECD case in South Asia. Furthermore, apart from these exceptions the mutations causing CHED2 and FECD appear to be distinct<sup>26</sup>. While the carriers within this family have not been assessed formally for early clinical signs of FECD, none have reported visual disturbances to date.



Interestingly, one study has reported a significant association between hearing loss and sporadic FECD <sup>27</sup>.

Genetic heterogeneity may exist for CHED2 as some families have no *SLC4A11* mutation detected <sup>12, 13, 22, 25, 28</sup>, however, only one CHED2 locus has been mapped <sup>3, 4, 29</sup>. Recently in a study of a non-consanguineous CHED2 Thai family all affected individuals were found to be compound heterozygotes for a novel missense mutation (K260E) and a 68kb deletion which encompasses the *SLC4A11* gene <sup>30</sup>; this type of mutation may be the cause of disease in families in which an *SLC4A11* gene mutation has not been identified to date. Sultana et al showed no phenotype- genotype correlation between clinical and histological parameters and the type or location of *SLC4A11* mutation <sup>12</sup>.

The *SLC4A11* gene encodes a plasma membrane borate transporter and mutations cause a loss of function either by preventing membrane targeting or by nonsense-mediated decay <sup>4, 8</sup>. Of the more than 80 *SLC4A11* mutations reported to date, the majority are located in the transmembrane region. *SLC4A11* exists as a dimer and studies demonstrate that heteromeric interactions occur between wildtype (WT) and mutants. Furthermore, cell surface processing of CHED2 mutations was increased when co-expressed with WT, while FECD mutants were unaltered by WT protein <sup>31</sup>. These findings provide an explanation for the dominant nature of FECD and recessive pattern seen in CHED2. It also presents the basis of a therapeutic strategy to target compounds that might release the intracellularly retained mutant proteins similar to the approach being used for cystic fibrosis. Cells transfected with various disease causing *SLC4A11* gene mutations have established that homozygous CHED2 mutations have less than 5% of wild type activity, CHED2 heterozygotes have 50% activity and FECD mutant displayed approximately 27% WT activity <sup>32</sup>. These values define the relative levels of function required to delay or prevent visual symptoms.

Studies have demonstrated that endoplasmic reticulum-retained CHED2 mutant SLC4A11 protein, including Leu843Pro, could be rescued to the plasma membrane where at least partial restoration of function was observed <sup>32</sup>.

While originally identified as a bicarbonate/ borate transporter <sup>4</sup>, recent studies have confirmed the role of SLC4A11 in forming a basolateral trans-endothelial cell water pathway without a coupled solute transport <sup>33,34</sup>. Defects in this protein give rise to the fluid accumulation observed in the corneal stroma in CHED patients <sup>34</sup>.

At present the main treatment option for CHED is corneal transplantation which carries a risk for infection and graft rejection. Therefore alternative therapies targeted at the underlying defective water movement should be further investigated to minimise or restore lost capacity, thereby reducing fluid accumulation and the resulting visual impairment.

<b>Patient (Position Figure 1)</b>	<b>Age (y)</b>	<b>Clinical history</b>
1 (IV:4)	52	Bilateral PKP, right corneal graft failure, glaucoma, nystagmus, bilateral cataract surgery, VA 20/200 OU
2 (IV:25)	50	Bilateral PKP, left corneal graft failure, glaucoma, nystagmus, retinal detachment repair, bilateral cataract surgery, VA 20/200 OD, CF OS
3 (V:16)	18	Left PKP, corneal graft failure, congenital glaucoma, nystagmus, VA 20/100 OD, CF OS
4 (V:21)	25	Bilateral PKP, left penetrating eye injury, nystagmus, VA 20/400 OD, NPL OS
5 (V:9)	24	Bilateral PKP, nystagmus, VA 20/200 OU
6 (V:22)	21	Bilateral PKP, left corneal graft failure, glaucoma, nystagmus, bilateral sensorineural hearing loss, VA CF OD, 20/200 OS
7 (IV:21)	62	Bilateral PKP, glaucoma, nystagmus, VA 20/200 OD, CF OS
8 (V:15)	25	Bilateral PKP, left corneal graft failure, right cataract surgery, nystagmus, VA 20/200 OD, HM OS

**Table 1 Demographics and clinical characteristics of CHED2 patients.** The position of these individuals in Figure 1 is indicated.

Abbreviations: PKP penetrating keratoplasty, OU both eyes; OD right eye; OS left eye; NPL no perception of light; CF counting fingers; HM hand movements; VA visual acuity

## Figure Legend

**Figure 1** Pedigree structure of CHED2 family indicating disease haplotype and mutation screening results.

Circles and squares represent females and males respectively; filled symbols denote affected individuals and clear symbols with the letter N denote unaffected individuals, carriers are indicated by a dot within the symbol; a diagonal line indicate that the person is deceased.

CHED2 refers to the presence of the disease haplotype as determined in the original mapping study<sup>3</sup>:  $+/+$  indicates an individual homozygous for the disease haplotype,  $+/-$  is a heterozygous carrier and  $-/-$  is homozygous wild type. Similarly, the presence of the *SLC4A11* L843P mutation is indicated in the individuals who participated in this mutation screening study.

Several additional family members were removed for clarity.

There are 8 affected individuals homologous for the mutation (IV:4, IV:21, IV:25, V:9, V:15, V:16, V:21, V:22), 4 of which were part of the original mapping study and have haplotype data available.

The three heterozygous carriers (IV:16, IV:17, IV:26) had a single copy of the disease haplotype; the homozygous wildtype individual (V:24) does not have haplotype data available.

## Declaration of interest

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Figure 1

