LETTER TO JMG

Autosomal recessive corneal endothelial dystrophy (CHED2) is associated with mutations in SLC4A11

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utosomal recessive corneal endothelial dystrophy, also called congenital hereditary endothelial dystrophy (CHED2, MIM 217700), is an uncommon hereditary corneal disorder clinically presenting at birth or in early childhood. It commonly presents as a corneal haze that has a ground-glass appearance. The cornea is also thickened overall and shows epithelial and stromal oedema. It is clinically similar to autosomal dominant corneal endothelial dystrophy (CHED1, MIM 121700), although it may present earlier. The age of onset and progression of the disease and the degree of visual impairment can be variable, although some children with CHED can develop nystagmus. Histologically, corneal endothelial dystrophy is characterised by irregular and multinucleated corneal endothelial cells with a normal or reduced density and markedly thickened Descemet’s membrane showing a trilaminar structure with an anterior banded zone, a posterior non-banded zone and a posterior collagenous layer having multiple focal areas of abnormal fibrillar deposits in the posterior half. These fibrillary changes are more common in autosomal dominant CHED, whereas recessive CHED shows failure of growth regulation characterised by a more marked thickening of the anterior banded zone. The disorder is bilateral and has a high degree of penetrance. The preferred treatment is penetrating keratoplasty, which can provide marked visual improvement even in advanced stages of the disease. CHED2 has been mapped to an 8 cM region on chromosome 20p12 flanked by D20S113 and D20S882. This region is distinct from the CHED1 locus in the pericentromeric region of chromosome 20, which colocalises with VSX1.

SLC4A11, sodium bicarbonate transporter-like solute carrier family 4 member 11, is a member of the SLC4 family of bicarbonate transporters, which, along with the SLC26 family, are the main bicarbonate transporter proteins in humans. The SLC4 gene family classically includes three types of bicarbonate exchangers: Cl-HCO₃, Na/HCO₃ cotransporters and Na-driver Cl-HCO₃ exchangers. SLC4A11, also called BTR1 or NaBC1, is phylogenetically the most distant member of the SLC4 gene family. Mutations in SLC4A11 result in distal and proximal renal tubular acidosis, respectively. However, SLC4A11 is an electrogenic Na/borate cotransporter that stimulates cell growth and proliferation by increasing intracellular borate and activating the MAPK pathway. The SLC4A11 gene on chromosome 20p12 has 13 exons, all containing coding sequences and encoding 891 amino acids. As shown in the National Center for Biotechnology Information UniGene expression profile, it is highly expressed in the eye, blood cells, ovary, tongue, lung, skin and colon, and to a lesser extent in the brain, pancreas, kidney and skin. It is also expressed in some tumours, including gastrointestinal, oral, ovarian, respiratory and skin tumours, as well as in leukaemia and retinoblastoma.

We studied 16 families with autosomal recessive CHED by mapping and candidate gene screening to identify the gene associated with the disease. Our results confirm those of a recent study, and show mutations in the SLC4A11 gene in 12 of 16 families.

METHODS

A diagnosis of CHED was made in all patients by clinical and histopathological evaluation. Patients were examined using indirect ophtalmoscopy and slit lamp examination. Affecte members were diagnosed at ages <1–10 years. Inclusion criteria for diagnosis of CHED were the presence of a cloudy cornea from birth to 10 years of age, with increased corneal thickness and bilateral corneal oedema in the presence of a normal corneal diameter and normal intraocular pressure. Exclusion criteria were the presence of Haab’s striae or any sign of primary congenital glaucoma, and a history of prior ocular surgery. Histopathological criteria for CHED were thickening of the cornea, thickening of Descemet’s membrane and normal/reduced endothelial cell count and morphology. This study was approved by the institutional review boards of The LV Prasad Eye Institute, Hyderabad, India, and the National Eye Institute, Bethesda, Maryland, USA, and informed consent was obtained from each person studied, consistent with the tenets of the Declaration of Helsinki. DNA samples were collected from 16 families, all of which were consanguineous. Twelve families fulfilled all criteria listed earlier. Four families, 73019, 73029, 73035 and 73089, were excluded from linkage analysis because previous surgery or signs of primary congenital glaucoma.

Abbreviation: CHED, congenital hereditary endothelial dystrophy
was carried out with 13 markers in the region of chromosome 20q.

Modeling was performed with the FASTLINK implementation of the MLINK program package,

unaffected members) were used in linkage analysis; pedigrees of these are shown in Figure 1.

For linkage analysis, DNA was extracted directly from blood by standard phenol–chloroform protocols.

Linkage analysis was carried out with 13 markers in the region of chromosome 20p flanked by D20S113 and D20S882, to which CHED2 had been mapped by Hand et al. Markers in this region were genotyped using fluorescent-labelled microsatellite markers (ABI Linkage Mapping Set MD-10, Foster City, California, USA). Multiplexed polymerase chain reaction was carried out as previously described. Two-point linkage analyses were performed with the FASTLINK implementation of the MLINK program of the LINKAGE program package, modelling CHED2 as a fully penetrant autosomal recessive disease. Equal allele frequencies arbitrarily set at 0.05 were used for all markers.

The reference cDNA (NM 032034) and genomic sequences (NT 011387) for the SLC4A11 gene are available from the National Center for Biotechnology Information. For mutation screening, coding exons and adjacent intronic sequences of candidate genes in the critical interval were amplified from genomic DNA of 16 affected patients and two unaffected members. Table 1 shows the primers used for amplification and sequencing of the 19 exons of SLC4A11. Exons 18 and 19 were amplified using an Invitrogen High GC Kit (Carlsbad, California, USA). Products of the sequencing reactions were purified using the Edge Biosystem Performa TM DTR Gel Filtration System (Gaithersburg, Maryland, USA) or AmPure and CleanSeq reagents (Agencourt, Boston, Massachusetts, USA) on a Beckman Biomek NX Laboratory Automation Workstation (Fullerton, California, USA).

Figure 1 Pedigrees of families 73004, 73013, 73015, 73022, 73024, 73026, 73037, 73043, 73044 and 73049, used in linkage and haplotype analysis. The dark bars correspond to affected haplotypes, including alleles that cosegregate with the disease and are homozygotic in affected members. Grey bars indicate alleles not homozygotic in affected members and white bars indicate recombinant alleles.

RESULTS AND DISCUSSION

Evidence of significant linkage was obtained with markers in the chromosome 20p12 region. All markers telomeric of D20S835 gave LOD scores >3, the highest LOD score being observed with D20S117, which gave 11.1 at θ = 0 (table 2). Examination of the haplotypes did indicate a probable recombination with D20S835. D20S889 shows an obligate recombination, although the maximum LOD score at θ = 0.01 is 8.9. D20S115 and D20S177, which lie centromeric to D20S889, both show obligate recombinations, as do markers
centromeric to this region. D20S906 and D20S193, both within the included region, gave maximum LOD scores of 9.5 and 9.6, respectively, at θ = 0. D20S198, while not showing an obligate recombination, shows a lack of homozygosity in affected members from multiple families for markers centromeric to D20S889 support this interpretation. As this region lies near the telomere of chromosome 20, probable telomeric recombination events are less common, although a lack of homozygosity at D20S198 in member 4 of family 73037 suggests that this is the telomeric boundary. Conservation of the tightly linked haplotypes suggests that D20S117 also recombines, although this is not apparent from the two-point linkage data. Thus, examination of haplotypes in these families places the CHED2 locus between D20S198 and D20S889, a 1.3 Mb (2.2 cM) interval that contains the SLC4A11 gene.

Significant mutations identified in SLC4A11 as described later and in table 3 were not seen in 50 controls (100 chromosomes) of southern Indian ethnicity. All mutations identified in SLC4A11 cosegregated with the disease allele, except the polymorphism identified in family 73026.

CHED2 is a recessive disease, suggesting that causative mutations might result in a loss of SLC4A11 function. Mutation analysis in this set of families is simplified by the occurrence of all mutations in a homozygotic form owing to consanguinity. Some of the observed mutations would be expected to have dramatic effects on the structure or expression of the protein. These include two nonsense mutations: p.Arg605X (g.8295G→T, families 73015 and 73026), and p.Glu632X (g.8379G→A, family 73035). Three frameshift mutations (p.Arg82ArgfsX33 (g.2294delTT, family 73024), p.His568HisfsX177 (g.8118delCT, family 73013) and p.Leu807ArgfsX71 (g.9200delTinsGG, family 73004)) would also result in loss of a large number of amino acids from the carboxy terminus of the protein and would also replace these sequences with long novel amino acid sequences encoded by the shifted reading frame. In addition, four of these cause premature termination in an internal exon and are likely to be subject to nonsense-mediated decay. Thus, examination of haplotypes in these families recombines, although this is not apparent from the two-point linkage results. Conservation of the lack of homozygosity at D20S198 in member 4 of family 73037 suggests that this is the telomeric boundary. Conservation of the tightly linked haplotypes suggests that D20S117 also recombines, although this is not apparent from the two-point linkage data. Thus, examination of haplotypes in these families places the CHED2 locus between D20S198 and D20S889, a 1.3 Mb (2.2 cM) interval that contains the SLC4A11 gene.
Arg804His (family 73014), Thr833Met (families 73029 and 73044), and Arg869His (families 73039 and 73043). A fifth missense change of Ala160Thr was found to be homozygotic in two families (73026 and 73015) and was found in an unaffected relative in family 73026. This change is unlikely to have pathogenic significance, as it also occurred in homozygotic form in an unaffected member in one family (73026), and the residue is not conserved among SLC4A11 and CHED2.

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In summary, CHED2 has been mapped to a 2.2 cM (1.3 Mb) interval flanked by D20S198 and D20S889, including SLC4A11. This region includes SLC4A11, mutations which are shown to be associated with CHED2 in 12 of 16 families examined.

**Key points**

- Linkage and haplotype analysis of families with autosomal recessive congenital hereditary endothelial dystrophy mapped the disease to a 2.2 cM interval flanked by D20S198 and D20S889 on chromosome 20p12. The maximum limit of detection score of 11.1 was obtained with D20S117 at θ = 0.

- Evaluation of candidate genes in this interval showed mutations in the sodium bicarbonate transporter-like solute carrier family 4 member 11 (SLC4A11) gene in 12 of 16 families.

- Mutations consisted of three frameshift, two nonsense and four missense mutations.

**Table 3** Summary of SLC4A11 mutations found in families with CHED2

<table>
<thead>
<tr>
<th>Family</th>
<th>Nucleotide change</th>
<th>Exon</th>
<th>Protein mutation (Blosum 80 Score; % residue conservation)*</th>
<th>Location in protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>73024</td>
<td>g.2943delTTinsA†</td>
<td>2</td>
<td>p. Arg82ArgfsX33</td>
<td>Amino-terminal soluble polypeptide with a new sequence FLSMSTLRCRPPTL added after residue Arg82. Most of the cytoplasmic soluble domain and the transmembrane domain are truncated.</td>
</tr>
<tr>
<td>73026, 73015†</td>
<td>g.3552G→A†</td>
<td>4</td>
<td>p. Ala160Thr (0; 3%)</td>
<td>Located in the loop connecting two α-helices from opposite sides of the αβα sandwich.</td>
</tr>
<tr>
<td>73013</td>
<td>g.8118delCT†</td>
<td>13</td>
<td>p. His568HisfsX177</td>
<td>Truncates the transmembrane domain after residue His568 and adds a hydrophilic peptide of 28 residues at the C terminus [RPGDRAQPPPHAGHLAGHHPPLPQEE].</td>
</tr>
<tr>
<td>73026, 73015†</td>
<td>g.8298C→T†</td>
<td>14</td>
<td>p. Arg605X</td>
<td>Truncates transmembrane domain at residue Arg605.</td>
</tr>
<tr>
<td>73035†</td>
<td>g.8379G→T†</td>
<td>14</td>
<td>p. Gln562X</td>
<td>Truncates transmembrane domain at residue Gln562.</td>
</tr>
<tr>
<td>73037</td>
<td>g.9044G→A</td>
<td>17</td>
<td>p. Arg755Gln (1; 97%)</td>
<td>Located at the surface of transmembrane helix 9. Might affect interaction of transmembrane helix 9 and cytoplasmic membrane.</td>
</tr>
<tr>
<td>73014†</td>
<td>g.9191G→A</td>
<td>17</td>
<td>p. Arg804His (0; 100%)</td>
<td>Located in the loop connecting helices 11 and 12 in the predicted transmembrane domain structure. Mutation changes hydrophobic interaction of methyl groups located in ARG stem with those of P640 (distance increases by 1.2 Å). This might change the loop stability.</td>
</tr>
<tr>
<td>73004</td>
<td>g.9200delTinsGG</td>
<td>17</td>
<td>p. Leu807ArgfsX71</td>
<td>Truncation of the C-terminal part after residue L807 and after addition of the six-residue peptide (RAAQGA).</td>
</tr>
<tr>
<td>73044, 73029†</td>
<td>g.9361C→T</td>
<td>18</td>
<td>p. Thr833Met (−1; 91%)</td>
<td>Predicted to be located in transmembrane helix 11. The mutation might destabilise the polar cluster formed by residues Thr833, Gln826, Arg827 and Lys828, and affect the loop stability.</td>
</tr>
<tr>
<td>7039†, 7043 g.9469G→A</td>
<td>18</td>
<td>p. Arg869His (0; 94%)</td>
<td>Located at the surface of transmembrane helix 12. Might affect interaction of transmembrane helix 12 and cytoplasmic membrane.</td>
<td></td>
</tr>
</tbody>
</table>

*The Blosum 80 substitution matrix is based on a threshold of 80% sequence identity. The percentage residue conservation was calculated by comparing corresponding amino acids in all available members (35) of the SLC4A protein family from the UniProtKB/Swiss-Prot database. |

†Subject to potential nonsense-mediated decay.

These families were not used in linkage analysis.

VA polymorphism that occurs with g.8298C→T (p. Arg605X) in families 73026 and 73015 and was found in an unaffected relative in family 73026.

Note: Mutations were not observed in families 73022 and 73049, both used in linkage analysis.

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**Electronic database information**


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Warning family members of individuals with an HNPCC-causing mutation

permission. The best course of action is for health professionals to engage probands in a discussion of familial implications before genetic testing. An agreement can be formulated between provider and patient as to which relatives will need to know the information and how they should be informed. Owing to the likely unrealistic resources required for provider notification, patients should be counselled and encouraged about the personal duty to warn family members.

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CORRECTION

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The journal apologises for an error that has occurred within this paper. In the abstract on page 64 (under Results, line 3) and in the key points box on page 67 (line 5) the term “limit of detection” should read as “LOD”.

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