Mutational Spectrum of \textit{SLC4A11} in Autosomal Recessive CHED in Saudi Arabia

Mohammed A. Aldabmesh,1 Arif O. Khan,1,2 Brian F. Meyer,1 and Fowzan S. Alkuraya1,3,4

\textbf{PURPOSE.} To determine the extent of allelic, and possibly locus, heterogeneity in congenital hereditary endothelial dystrophy (CHED, MIM 217700) in patients from a highly consanguineous Saudi population.

\textbf{METHODS.} Homozygosity was determined at the solute carrier family 4, sodium bicarbonate transporter-like, member 11 (\textit{SLC4A11}) locus followed by full sequencing of \textit{SLC4A11} in 10 patients representing seven unrelated families.

\textbf{RESULTS.} All 10 patients were homozygous at the \textit{SLC4A11} locus. Seven mutations were identified, five of which are novel, including one likely intronic splicing enhancer mutation, all predicted to result in reduction or loss of bicarbonate transporter-related protein 1 (BTR1).

\textbf{CONCLUSIONS.} In this small cohort, no evidence was found of genetic heterogeneity in CHED and that loss of BTR1 function is the most likely mutational mechanism. (\textit{Invest Ophthalmol Vis Sci.} 2009;50:4142–4145) DOI:10.1167/iovs.08-3006

Transparency of the cornea, a key to normal vision, is achieved by several mechanisms, not the least of which is how tightly regulated water content is in the stroma of the cornea, largely through action of the corneal endothelium (the innermost layer of the cornea).1 As the cornea is free of blood vessels, the corneal endothelium is not related to the vascular endothelium but rather to a monolayer of specialized neural crest-derived hexagonal cells. Evidence that supports the critical role played by the corneal endothelium in maintaining transparency of the cornea comes from a recent revelation that congenital hereditary endothelial dystrophy (CHED) can be caused by a mutation in the 

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First described by Maunence in 1960,3 CHED is a rare corneal genetic disorder characterized by diffuse bilateral haziness of the cornea of variable degree with no evidence of other ophthalmic disease. At the histopathologic level, CHED is characterized by degenerated and abnormal corneal endothelium with accompanying corneal edema.4 The condition is known to occur in two genetic forms: autosomal dominant (CHED1, MIM #121700; Mendelian Inheritance in Man; National Center for Biotechnology Information, Bethesda, MD) and autosomal recessive (CHED2, MIM #217700), the latter being more severe and usually of significantly earlier onset.

The loci for CHED1 and CHED2 have been mapped to the pericentromeric region of chromosome 20p11.2-q11.2 and 20p13,4 respectively, and mutations in \textit{SLC4A11} have recently been shown to underlie CHED2 as well as a rare syndromic form of CHED2 associated with deafness (Harboyan syndrome).3,7 \textit{SLC4A11} consists of 19 exons spanning 11,774 bp of genomic DNA and encodes an 891-amino acid protein BTR1 (bicarbonate transporter-related protein 1). Although the actual structure of BTR1 has not been solved yet, based on significant homology with bicarbonate transporters, it is predicted to contain 13 transmembrane domains (TMDs) and intracellular NH$_2$- and COOH termini.8 As BTR1 is an Na/HCO$_3$ cotransporter, it seems logical that loss of function mutations in \textit{SLC4A11} would result in perturbation of the regulation of water content in the cornea. However, the latter view may be too simplistic as it fails to incorporate the role BTR1 plays in regulating intracellular borate concentration. Indeed, recent evidence suggests that the latter role may be involved in MAPK (mitogen-activated protein kinase) signaling thus adding an extra layer of complexity to the pathogenesis of CHED.9

Since the first description of \textit{SLC4A11} mutations, several case reports and small case series have been published.10–15 Of interest, most of the patients in those reports are from the Indian subcontinent, and very little is known about the role played by \textit{SLC4A11} mutations in patients with CHED from the Middle East. In this study, we report seven different mutations, five of which are novel, from several independent Saudi families. Contrary to a recent report,12 we found no evidence of genetic heterogeneity of CHED2 in our Saudi cohort.

\textbf{MATERIALS AND METHODS}

Approval for publication of these data was granted by the Institutional Review Board of the King Faisal Specialist Hospital and Research Center, on the basis that these were results of diagnostic tests in which the identity of patients was not disclosed.

\textbf{Human Subjects}

Children with CHED diagnosed on the basis of a complete clinical ophthalmic examination (including slit-lamp examination, intraocular pressure measurement, cycloplegic retinoscopy, and indirect ophthalmoscopy) had venous blood samples collected for confirmatory diagnostic testing after informed consent was obtained in accordance with the Declaration of Helsinki. Patient ranged in age from 4 to 10 years. Clinical data were collected with the child under chloral hydrate sedation when necessary.

The diagnosis of CHED was made on the basis of the following: characteristic mosaic corneal haze with corneal edema (present since birth by history), increased central corneal thickness (>0.7 mm in all cases), normal horizontal corneal diameter (10-11 mm), and no evidence for glaucoma (e.g., no Haab’s striae, normal-appearing optic nerve heads, no buphthalmos).

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Supported by the King Faisal Specialist Hospital and Research Center and the King Saud University.

Submitted for publication October 16, 2008; revised December 21, 2008, and January 21, 2009; accepted June 18, 2009.

Disclosure: M.A. Aldabmesh, None; A.O. Khan, None; B.F. Meyer, None; F.S. Alkuraya, None

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DNA Extraction

Genomic DNA was extracted from whole blood anticoagulated with EDTA (Purgene Gentra DNA Extraction Kit; cat. No. D-5000, Gentra Systems, Minneapolis, MN) according to the manufacturer’s instructions. The DNA was quantified spectrophotometrically and stored in aliquots at −20°C until used.

PCR, Genotyping, and Direct Sequencing

PCR amplification was performed on a thermocycler (DNA Engine Tetrad; MJ Research, Inc., Waltham, MA) in a total volume of 25 µL, containing 10 ng DNA, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 1.5 mM MgCl₂, 0.1% Triton X-100, 0.25 mM of each dNTP, 0.8 µM of each primer, and 0.5 units of Taq polymerase (D-40724; Qiagen, Hilden, Germany). For PCR, an initial denaturation step at 95°C for 10 minutes was followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 59°C for 30 seconds, and extension at 72°C for 30 seconds, followed by a final extension step of 72°C for 10 minutes. The primers used for amplification of the coding regions of SLC4A11 gene were as described before.15 Genomic DNA of each patient was screened for mutations in the exons and splice sites of SLC4A11 (BigDye Terminator ver. 3.1 Cycle Sequencing Kit; Applied Biosystems, Inc. [ABI], Foster City, CA), and samples were loaded onto a DNA analyzer (ABI Prism 3730XL; ABI), according to the manufacturer’s instructions. Sequence analysis was performed (SeqManII module of the Lasergene; DNA Star Inc., Madison, WI) software package, then compared to a reference sequence. Homozygosity at the SLC4A11 locus was established with microsatellite markers, and genotyping was also performed with the DNA analyzer (Fig. 1).

RESULTS

We screened the coding regions of SLC4A11 using PCR-based amplification followed by direct sequencing. Mutations were identified in all 10 affected individuals from seven unrelated Saudi CHED families who were all found to be homozygous at the SLC4A11 locus. We found seven different mutations, including five previously unreported, which were absent in a control population of the same ethnic origin as determined by direct sequence analysis on at least 100 chromosomes. The mutation spectrum included missense, nonsense, splice site, and small deletions (one out-of-frame, and one intronic deletion; Table 1). In addition, we identified one silent variant p.Thr833Thr that most likely represents a previously unreported rare SNP. The homozygous missense mutation p.Gly394Arg was caused by a c.1228G→C transversion in a single proband in family DG-2. In family DG-3, a previously reported missense mutation (c.1253G→A, p.Gly418Asp) was identified and is predicted to disrupt TMD 2.11 A splice-site mutation (c.2114+1G>A) was identified in all affected members of family DG-7. The homozygous small deletion of 19 nt in intron 7 identified in family DG-4 contained the fairly conserved CGGCGGG sequence which suggests it may represent a

Table 1. Summary of the Mutations Identified in the SLC4A11 Gene

<table>
<thead>
<tr>
<th>Family ID</th>
<th>Number of Affected</th>
<th>Mutation</th>
<th>SNP</th>
<th>Type of Mutation</th>
<th>Position</th>
<th>Mutation Effect</th>
<th>Amino Acid Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>DG-1</td>
<td>1</td>
<td>c.520delGCTTCGCC</td>
<td></td>
<td>Out-of-frame deletion</td>
<td>Exon 4</td>
<td>Truncation protein</td>
<td>Arg158fs</td>
</tr>
<tr>
<td>DG-2</td>
<td>1</td>
<td>c.1228G&lt;177C</td>
<td></td>
<td>Missense</td>
<td>Exon 9</td>
<td>Disruption of TMD1</td>
<td>Gly394Arg</td>
</tr>
<tr>
<td>DG-3</td>
<td>1</td>
<td>c.1253G&lt;177A</td>
<td></td>
<td>Missense</td>
<td>Exon 10</td>
<td>Disruption of TMD2</td>
<td>Gly418Asp</td>
</tr>
<tr>
<td>DG-4</td>
<td>1</td>
<td>c.1044+25del19nt</td>
<td>2289-4G&gt;A/18C&gt;T c.2485-9C&gt;T</td>
<td>Deletion SNP SNP</td>
<td>Intron 7</td>
<td>Not known</td>
<td>—</td>
</tr>
<tr>
<td>DG-5</td>
<td>1</td>
<td>2236C&gt;T</td>
<td>c.2547G&gt;C</td>
<td>Silent Nonsense</td>
<td>Exon 16</td>
<td>Truncation protein</td>
<td>Arg757X</td>
</tr>
<tr>
<td>DG-6</td>
<td>2</td>
<td>2236C&gt;T</td>
<td>c.2547G&gt;C</td>
<td>Silent Nonsense</td>
<td>Exon 18</td>
<td>No effect</td>
<td>Thr833Thr</td>
</tr>
<tr>
<td>DG-7</td>
<td>3</td>
<td>c.2114+1G&gt;A</td>
<td>c.2485-9C&gt;T</td>
<td>Silent SNP Donor splice site</td>
<td>Exon 15</td>
<td>Inclusion of intron 15</td>
<td>—</td>
</tr>
</tbody>
</table>

FIGURE 1. Sequencing data on the five novel mutations reported in this study (wild-type is shown on top of each panel for comparison).
GGG-containing intronic splicing enhancer. The synonymous SNP (c.2547G>C) demonstrated a segregation pattern that suggests that it is in linkage disequilibrium with the nonsense mutation 2236C>T. In addition, three homoallelic SNPs were detected in affected individuals (c.2424+4G>A, c.2424+18C>T, and c.2485-9C>T).

**DISCUSSION**

This review of diagnostic data from our patients with CHED was motivated by a recent report describing the lack of SLC4A11 mutations in some patients with CHED. We were interested in elucidating the potential role of other genes in the causation of CHED among Saudis. Taking advantage of the fact that all patients were products of consanguineous marriages, we were able to demonstrate homozygosity in all patients at the SLC4A11 locus. Indeed, our mutational analyses confirmed that all patients were homozygous for different SLC4A11 mutations, all predicted to result in reduction or loss of BTR1 function. A recent study had sought to map the gene responsible for CHED in a large Saudi family revealed that SLC4A11 was again the culprit gene. 17 Hemadevi et al. indicated that the lack of SLC4A11 mutations in some of their patients suggests that other loci may be involved. Unfortunately, they did not comment on whether they were able to include the SLC4A11 locus at least in consanguineous families by using markers to test homozygosity. All our patients were products of consanguineous unions, which explains why all mutations were homoallelic. We describe seven mutations and four SNPs detected in 10 affected individuals from seven unrelated families. One of the two missense mutations identified in this work (Gly418Asp) has been reported. A second missense mutation Gly594Arg was not found in 180 chromosomes from Saudi control subjects. In addition, multiple sequence alignment demonstrated that glycine in that position is fully conserved across species, including the plant homolog BOR-1, which demonstrated that glycine in that position is fully conserved across species, including the plant homolog BOR-1, which supports the pathogenicity of this mutation (Fig. 2).

The splice site mutation, c.2114+4G>A, identified in the Saudi family with CHED affects the invariant G at the consensus donor site and such mutations are almost always pathogenic. Splice site mutations have been reported before in patients with CHED. The frameshift mutation (c.520delGCTTCGCC) in exon 4 was again the culprit gene. The synonymous SNP, which we identified in this study (c.2547G>C) in association with Arg757X, has no effect on the protein function and is in linkage disequilibrium with Arg757X. The intronic deletion mutation that was found in our study is likely pathogenic given its segregation pattern and absence in 180 normal controls. We hypothesize that an intronic splice enhancer lies within the deleted segment and this would cause skipping of the upstream exon with resulting frameshift. This hypothesis is further supported by previous data that show that intronic splicing enhancers tend to be observed in small introns (the involved intron is only 87 bp long). Unfortunately, the ability to predict intronic splice enhancers in silico is limited and we were unable to experimentally prove the hypothesized effect on exon skipping on RT-PCR because of lack of reliable expression of the gene in lymphocytes. This study is the largest so far to examine the spectrum of mutations in SLC4A11 gene among patients with CHED outside the Indian subcontinent. Our ability to identify mutations in all affected patients argues for the genetic homogeneity of this disorder, at least among Saudi patients. However, we are cautious about drawing such a conclusion without examining a larger cohort of patients with CHED. Our findings are in agreement with those of others with regard to loss of BTR1 function as the mechanism that underlies CHED.

**References**


