

Identification of Mutations in the *SLC4A11* Gene in Patients With Recessive Congenital Hereditary Endothelial Dystrophy

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Objective: To identify Solute Carrier family 4 (sodium borate cotransporter) member 11 (*SLC4A11*) gene mutations associated with autosomal recessive congenital hereditary endothelial dystrophy (CHED2).

Methods: DNA extraction from blood, polymerase chain reaction amplification, and direct sequencing of all the exons of the *SLC4A11* gene were performed for 26 affected members of 20 unrelated families with CHED2.

Results: Of 10 mutations observed, 6 were novel, 1 of which involves a complete deletion of exon 6, identified for the first time, to our knowledge, in *SLC4A11*. The mutations cosegregated with the disease phenotype and were absent in 200 ethnically matched control chromosomes analyzed.

Conclusions: This study increases the number of *SLC4A11* gene mutations and confirms the role of this gene in causing CHED2. Clinical examination did not reveal any considerable variability in disease expressivity in patients carrying *SLC4A11* mutations. Extensive linkage analysis may reveal the modifier genes involved in causing CHED2 in the *SLC4A11* mutations unidentified in 9 families.

Clinical Relevance: In India, there is a high frequency of CHED2, possibly related to consanguineous marriages. Counseling could be provided to explain the drawbacks of consanguineous marriages to assist in reducing this devastating disorder.

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CONGENITAL HEREDITARY endothelial dystrophy (CHED) is a rare inheritable disorder of the corneal endothelium characterized clinically by a bilateral, symmetrical, noninflammatory corneal clouding (edema) without other anterior segment abnormalities, usually evident at birth or in the early years of life. It is due to a malfunction and degeneration of the corneal endothelium and the posterior nonbanded part of the Descemet membrane (DM), which results in a diffuse ground glass-like corneal edema especially of the corneal stroma, which is 2 to 3 times the normal thickness.^{1,2}

Autosomal dominant (CHED1) (OMIM %121700) and autosomal recessive (CHED2) (OMIM 217700) modes of inheritance have been described for CHED. The high incidence of CHED2 in southern India is probably related to the prevalence of consanguineous marriages and marriages within a distinctive caste or community. Clinically, both forms of the disorder are similar; the distinction between them is made by the age at onset and the presence or absence of

associated symptoms. The autosomal recessive type (CHED2) is more severe, is present at birth, and is relatively stationary, with the only symptoms being defective vision with nystagmus and, at times, an associated hearing deficiency (Harboyan syndrome)^{3,4}; penetrating keratoplasty is required to restore the vision of these patients.⁵ The dominantly inherited form (CHED1) is of later onset³ (first or second year of life), is progressive, and is associated with not only defective vision but also troublesome tearing and photophobia.

The loci for CHED1 and CHED2 have been mapped to 20p11.2-q11.2⁶ and 20p13,⁷ respectively. We identified the candidate gene for CHED2 as Solute Carrier family 4 (sodium borate cotransporter) member 11 (*SLC4A11*).⁸⁻¹⁰ According to the National Center for Biotechnology Information UniGene expression profile, *SLC4A11* is expressed in several organs and tissues, including the eyes, blood, lungs, ovaries, colon, mouth, embryonic tissue, pancreas, kidneys, skin, cranial nerves, prostate, and brain. This gene has 19 exons encoding the bicarbonate transporter-related protein 1

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(BTR1) of 891 amino acids; BTR1 contains 14 transmembrane domains and intracellular amino and carboxyl terminals and also multiple intracellular phosphorylation sites and 2 extracellular *N*-glycosylation sites.¹¹ The BTR1 was recently characterized as a sodium borate cotransporter (NaBC1) essential for cell growth and proliferation by increasing intracellular borate and activating the mitogen-activated protein kinase pathway.¹² Vithana et al⁸ demonstrated that owing to *SLC4A11* mutations, the mutant BTR1 fails to reach the cell surface to perform its function, and they speculated that some of the morphologic features observed in CHED are achieved through a deregulated mitogen-activated protein kinase pathway. Other reports¹³⁻¹⁷ have also revealed the role of *SLC4A11* in causing CHED2. An association of *SLC4A11* with Harboyan syndrome (CHED2 with hearing loss) has also been reported.¹⁸

To improve our understanding of the involvement of *SLC4A11* in the pathogenesis and expressivity of CHED2 and to potentially refine the correlation of genotype-phenotype, we studied 20 Indian families with CHED2.

METHODS

CLINICAL EVALUATION OF CHED AND EXCLUSION CRITERIA

The patients and their families analyzed in this study underwent detailed ophthalmic evaluations, including slitlamp biomicroscopy, indirect ophthalmoscopy, and corneal pachymetry, by pediatric ophthalmologists and corneal specialists at the Cornea & External Disease Service of Aravind Eye Hospital. A clinical diagnosis of CHED was made, and other dystrophies, such as congenital hereditary stromal dystrophy, posterior polymorphous corneal dystrophy, and congenital glaucoma, were specifically excluded as follows. The CHED is characterized by bilaterally symmetrical noninflammatory corneal edema seen as diffuse ground glass-like blue-gray corneal opacification (cloudiness) present from birth in an otherwise healthy term newborn with an otherwise healthy eye. The DM is uniformly thickened with a gray peau d'orange texture. The corneal stroma is 2 to 3 times the normal thickness, resulting in gross corneal thickening. However, there is only fine epithelial edema and no bullae (hence, no tearing or photophobia). The horizontal corneal diameter is normal. The anterior and posterior segments are otherwise normal. The patients have diminution of vision and sensory (rotatory) nystagmus with or without esotropia. In addition, patients with congenital glaucoma have tearing, photophobia, inflammation, elevated intraocular pressure with glaucomatous optic nerve damage, and a progressive corneal enlargement with breaks in the DM (Haab striae).

Congenital hereditary stromal dystrophy is characterized by bilateral, symmetrical, nonprogressive flaky or feathery clouding of the superficial central corneal stroma that causes decreased vision and nystagmus. The differentiating features from CHED are normal corneal thickness, a normal endothelium with the absence of epithelial edema, and a clinically thickened DM. Posterior polymorphous corneal dystrophy is an asymptomatic, slowly progressive, bilateral endothelial dystrophy, usually with no visual loss. Slitlamp biomicroscopy shows vesicular, curvilinear, or placoid irregularities, with a gray halo at the level of the DM. There is no stromal edema, unlike in CHED, but there are other associated anterior segment abnormalities, such as peripheral anterior synechiae (iridocorneal adhesions), glaucoma, and band keratopathy.

PARTICIPANTS

Blood samples were collected from 26 affected individuals in 20 families with CHED2 and their available unaffected family members. Control subjects, matched to probands by ethnic background, were recruited from the General Ophthalmology Department of Aravind Eye Hospital. Informed consent was obtained from all the study participants. Ethical approval for the study was obtained from the institutional review board of Aravind Eye Hospital, and the study was performed in accordance with the tenets of the Declaration of Helsinki.

MUTATION SCREENING

Genomic DNA was isolated from peripheral blood using the salt precipitation method.¹⁹ For mutational analysis, all the coding exons of *SLC4A11* and their flanking splice junctions were amplified by means of polymerase chain reaction (PCR) using the primer reported elsewhere.⁸ The PCR products were purified using a gel extraction kit (Amersham Biosciences, Piscataway, New Jersey), and bidirectional sequencing was performed using an ABI 3100 sequencer (Applied Biosystems, Foster City, California). A primer pair positioned near the exon/intron boundaries of exon 6 could not amplify the expected PCR product in affected individuals in family 1. Suspicious of a large deletion, a new primer pair was designed farther away from exon 6 to amplify a 990-base pair (bp) product (F-5'CCAACCAACTGGGAGAAGA3' and R-5'GCACCAG-GCTTTAACTCAGC3').

SCREENING OF THE PROMOTER SEQUENCE

The families in which no mutation was identified in the exonic region were screened for *SLC4A11* mutations in the putative promoter. Because the *SLC4A11* promoter was uncharacterized, we predicted the promoter region using the Ensembl genome browser (<http://www.ensembl.org/index.html>) (ie, the region directly upstream of the 5'UTR of the transcript RefSeq [manually curated] complementary DNA, with GenBank NM_032034). Specific primers were designed to amplify the predicted promoter region, and the relevant PCR products were subjected to direct sequencing: P1F-5'GCCTTACTCAC-CCAATCTATGC3', P1R-5'CCCTGTCTCCTCCTTTC-GAC3', P2F-5'GGAGGAGGAGAAGGACTTGC3', and P2R-5'GCACACTCGCGCACTCAC3'.

RESTRICTION FRAGMENT LENGTH POLYMORPHISM ANALYSIS

The PCR products were restriction digested using 2 U of enzymes: *MspI* for p.P773L (Bangalore Genei, Bangalore, India); *SmaI* for p.C386R; *TauI* for p.R158PfsX3, p.R755W, and p.L873P; *PstI* for p.Q836X; and *PauI* for p.A269V in a 10- μ L setup and visualized on 2% agarose gel. All the enzymes (except *MspI*) were purchased from MBI Fermentas, Burlington, Ontario, Canada.

THE SIFT TOOL

To assess whether missense mutations were likely to have a phenotypic effect, we used the SIFT (Sorting Intolerant From Tolerant) tool (<http://blocks.fhrc.org/sift/SIFT.html>). This tool produces a multiple sequence alignment of *SLC4A* members from different species to assess the degree of evolutionary conservation of positions where the missense mutations lie. Then, the SIFT tool assigns a score to the mutations (if the score is <0.05, the change is potentially damaging).

Table 1. Clinical Information for Probands With the Mutation^a

| Family No./ Proband Age, y | Mutation Found | SIFT Tool Score ^b | Age at Onset | Surgery (PKP) | Age at Surgery, y | Other Clinical Signs | IOP, OD/OS, mm | Visual Acuity | | | | Nystagmus/ Consanguineous |
|----------------------------|--|------------------------------|--------------|---------------|-------------------|---|-----------------------|--|-------|---------------|-----|---------------------------|
| | | | | | | | | Before Surgery | | After Surgery | | |
| | | | | | | | | OD | OS | OD | OS | |
| 1/9 | c.654(-97)_c.778(-1488) del698nucleotides (C218KfsX49) | NA | Since birth | Yes | 7 | Spheroidal degeneration | 27/21 | 2/60 | 6/60 | 4/60 | -/+ | |
| 2/17 | c.2318C>T(P773L) | 0.17 | 3 y | No | NA | None | 21/23 | 6/18 | 6/12p | NA | NA | -/+ |
| 3/12 | c.473_480delGCTTCGCC c.481A>C (R158PfsX3) | NA | Since birth | No | NA | Microcysts with epithelial bullae | 22/18 | 1/60 | NA | NA | NA | +/+ |
| 4/5.5 | c.2618T>C (L873P) | 0.01 | Since birth | No | NA | Intermittent squint with 30° of exotropia | 28/25 | 3/60 | 2/60 | NA | NA | +/+ |
| 5/12 | c.2318C>T+ c.2506 C>T (P773L+ Q836X) | NA | Since birth | Yes (OS) | 11 | None | 18/20 | 4/60 | NA | 6/24 | NA | +/- |
| 6/10 | c.806C>T(A269V) | 0.04 | Since birth | No | NA | Pigments on lens anterior capsule | 15/12 | 6/36 | 6/60 | NA | NA | +/+ |
| 7/11 | c.478G>A(A160T) | 0.03 | Since birth | Yes | 7 | Corticosteroid-induced cataract | 15/21 | Finger counting | 6/12p | 6/36 | NA | +/+ |
| 8/36 | c.1156T>C (C386R) | 0.03 | 5 y | No | NA | Epithelial iron line | 12/10 | 6/36 | 6/18p | NA | NA | +/- |
| 13/7 | c.374G>A(R125H) | 0.00 | Since birth | Yes | 6 | None | Could not be recorded | 1/60 | NA | 6/36 | NA | +/+ |
| 14/28 | c.806C>T(A269V) | 0.04 | Since birth | Yes | 17 | Esotropia OS | Could not be recorded | 1/60 | 6/18 | 6/24 | NA | +/+ |
| 18/25 | c.2263C>T(R755W) | 0.00 | Since birth | No | NA | Secondary spheroidal degeneration | Could not be recorded | Hand movements; projection of light accurate | NA | NA | NA | +/+ |

Abbreviations: IOP, intraocular pressure; NA, not applicable; PKP, penetrating keratoplasty; SIFT, Sorting Intolerant From Tolerant; +, presence; -, absence.

^aProband identification is given according to the markings given in the pedigree.

^bA SIFT score less than 0.05 is predicted to be deleterious, whereas a SIFT score of 0.05 or greater is tolerated.

RESULTS

All index patients showed the typical CHED features described in the previous clinical evaluation section except that in families 2 and 8, the disease onset in the probands was at ages 3 and 5 years, respectively, and the probands of families 1 and 2 did not have nystagmus. None of the parents have CHED, proving an autosomal recessive inheritance. The clinical details of the probands with mutations are summarized in **Table 1**.

The present analysis of *SLC4A11* revealed potential changes in 11 of 20 probands screened. All the mutations cosegregated with the disease phenotype, and none were observed in 200 control chromosomes. Mutations were homozygous in the 9 consanguineous families (families 1-4, 6, 7, 13, 14, and 18) and in a nonconsanguineous family (family 8) (**Figure 1**). The unaffected parents of all the probands were heterozygous for the identified mutations, genetically confirming autosomal recessive inheritance of the disease. In family 8, the parents were not cooperative with the study.

Agarose gel electrophoresis of exon 6 PCR products in individuals in family 1 revealed the expected 990-bp product and an additional 292-bp fragment for unaffected individuals II:2 and III:1 and only a 292-bp

product for affected individuals IV:1 and IV:2. Sequencing of the 292-bp product eluted from the gel revealed a deletion of 698 nucleotides that encompassed exon 6 (**Figure 2**). Owing to this 698-bp homozygous deletion in agarose gel, affected individuals showed only the 292-bp product, whereas the unaffected parents showed the wild-type 990-bp product and the 292-bp mutated product, clearly stating their heterozygous nature.

In family 2, the proband carried a C>T transition at c.2318 that results in the substitution of p.Pro773Leu. In family 3, an Indel mutation (c.473_481delGCTTCGCCAinsC) was identified in exon 4. This mutation is predicted to truncate *SLC4A11* protein with the introduction of 3 novel amino acid residues, beginning from position 158 (p.Arg158ProfsX3). In family 4, a missense mutation (p.Leu873Pro) was identified owing to the transition of T>C at c.2618. Missense mutations p.Arg125His (c.374G>A), p.Ala160Thr (c.478G>A), p.Cys386Arg (c.1156T>C), and p.Arg755Trp (c.2263C>T) were identified in families 13, 7, 8, and 18, respectively. p.Ala269Val (c.806C>T) was found in families 6 and 14. In family 5, in which there was no consanguineous marriage, the proband proved to be a compound heterozygote for the change c.2318C>T in exon 17 (leading to p.Pro773Leu) and a premature stop mutation

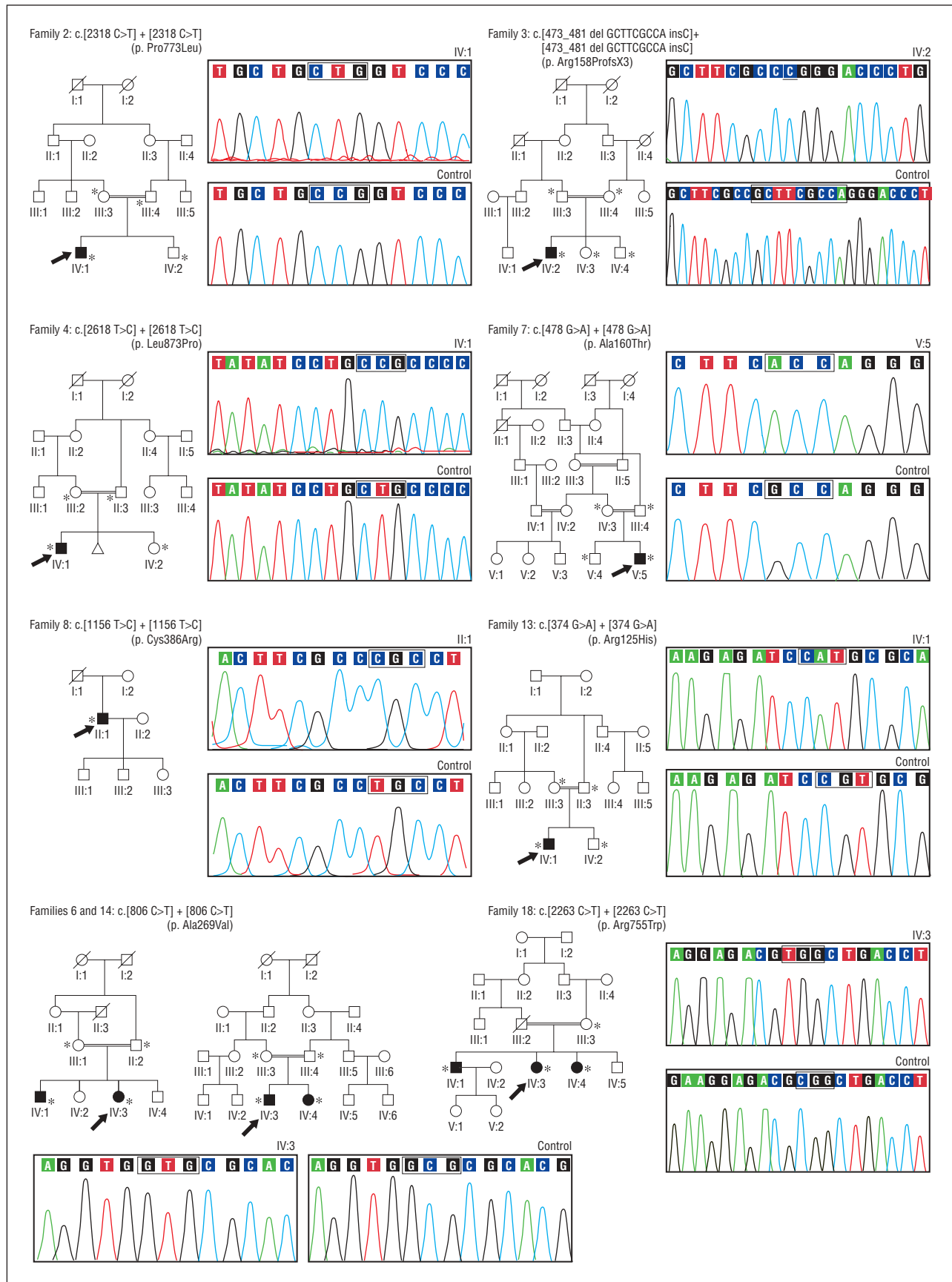
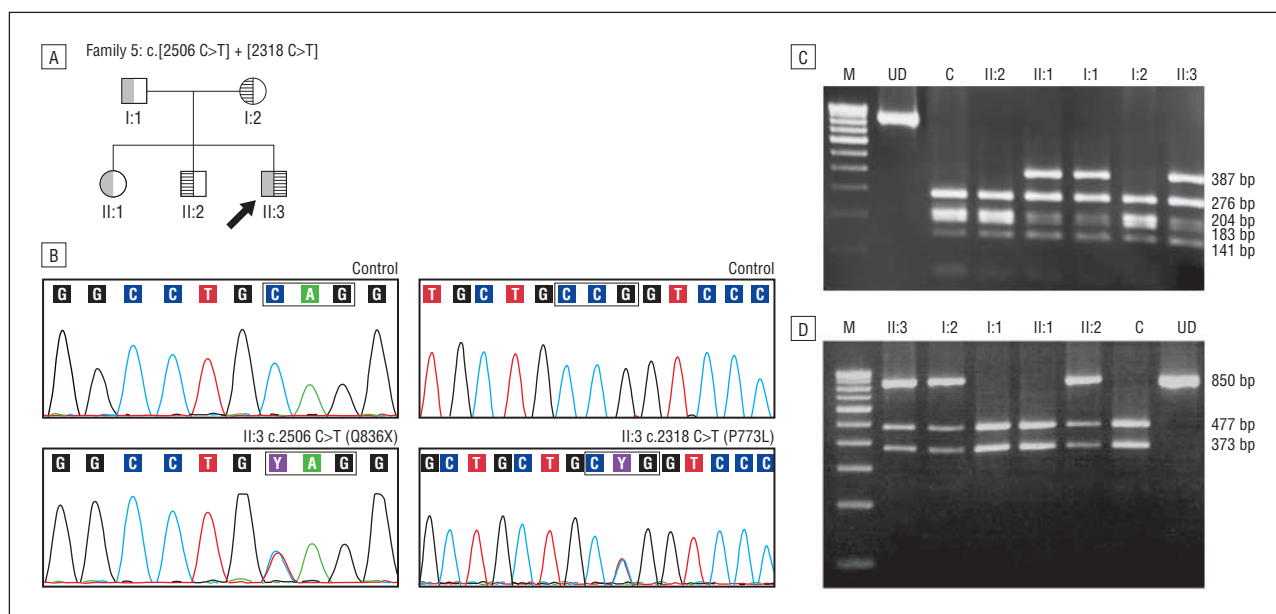
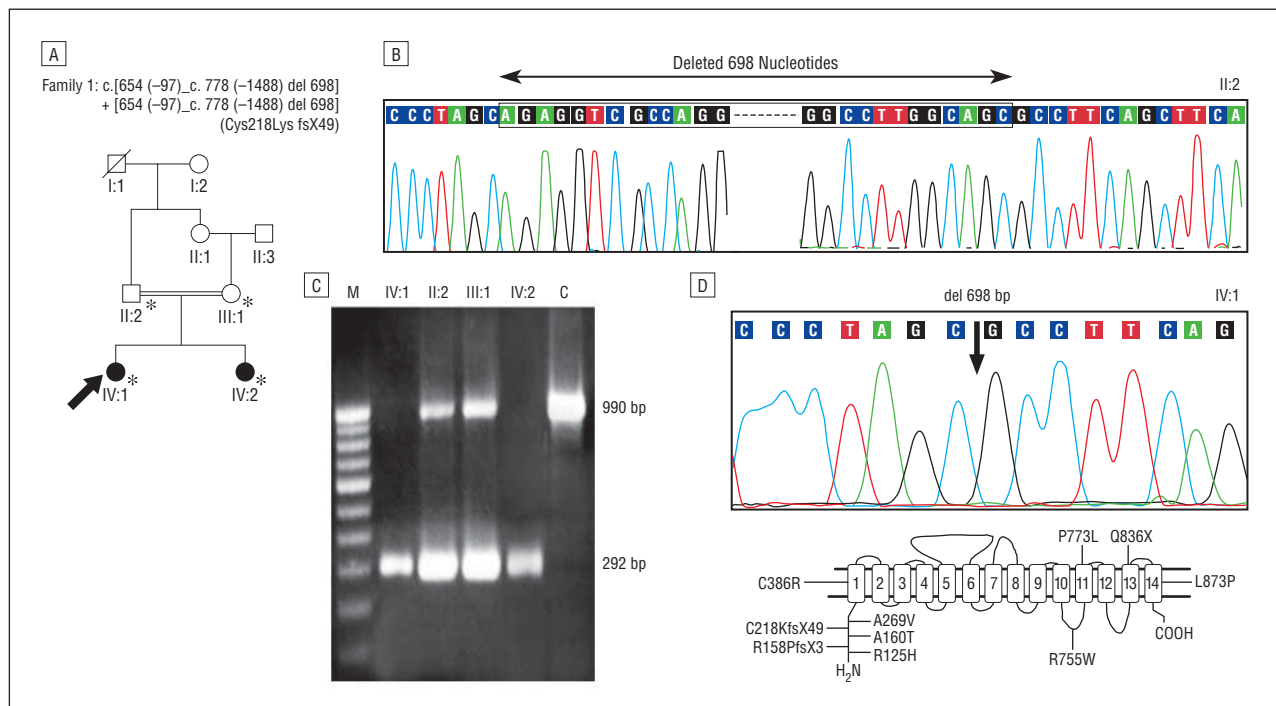


Figure 1. Mutation analysis by sequencing. Squares indicate males; circles, females; solid squares and circles, affected individuals; open squares and circles, unaffected individuals; arrow, proband; parallels, consanguinity; a slash through a square or circle, deceased individual; asterisk, available sample for analysis; and box in chromatogram, codon subjected to change.



(p.Gln836Stop) in exon 18 (c.2506C>T). The mutations were contributed by the healthy parents (**Figure 3**).

Silent variation–like (c.1659C>T) Asn553Asn was found in families 4 and 17, and (c.405G>A) Ala135Ala and (c.2499G>A) Thr833Thr were found in families 16

Table 2. Summary of *SLC4A11* Mutations Identified to Date

| Mutation ^a | Exon/ Intron | Type of Mutation | Effect on Protein | Ethnicity of Families | Source |
|--|-----------------|---------------------|--|---------------------------------|---|
| c.140delA (Y47SfsX69) | 2 | Deletion | Truncation of protein and addition of novel amino acids | 1 Indian family | Sultana et al, ¹⁶ 2007 |
| c.246_247delTTinsA (R82RfsX33) | 2 | Indel | Truncation of protein and addition of novel amino acids | 1 Indian family | Park et al, ¹² 2004 |
| c.306delc (G103VfsX13) ^b | 3 | Deletion | Truncation of protein and addition of novel amino acids | 1 Indian family | Sultana et al, ¹⁶ 2007 |
| c.334C>T (R112X) ^b | 3 | Nonsense | Truncation of protein | 3 Indian families | Sultana et al, ¹⁶ 2007 |
| c.353_356delAGAA (K118 TfsX11) | 4 | Deletion | Truncation of protein | 1 Indian family | Toma et al, ⁶ 1995 |
| c.374G>A(R125H) | 4 | Missense | May have an effect on N-terminal cytoplasmic domain | 1 Indian family | Present study |
| c.427G>A(E143K) | 4 | Missense | May have an effect on N-terminal cytoplasmic domain | 1 Indian family | Parker et al, ¹¹ 2001 |
| c.473_481delGCTTCGCCAinsC (R158PfsX3) | 4 | Indel | Truncation of protein and addition of novel amino acids; absence of all TMDs | 1 Indian family | Present study |
| c.473_480del8 bp (R158QfsX4) | 4 | Deletion | Truncation of protein and addition of novel amino acids | 2 Indian families | Sultana et al, ¹⁶ 2007 |
| c.478G>A(A160T) | 4 | Missense | May have an effect on N-terminal cytoplasmic domain | 2 Indian families | Present study; Park et al, ¹² 2004 |
| c.618_619delAG (V208AfsX38) | 5 | Deletion | Truncation of protein and addition of novel amino acids | 2 Indian families | Sultana et al, ¹⁶ 2007 |
| c.625C>T (R209W) | 5 | Missense | May have an effect on N-terminal cytoplasmic domain | 2 Indian families | Sultana et al, ¹⁶ 2007 |
| c.638C>T (S213L) | 5 | Missense | May have an effect on N-terminal cytoplasmic domain | 1 Indian family | Sultana et al, ¹⁶ 2007 |
| c.654 (-97)_c.778 (-1488)del698 (C218KfsX49) | 5-6 | Deletion | Truncation of protein and addition of novel amino acids; absence of all TMDs | 1 Indian family | Present study |
| c.695G>A (S232N) ^b | 6 | Missense | May have an effect on N-terminal cytoplasmic domain | 1 US family of Chinese ancestry | Jiao et al, ¹⁴ 2007 |
| c.697C>T (R233C) | 6 | Missense | May have an effect on N-terminal cytoplasmic domain | 1 Indian family | Sultana et al, ¹⁶ 2007 |
| c.720G>A (W240X) | 6 | Nonsense | Truncation of protein | 1 British family | Parker et al, ¹¹ 2001 |
| c.806C>T(A269V) | 7 | Missense | May have an effect on N-terminal cytoplasmic domain | 2 Indian families | Present study |
| c.859_862delGAGAGinsCCT (E287PfsX21) | 7 | Indel | Truncation of protein and addition of novel amino acids; absence of all TMDs | 1 Indian family | Ramprasad et al, ¹³ 2007 |
| c.878_889del12 (E293_E296del) | 7 | Deletion | May have an effect on N-terminal cytoplasmic domain | 1 Indian family | Sultana et al, ¹⁶ 2007 |
| c.985A>T (R329X) ^b | 7 | Nonsense | May have an effect on N-terminal cytoplasmic domain | 1 US family of Chinese ancestry | Jiao et al, ¹⁴ 2007 |

(continued)

and 5. In the 9 families in which no mutation was identified in the coding region, no significant changes in the promoter region of *SLC4A11* were detected.

COMMENT

Screening of 20 families with CHED2 revealed 10 mutations. Of the mutations identified, 7 were missense, 1 was nonsense, and 2 led to frameshifts. The relative po-

sitions of the mutations identified in this study are shown in comparison with recently reported mutations in *SLC4A11* (**Table 2**). This work extends considerably the number of *SLC4A11* mutations shown to cause CHED2. The mutations observed in these cases consolidate the notion that CHED2 seems to arise from apparent null alleles or from alleles causing severe loss of function.

Of the 7 missense mutations identified, 4 had already been reported^{13,14,16} and 3 were novel. Missense mutations

Table 2. Summary of *SLC4A11* Mutations Identified to Date (cont)

| Mutation ^a | Exon/ Intron | Type of Mutation | Effect on Protein | Ethnicity of Families | Source |
|---|-----------------|---------------------|--|---|---|
| c.996 + 26C_+44Cdel19 | 7 | Deletion | Not known | 2 Indian families | Sultana et al, ¹⁶ 2007 |
| c.1091- 1G>C | 8 | Point mutation | Not known | 1 Indian family | Sultana et al, ¹⁶ 2007 |
| c.1156T>C (C386R) | 9 | Missense | Disruption of TMD 1 | 2 Indian families | Present study; Parker et al, ¹¹ 2001 |
| c.1202C>A (T401L) ^b | 9 | Missense | Not known | 1 Indian family | Sultana et al, ¹⁶ 2007 |
| c.1253G>A (G418D) | 10 | Missense | Disruption of TMD 2 | 1 Indian family | Sultana et al, ¹⁶ 2007 |
| c.1317_1322del6ins8 (L440VfsX6) | 10 | Indel | Truncation of protein and addition of novel amino acids | 1 Indian family | Sultana et al, ¹⁶ 2007 |
| c.1391G>A (G464D) | 11 | Missense | Conformation change | 3 Pakistani families | Toma et al, ⁶ 1995 |
| c.1418T>G (L473R) ^b | 11 | Missense | Disruption of TMD 4 | 1 Indian family | Sultana et al, ¹⁶ 2007 |
| c.1466C>T (S489L) | 12 | Missense | Conformation change | 1 Pakistani family and 1 Indian family | Toma et al, ⁶ 1995; and Sultana et al, ¹⁶ 2007 |
| c.1704_1705delCT (H568HfsX177) | 13 | Deletion | Truncation of protein and addition of novel amino acids | 1 Indian family | Park et al, ¹² 2004 |
| c.1751C>A (T584K) ^b | 13 | Missense | Disruption of TMD 6 | 2 Indian families | Sultana et al, ¹⁶ 2007 |
| c.1813C>T (R605X) ^a | 14 | Nonsense | Truncation of protein | 6 Indian families | Park et al, ¹² 2004; Toma et al, ⁶ 1995; and Sultana et al, ¹⁶ 2007 |
| c.1894G>T (E632X) | 14 | Nonsense | Truncation of protein | 2 Indian families | Park et al, ¹² 2004; and Sultana et al, ¹⁶ 2007 |
| c.2067-6_-16delins GGCCGGCCGG | 15 | Indel | Inactivation of an acceptor splice site | 1 Indian family | Toma et al, ⁶ 1995 |
| c.2014_2016delITTC or c.2017_2019delITTC (F672del or F673del) | 15 | In-frame deletion | Disruption of TMD 8 | 1 Indian family | Ramprasad et al, ¹³ 2007 |
| c.2240 + 1G>A ^b | 16 | Point mutation | Inactivation of splice donor site | 1 British family | Parker et al, ¹¹ 2001 |
| c.2263C>T (R755W) | 17 | Missense | Disruption of TMD 11 | 3 Indian families | Present study; Parker et al, ¹¹ 2001; and Sultana et al, ¹⁶ 2007 |
| c.2264G>A (R755Q) ^b | 17 | Missense | Conformation change | 4 Indian families and 1 Myanmar family | Toma et al, ⁶ 1995; Park et al, ¹² 2004; Parker et al, ¹¹ 2001; and Sultana et al, ¹⁶ 2007 |
| c.2318C>T (P773L) ^b | 17 | Missense | Disruption of TMD 11 | 3 Indian families | Present study and Sultana et al, ¹⁶ 2007 |
| c.2398C>T (Q800X) ^b | 17 | Nonsense | Truncation of protein | 1 British family | Parker et al, ¹¹ 2001 |
| c.2389_2391delGAT (D797del) | 17 | Deletion | Disruption of TMD 12 | 1 Indian family | Sultana et al, ¹⁶ 2007 |
| c.2407 C>T (Q803X) | 17 | Nonsense | Truncation of protein | 1 Indian family | Sultana et al, ¹⁶ 2007 |
| c.2411G>A (R804H) | 17 | Missense | Conformation change | 1 Indian family | Park et al, ¹² 2004 |
| c.2420delTinsGG (L807RfsX71) | 17 | Indel | Truncation of protein and addition of novel amino acids | 1 Indian family | Park et al, ¹² 2004 |
| c.2437-1G>A ^b | 17 | Point mutation | Inactivation of splice acceptor site | 1 British family | Parker et al, ¹¹ 2001 |
| c.2470G>A (V824M) | 18 | Missense | Not known | 2 Indian families | Sultana et al, ¹⁶ 2007 |
| c.2498C>T (T833M) | 18 | Missense | Conformation change | 2 Indian families | Park et al, ¹² 2004 |
| c.2506 C>T (Q836X) ^b | 18 | Nonsense | Truncation of protein | 1 Indian family | Present study |
| c.2605C>T (R869C) | 18 | Missense | Conformation change | 3 Indian families and 1 Middle Eastern family | Toma et al, ⁶ 1995; Parker et al, ¹¹ 2001; and Sultana et al, ¹⁶ 2007 |
| c.2606G>A (R869H) | 18 | Missense | Conformation change | 2 Indian families | Park et al, ¹² 2004 |
| c.2618T>C (L873P) | 19 | Missense | Disruption of TMD 14 | 1 Indian family | Present study |
| c.2623C>T (R875X) ^b | 19 | Nonsense | Truncation of protein | 1 Indian family | Sultana et al, ¹⁶ 2007 |

Abbreviation: TMD, transmembrane domain.

^aThe numbering is based on the complementary DNA sequence, with +1 corresponding to the A of the ATG translation initiation codon.

^bCompound heterozygous changes.

| | R125H |
|--------------|---|
| BTR1-HUMAN | I R A H R D L D G F L A Q A S I V L N E T A T S L |
| BTR1-MOUSE | V R A H R D L D G F L A Q A S I I L N E T A T S L |
| BTR1-DOG | I R A H R D L D G F L A R A S I I L N E T A T S L |
| BTR1-Xenopus | L H S L F E L R S C I L N G T I M L D M R A N T I |
| BTR1-CHICK | V R A H R D L D G F L A R A S I I L D E T A T S L |
| SLC4A7/NBCn1 | L H S L F E L R S C I L N G T V M L D M R A S T L |
| SLC4A8/NDCBE | L H S L F E L R S C L I N G T V L L D M H A N S I |
| SLC4A9/AE4 | L P S L Q K L R S L L A E G L V L L D C P A Q S L |
| SLC4A4/NBC1 | L H S L F E L R T C M E K G S I M L D R E A S S L |
| SLC4A1/AE1 | F W S L L E L R R V F T K G T V L L D L Q E T S L |

| | A269V |
|--------------|---|
| BTR1-HUMAN | M E V A R T F A T M F S D I A F R Q K L L E T R T |
| BTR1-MOUSE | M E V A R T F A T M F S D I T F R Q K L L K T R T |
| BTR1-DOG | T E V G R T F A T M F L D I T F R Q K L L N T R T |
| BTR1-Xenopus | H E I G R S I A T L M T D E I F H D V A Y K A K D |
| BTR1-CHICK | T E V G R T F A T M F S D I T F R Q K L L E T K T |
| SLC4A7/NBCn1 | H E I G R S I A T L M T D E I F H D V A Y K A K D |
| SLC4A8/NDCBE | H E I G R S M A T I M T D E I F H D V A Y K A K E |
| SLC4A9/AE4 | H E M G R A A A V L L S D P Q F Q W S V R R A S N |
| SLC4A4/NBC1 | H E I G R A I A T L M S D E V F H D I A Y K A K D |
| SLC4A1/AE1 | T Q L G R A A A T L M S E R V F R I D A Y M A Q S |

| | L873P |
|--------------|---|
| BTR1-HUMAN | S S L P Y M K M I F P L I M I A M I P I R Y I L L |
| BTR1-MOUSE | S S L P Y M K M V F P L I M I A M I P I R Y N L L |
| BTR1-DOG | S T L P Y M K M I F P L I M I A M I P I R Y N L L |
| BTR1-Xenopus | S --- S A A V V F P M M V L A L V F I R - K L L |
| BTR1-CHICK | S P L P Y M K M I F P L I M I G M I P I R --- |
| SLC4A7/NBCn1 | S --- A A A V V F P M M V L A L V F V R - K L M |
| SLC4A8/NDCBE | S --- P A A I V F P M M V L A L V F V R - K V M |
| SLC4A9/AE4 | T --- P A A I I F P L M L L G L V G V R - K A L |
| SLC4A4/NBC1 | T --- V A A I I F P V M I L A L V A V R - K G M |
| SLC4A1/AE1 | T --- P A S L A L P F V L I L T V P L R R V L L |

Figure 4. Multiple alignment of the amino acid sequence of *SLC4A11* with different species and 5 other transporters from humans. The box covers up the position of mutated residue in orthologs and paralogs.

were preponderant in the present study (72.72%) and in studies by Ramprasad et al¹³ (62.5%), Jiao et al¹⁴ (50%), and Sultana et al¹⁶ (69.23%). Conservation of the amino acid residues involved in the novel missense mutations is shown in **Figure 4**. The novel missense mutation p.Ala269Val lies in an unconserved site, its SIFT score is less than 0.05 and its Blosom 80 score is -1, and it is absent in 200 control chromosomes, which suggests that this substitution could be a pathogenic mutation.

A schematic diagram of the topology of BTR1 with its predicted transmembrane spans is shown in Figure 2D. Residues Arg125, Ala160, and Ala269 are located in the N-terminal cytoplasmic domain. Residues Cys386 and Leu873 are situated in transmembrane domains 1 and 14, respectively, whereas Pro773 and Arg755 residues are present in transmembrane domain 11. Mutations in these 7 residues may disrupt the localization or proper assembly of this protein in the membrane.

Two deletion mutations identified are predicted to result in null alleles as a result of a frameshift. We report the first deletion of an entire exon in *SLC4A11*: in family 1, the deletion of exon 6 leads to p.Cys218LysfsX49. The deletion mutation c.473_481delGCTTCGCCAinsC leads to premature protein termination (R158PfsX3) in CHED2 family 3 (member IV:2). Both of these deletions were predicted to result in a protein that lacks all 14 transmembrane domains. The R158PfsX3 mutation seems to have presented with different phenotypes. Desir et al¹⁸ reported this change in association with Harboyan syndrome (CHED2 with hearing loss), whereas the change resulted only in an isolated

case of CHED2 in this study. Hearing loss was diagnosed at age 5 years in the study by Desir et al, whereas in the present study, the 12-year-old individual does not show any symptoms of hearing loss to date; it may occur later in life and deserves follow-up.⁴

In both frameshift mutations (p.Cys218LysfsX49 and p.Arg158PfsX3) and 1 nonsense mutation (p.Gln836Stop), the premature termination codons occur more than 50 nucleotides upstream of the final splice junction of *SLC4A11*, making these messenger RNAs potential targets for nonsense-mediated decay.²⁰

The compound heterozygosity observed in family 5 confirms the allele dose effect: 2 copies of p.Pro773Leu lead to disease in family 2 (member IV:1), and 1 copy of p.Pro773Leu combined with 1 copy of p.Gln836Stop results in the disease in family 5. Recently, compound heterozygosity in *SLC4A11* has been reported as the cause of CHED2 in 2 white British families and 1 US family of Chinese ancestry.^{13,17}

The earlier report⁸ of the effect of CHED2 causing *SLC4A11* mutations on BTR1 protein states that the mutant BTR1 proteins fail to modify into the mature size (120 kDa), could not reach the cell surface, and are consequently degraded. In the same way, the mutations identified in this study may lead to lack of the mutant proteins in the membrane and could cause the disease phenotype.

The 6 novel mutations reported herein, together with the previously reported mutations by other groups,^{8,13-17} bring the total number of reported *SLC4A11* mutations associated with CHED2 to 52. Screening for *SLC4A11* mutations is essential for identifying individuals at risk for transmitting the disease. The development of strategies that are noninvasive, rapid, and cost-effective will be useful for screening populations with a high incidence of this disease, especially in populations that practice consanguineous marriages. Counseling must take into account the merits and demerits of consanguineous marriages in an attempt to reduce the incidence of this disease.

Of the 20 families studied, we detected pathogenic mutations in only 11; the remaining 9 families did not show any changes in the coding region of *SLC4A11*. In addition, screening of the promoter region did not reveal any significant changes in these families. Jiao et al¹⁴ also did not identify *SLC4A11* changes in 4 of 16 families screened. A plausible explanation could be that the phenotype is caused by another gene or genes or by the genes that interact with *SLC4A11*, such as a transcription factor; overall, CHED2 displays allele (perhaps locus) heterogeneity, which can be explored by means of extensive linkage analysis.

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REFERENCES

1. Maumenee AE. Congenital hereditary corneal dystrophy. *Am J Ophthalmol.* 1960; 50:1114-1124.
2. Ehlers N, Mødis L, Møller-Pedersen T. A morphological and functional study of congenital hereditary endothelial dystrophy. *Acta Ophthalmol Scand.* 1998; 76(3):314-318.
3. Judisch GF, Maumenee IH. Clinical differentiation of recessive congenital hereditary endothelial dystrophy and dominant hereditary endothelial dystrophy. *Am J Ophthalmol.* 1978;85(5, pt 1):606-612.
4. Harboyan G, Mamo J, der Kaloustian V, Karam F. Congenital corneal dystrophy: progressive sensorineural deafness in a family. *Arch Ophthalmol.* 1971;85(1):27-32.
5. Thompson RW Jr, Price MO, Bowers PJ, Price FW Jr. Long-term graft survival after penetrating keratoplasty. *Ophthalmology.* 2003;110(7):1396-1402.
6. 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(12):2395-2398.
7. 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):1-4.
8. Vithana EN, Morgan P, Sundaresan P, et al. Mutations in sodium-borate co-transporter *SLC4A11* cause recessive congenital hereditary endothelial dystrophy, CHED2. *Nat Genet.* 2006;38(7):755-757.
9. Alper SL, Darman RB, Chernova MN, Dahl NK. The AE gene family of Cl/HCO₃-exchangers. *J Nephrol.* 2002;15(suppl 5):S41-S53.
10. Romero MF, Fulton CM, Boron WF. The SLC4 family of HCO₃ – transporters. *Pflugers Arch.* 2004;447(5):495-509.
11. Parker MD, Ourmozdi EP, Tanner MJ. Human BTR1, a new bicarbonate transporter superfamily member and human AE4 from kidney. *Biochem Biophys Res Commun.* 2001;282(5):1103-1109.
12. 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(3):331-341.
13. Ramprasad VL, Ebenezer ND, Aung T, et al. Novel SLC4A11 mutations in patients with recessive congenital hereditary endothelial dystrophy (CHED2). *Hum Mutat.* 2007;28(5):522-523.
14. Jiao X, Sultana A, Garg P, et al. Autosomal recessive corneal endothelial dystrophy (CHED2) is associated with mutations in *SLC4A11*. *J Med Genet.* 2007; 44(1):64-68.
15. Kumar A, Bhattacharjee S, Prakash DR, Sadanand CS. Genetic analysis of two Indian families affected with congenital hereditary endothelial dystrophy: two novel mutations in SLC4A11. *Mol Vis.* 2007;13:39-46.
16. 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;13:1327-1332.
17. Aldave AJ, Yellore VS, Bourla N, et al. Autosomal recessive CHED associated with novel compound heterozygous mutations in *SLC4A11*. *Cornea.* 2007;26(7): 896-900.
18. Desir J, Moya G, Reish O, et al. Borate transporter *SLC4A11* mutations cause both Harboyan syndrome and non-syndromic corneal endothelial dystrophy. *J Med Genet.* 2007;44(5):322-326.
19. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* 1988;16(3):1215.
20. Wilusz CJ, Wormington M, Peltz SW. The cap-to-tail guide to mRNA turnover. *Nat Rev Mol Cell Biol.* 2001;2(4):237-246.

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