Sponsor-Investigator: Anthony J. Aldave, MD

Recombinant Adeno-Associated Virus 8 Human Solute Carrier Family 4 Member 11 (AAV8-hSLC4A11) for Congenital hereditary endothelial dystrophy (CHED)

Rare Pediatric Disease Designation Request

June 19, 2024

- CONFIDENTIAL –

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LIST OF ABBREVIATIONS

bGH	Bovine Growth Hormone
BSS	Balanced Salt Solution
CAG	Chicken β-actin
CCO	Congenital Corneal Opacification
CCT	Central Corneal Thickness
cDNA	Complementary DNA
CD	Cell Density
CDS	Corneal Densitometry Score
CFR	Code of Federal Regulations
CMV	Cytomegalovirus
CHED	Congenital Hereditary Endothelial Dystrophy
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
DM	Descemet membrane
EF1$\boldsymbol{\alpha}$	Eukaryotic Translation Elongation Factor 1 Alpha 1
FDA	U.S. Food And Drug Administration
GFP	Green Fluorescent Protein
GSU	Greyscale Unit
HCEnC-21T	Immortalized Human Corneal Endothelial Cell
HEK293	Human Embryonic Kidney 293 Cells
hSLC4A11	Human Solute Carrier Family 4 Member 11
ITR	Inverted Terminal Repeat
KO mice	Knock-Out mice
Na+/K+-ATPase	Sodium/Potassium-Adenosine Triphosphatase
LI	Laser Interferometer
LogMAR	Logarithm of the Minimum Angle of Resolution
LV	Lentivirus
mRNA	Messenger Ribonucleic Acid
OMIM	Online Mendelian Inheritance In Man
ORPHA	Orphanet nomenclature of rare diseases
pA	Poly Adenylation
rAAV	Recombinant Adeno-Associated Virus
tBH	Tert-butyl hydroperoxide
UCLA	University Of California, Los Angeles
US	United States
VA	Visual Acuity
vg	Vector Genome

1 RARE PEDIATRIC DISEASE DESIGNATION REQUEST STATEMENT

Pursuant to 21 CFR 316.20, the sponsor-investigator Anthony J. Aldave, MD requests designation for the Recombinant Adeno-Associated Virus 8 Human Solute Carrier Family 4 Member 11 (AAV8-hSLC4A11) as a rare pediatric drug product for treatment of patients with Congenital hereditary endothelial dystrophy (CHED) associated with biallelic mutations in *SLC4A11*.

CHED is an autosomal recessive condition affecting the corneal endothelial cells, which function to maintain corneal clarity. CHED is characterized by bilateral corneal edema and opacification presenting at birth or in the first decade of life. Symptoms consist of bilateral visual impairment, which is typically significant, and secondary depravation amblyopia and nystagmus. The diffuse corneal edema may be associated with increased corneal thickness up to twice normal, although the remainder of the ocular structures typically develop normally.

Approximately 80% of individuals with CHED screened to date demonstrate biallelic coding region mutations in the solute carrier family 4 member 11 gene (*SLC4A11*). To date, more than 90 distinct mutations throughout exons 2 to 19 of *SLC4A11* have been identified in individuals with CHED, including missense and nonsense point mutations, insertion or deletion-associated frameshift mutations, as well as canonical splice-site mutations.

CHED is a rare disease in the United States, where neither the birth incidence nor the population prevalence has been reported, and is an uncommon domestic indication for corneal transplantation. However, CHED is one of the most frequently encountered corneal dystrophies in countries where consanguineous marriages are more common (Middle East, South Asia and Southeast Asia), and is a well-recognized cause of congenital corneal opacification (CCO) due to the presence of corneal edema at birth. The incidence of CCO in the United States is estimated to be approximately 2.2 per 100,000 live births.[1] A determination of the percentage of the cases of CCO that are due to CHED can be performed by reviewing the published series of the causes of CCO in the US. The largest series include between 14 and 56 infants. In none of these series was CHED identified as the case of CCO in an infant. However, the percentage of eyes in each of these series in which the cause of the CCO was idiopathic (0%, 4%, 10%) or was not specified (33%) was provided. Therefore, if one assumes that all of the infants with idiopathic CCO or in whom the cause was not specified had CHED, the birth incidence can be calculated by multiplying the incidence of CCO per live births in the US by the lowest and highest percentages of cases of CCO attributable to CHED in these series by the US population in the 2021 census: (2.2/100,000) x 0.04 x 330,218,929 to (2.2/100,000) x 0.33 x 330,218,929 = 291 to 2312. Given an average life expectancy of 76.4 years for both sexes in the US, the estimated number of individuals with CHED in the US would then be 22,232 – 176,637, below the 200,000 prevalence threshold to qualify for Orphan Disease designation. Therefore, we request that AAV8-hSLC4A11 be designated as an orphan drug product for the treatment of patients with Congenital hereditary endothelial dystrophy (CHED).

2 ADMINISTRATIVE INFORMATION

2.1 Sponsor and Principal Investigator

[redacted]
The Stein Eye Institute
Department of Ophthalmology, David Geffen School of Medicine, University of California, Los Angeles
200 Stein Plaza, UCLA
Los Angeles, CA 90095-7003

2.2 Contacts

Regulatory Contact

[redacted]

Sponsor Contact

[redacted]

2.3 Drug Name

2.3.1 Chemical Name - Drug Substance

Recombinant Adeno-Associated Virus 8 Human Solute Carrier Family 4 Member 11 (AAV8-hSLC4A11)

2.3.2 Generic/Trade Name - Drug Product

rAAV8-EF1α-hSLC4A11 is a non-replicating, rep/cap-deleted, rAAV vector containing wild type human single stranded cDNA encoding Solute Carrier Family 4 Member 11 (*SLC4A11*) variant B protein. Details describing the drug product are found in Section 4.1.

2.4 Manufacturer's Name and Address

2.4.1 Drug Substance Manufacturer

Andelyn Biosciences
1180 Arthur E. Adams Dr.
Columbus, OH 43221
FEI number 3026042970

2.4.2 Drug Product Manufacturer

Andelyn Biosciences
1180 Arthur E. Adams Dr.
Columbus, OH 43221
FEI number 3026042970

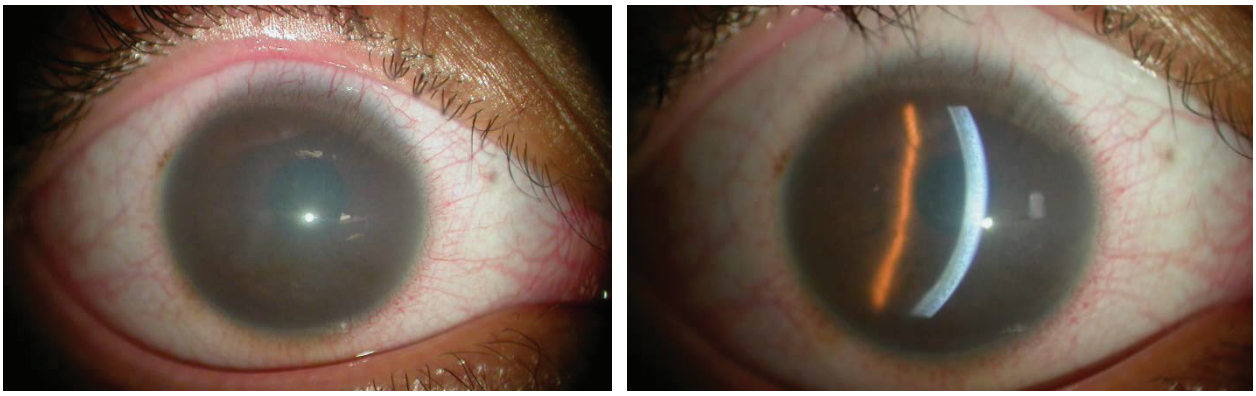
3 DESCRIPTION OF RARE DISEASE OR CONDITION, PROPOSED INDICATION, AND NEED FOR THERAPY

3.1 Description of Rare Disease or Condition

Clinical Manifestations

Congenital Hereditary Endothelial Dystrophy (CHED, OMIM# 217700, ORPHAcode 293603) is an autosomal recessive condition affecting the corneal endothelial cells, which function to maintain corneal clarity. CHED is characterized by bilateral corneal edema and opacification presenting at birth or in the first decade of life **(Figure 1)**. Symptoms consist of bilateral visual impairment, which is typically significant, with affected individuals commonly developing secondary deprivation amblyopia and nystagmus. The diffuse corneal edema is typically severe at the time of diagnosis and may result in an increased corneal thickness up to twice normal, although the remainder of the ocular structures typically develop normally.[2] A subset of affected individuals will develop a progressive sensorineural hearing loss (Harboyan syndrome, OMIM# 217400) that typically presents in the first two decades of life.[3-5] At present, there is no effective pharmaceutical treatment for CHED and thus corneal transplantation is the only treatment option for restoration of vision in infants and children with CHED.

Figure 1. Clinical presentation of Congenital hereditary endothelial dystrophy (CHED). Slit-lamp



images of 16-year-old-boy with CHED associated with compound heterozygous *SLC4A11* mutations, demonstrating diffuse corneal stromal edema seen on direct and slit illumination.

Molecular Etiology

CHED is associated with mutations in the solute carrier family 4 member 11 gene (*SLC4A11*). To date, more than 90 distinct mutations throughout exons 2 to 19 of *SLC4A11* have been identified in individuals with CHED, including missense and nonsense point mutations, insertion or deletion-associated frameshift mutations, as well as canonical splice-site mutations.[3,6-19] Approximately 80% of individuals with CHED screened to date demonstrate biallelic coding region mutations in *SLC4A11*. In CHED cases without *SLC4A11* coding region mutations, an intronic *SLC4A11* mutation was reported to result in aberrant pre-mRNA splicing and functionally null allele, whereas no pathogenic variants have been identified in the *SLC4A11* promoter region thus far.[12,15,20]

Pathophysiology

SLC4A11 is one of the highly expressed differentiation markers for corneal endothelium and is essential in facilitating energy producing glutaminolysis by maintaining ammonia homeostasis, reducing

glutaminolysis-associated oxidative stress, maintaining antioxidant signaling and preventing apoptosis in the corneal endothelium.[21-28] The progressive corneal stromal edema observed in CHED is evidence of corneal endothelial dysfunction, either from cell loss or dysfunction of the corneal endothelial "pump-leak" system.[29-31] The endothelial pump activity is driven by an ionic electrical-chemical gradient set up by the highly expressed Na+/K+-ATPase.[32] As such, corneal endothelial cells have the second highest density of mitochondria among any cell types in the body (second to photoreceptors), necessary to generate sufficient ATP to fuel the Na+/K+-ATPase driven endothelial pump.[33] Glucose and glutamine each contribute about half of the energy needed to fuel the corneal endothelial "pump" activity.[34] Multiple lines of evidence support dysfunction of the corneal endothelial "pump" activity secondary to loss of SLC4A11 function as the direct cause of corneal edema in CHED.[20,33,35-39]

Natural History

To determine the natural history of CHED, we collected demographic and clinical data on 166 individuals (age range: 4 months – 60 years old) with CHED, including 62 published cases (42 male, 20 female) and 104 unpublished cases (57 male, 47 female).[15,35,40-47] Visual acuity was measured on Snellen visual acuity chart or age-appropriate Cardiff chart for young children. If preoperative VA of both eyes was available at a given age, only the VA of the better-seeing eye was included.

Progressive corneal edema (measured by an increase in CCT) and loss of vision (measured by a decrease in visual acuity) with increasing age were evident in individuals with CHED (**Figure 2A, D**). In 180 eyes with available preoperative corrected visual acuity (VA), the median VA was 20/400 (1.30 logMAR) (95% confidence interval (95% CI) of 20/317 – 20/604 (1.2 – 1.48 logMAR)), with 73.3% eyes reaching the threshold of legal blindness (**Figure 2A**). Preoperative laser interferometer (LI) visual acuity testing performed to assess the visual acuity potential after optical correction and vision therapy demonstrated the potential for visual recovery in 31 of 33 eyes with a mean potential VA improvement of 0.80 logMAR unit (paired t test, $p < 0.0001$) (**Figure 2B**). Similarly, preoperative pin hole visual acuity testing performed to assess visual acuity potential after optical correction revealed the potential for visual recovery in 13 of 14 eyes with a mean potential VA improvement of 0.26 logMAR unit (paired t test, $p < 0.0001$) (**Figure 2C**). These data suggest that individuals with CHED have the potential for significant recovery of vision if corneal edema is corrected with or without subsequent vision therapy for amblyopia.

CCT in individuals with CHED can be fit against age with a one-phase association curve, if it is assumed that corneas in CHED will reach maximum thickness with zero swelling pressure beyond a certain age (**Figure 2D, E**). Our data of 301 eyes in individuals with CHED with preoperative CCT data demonstrate an increase in CCT between birth and 3 years of age, with a stabilization of CCT after 3 years of age (**Figure 2E**). In addition to increased CCT, the corneas from children affected with CHED demonstrate decreased endothelial cell density **(Figure 2F)** and exhibit significantly increased mean corneal densitometry (37.2 $\pm$ 1.5 grayscale units (GSU)) compared with controls (15.0 $\pm$ 1.9 GSU, $p < 0.0001$) **(Figure 2G)**.

The chronic corneal stromal edema in CHED leads to irreversible stromal architectural changes that limit the degree of corneal clarity following surgical intervention to replace the dysfunctional host endothelium with healthy donor endothelium (endothelial keratoplasty). Therefore, the decision of whether to perform a full thickness replacement of the cornea (penetrating keratoplasty) or endothelial keratoplasty is dependent on the severity and chronicity of the corneal edema and the presence of stromal scarring.[48] In published case series of CHED, the mean age at the time of keratoplasty is reported to be between 8.1 – 13.5 years of age (excluding two case series reporting 42.7 and 3.3 years of age as the mean age at the time of keratoplasty).[49-55] The first of the two series was published in 1969 and described early experiences of keratoplasty in individuals with CHED, while the second was a case series that specifically described keratoplasty outcomes in young children.[54,55] Our unpublished data from 157 eyes that underwent corneal transplantation showed a median age at the time of initial keratoplasty of 9.5 years of age (95% CI, 8.3 – 10.6), similar to published series (**Figure 2H**).

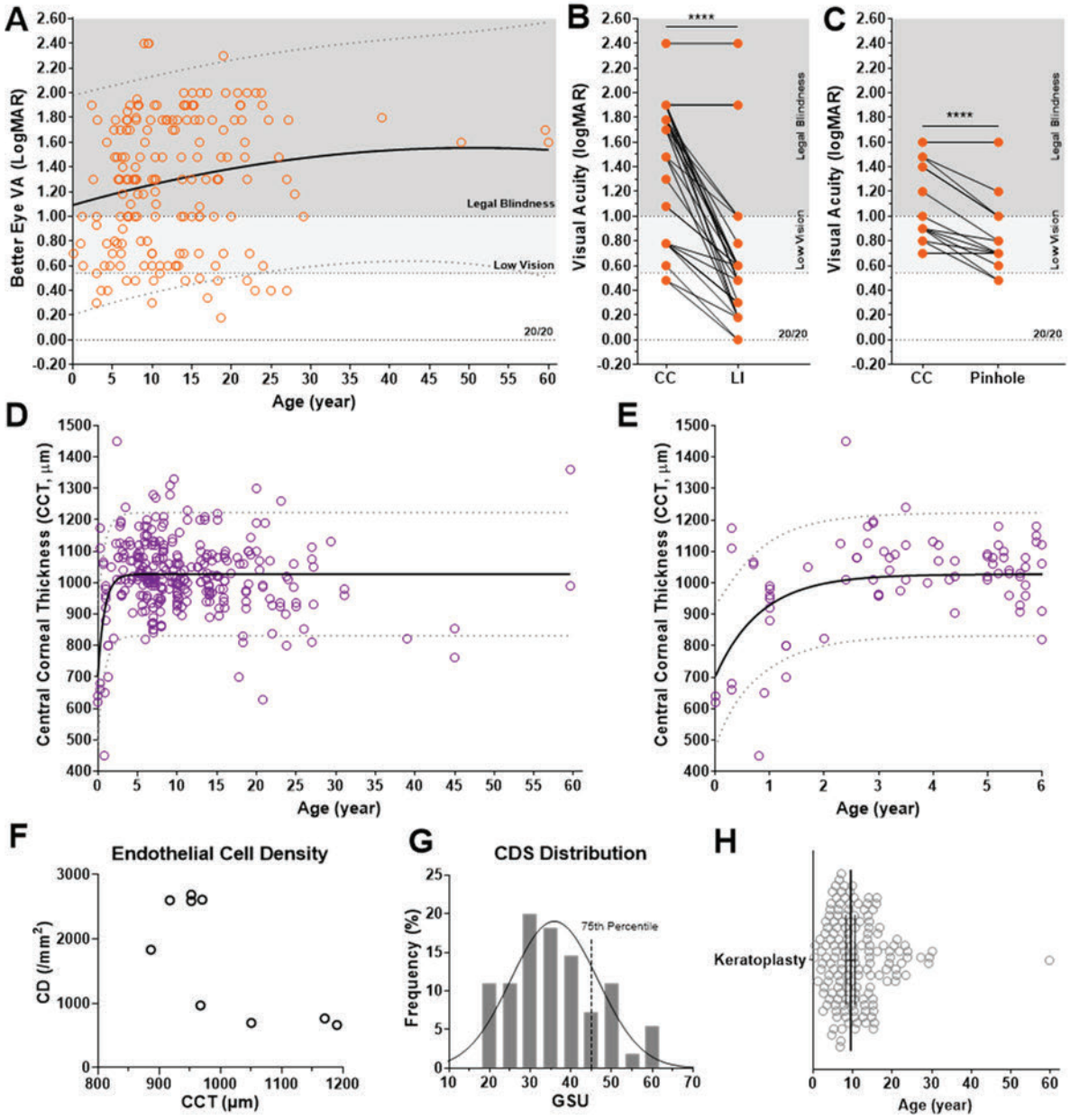


Figure 2. Natural History of CHED. A. Scatter plot of visual acuity (VA) in logMAR unit against age in individuals with CHED. Fitted second order polynomial curve (solid line) with 95% CI (dotted lines) of predicted VA against age were also plotted. Reference lines and shaded areas indicate 20/20 vision, VA range for low vision/moderate visual impairment (light grey) and VA range for legal blindness (dark grey). **B.** Slope graph of Snellen chart VA in 33 eyes of individuals with CHED measured with correction (CC) and laser interferometry (LI). ****, p<0.0001. **C**. Slope graph of Snellen chart VA in 14 eyes of individuals with CHED measured with correction (CC) and with pinhole. ****, p<0.0001. **D**. Scatter plot of CCT against age in individuals with CHED. Fitted one phase association curve (solid line) with 95% CI (dotted lines) of predicted CCT against age were also plotted. **E**. Zoomed-in figure of scatter plot *D*, showing CCT plotted against age within first 6 years of life in individuals with CHED. **F**. Scatter plot showing corneal endothelial cell density (CD) plotted against CCT in children 6 – 13 years of age with CHED. **G**. Histogram of CDS (in grayscale units (GSU)) in 55 eyes of children with CHED (GSU 10 indicates a relatively transparent cornea and 70 indicates a relatively opaque cornea). **H.** Scatter plot showing age at time of initial keratoplasty in individuals with CHED. Median with 95% CI was plotted as line with error bars.

3.2 Proposed Indication and Use of AAV8-hSLC4A11

We propose the use of recombinant Adeno-Associated Virus 8 Human Solute Carrier Family 4 Member 11 (AAV8-hSLC4A11) as a rare pediatric drug product for treatment of infants and children with Congenital hereditary endothelial dystrophy (CHED) associated with biallelic mutations in *SLC4A11*. Missense mutations in *SLC4A11* lead to loss of function of the encoded protein, with secondary dysfunction of the corneal endothelial "pump" activity and development of the progressive corneal edema that characterizes CHED. A single injection of AAV8-hSLC4A11 into the posterior corneal stroma will be performed to transduce the corneal endothelial cells and result in long-term expression of *SLC4A11* in the endothelial cells. Studies in Slc4a11-deficient mice have shown that recombinant adeno-associated virus (rAAV) gene therapy vectors expressing wild type Slc4a11 can provide significant restoration of corneal endothelial function and reversal of corneal edema, supporting the feasibility of corneal intrastromal delivery of a rAAV8 vector expressing human SLC4A11 in individuals with CHED.[56] Inclusion criteria for treatment would be: an infant or child with CHED associated with biallelic mutations in *SLC4A11*; and a corneal densitometry score (CDS) less than 40 grey scale unit (GSU) and endothelial cell density greater than 500 cells per square millimeter (optimal profile) or a CDS less than 45 GSU and an undocumented endothelial cell density (threshold profile).

3.3 Reasons Why Such Therapy Is Needed

Pediatric corneal transplantation, which is the only available treatment for CHED, is associated with an increased risk of intraoperative and postoperative complications, including higher rates of transplant rejection and failure. Long term topical ocular corticosteroid use to decrease the risk of corneal graft rejection is associated with potentiation of infection and an increased risk of secondary cataract and glaucoma. As the mean corneal transplant survival for CHED is approximately 10 years, multiple corneal transplants will be needed to maintain vision for individuals with CHED, with increasing rates of complications and decreasing duration of survival with each subsequent corneal transplant.[57]

The long-term postoperative care after pediatric corneal transplantation requires dedication from caregivers to instill corticosteroid eye drops at least daily for months or years after surgery and to return for frequent office visits with a cornea specialist, pediatric ophthalmologist, and frequently, a glaucoma specialist. Thus, the prolonged visual rehabilitation following even a successful corneal transplant means that amblyopia and nystagmus still commonly develop.

Additionally, while CHED is an uncommon domestic indication for corneal transplantation, it is one of the most frequently encountered corneal dystrophies in countries where consanguineous marriages are more common (Middle East, South Asia and Southeast Asia), and is a well-recognized cause of congenital corneal edema. However, the vast majority of children affected with CHED in these regions do not have access to a trained corneal transplant surgeon or to donor corneal tissue due to limited supply and their families are unable to afford imported corneal tissue. Thus, for most infants and children with CHED living in resource-limited settings, there is no accessible treatment at present.

4 DESCRIPTION OF rAAV8-hSLC4A11 AND SCIENTIFIC RATIONALE FOR USE

4.1 Description of AAV8-hSLC4A11

4.1.1 Drug Product Naming Convention

Recombinant Adeno-Associated Virus 8 Human Solute Carrier Family 4 Member 11 (AAV8-hSLC4A11)

4.1.2 Drug Product Physical, Chemical, and Pharmaceutical Properties

AAV8-hSLC4A11 is a non-replicating, rep/cap-deleted, rAAV vector containing single stranded cDNA encoding wild type human SLC4A11 protein. The vector contains AAV serotype 2 ITRs and an expression cassette consisting of a eukaryotic translation elongation factor 1α promoter (EF1α), a Kozak sequence, the wild type human SLC4A11 variant B (NM_032034) cDNA and bovine growth hormone polyadenylation sequence and is packaged in wild type AAV8 capsid (**Figure 3**). The non-viral EF1α promoter was selected because of the ubiquitous expression of SLC4A11 in human cornea as well as other organs and tissues. SLC4A11 variant B (NM_032034) was selected as the transgene variant as it is the most abundantly expressed transcript variant at the mRNA level in the human cornea.[58] A single-stranded design was used given the 3089 bp size of human SLC4A11 variant B (NM_032034) cDNA (**Figure 4**). The complete nucleotide sequence of AAV8-hSLC4A11 is included as **Appendix A**.

Figure 3. Schematic of the plasmid vector used to produce single stranded AAV-hSLC4A11. ITR2 = inverted terminal repeat serotype 2, EF1 α = eukaryotic translation elongation factor 1 α promoter, hSLC4A11 = human solute carrier family 4 member 11, bGH pA = bovine growth hormone polyadenylation signal.

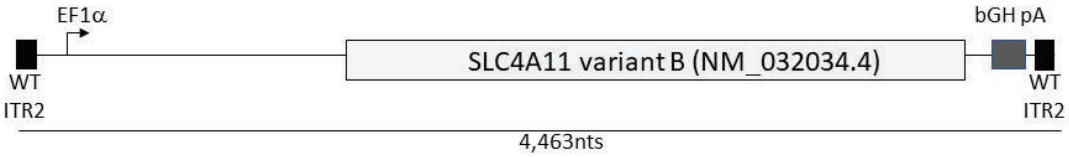


Figure 4. Organization of the AAV8-hSLC4A11 vector. ITR2 = inverted terminal repeat serotype 2, EF1α = eukaryotic translation elongation factor 1 α promoter, hSLC4A11 = human solute carrier family 4 member 11, bGH pA = bovine growth hormone polyadenylation signal.

4.1.3 Drug Product Description, Mechanism of Action and Route of Administration

AAV8-hSLC4A11 is a biologic modality for introducing SLC4A11 cDNA into the corneal endothelium in individuals with CHED. The formulation relies on the AAV8 capsid, which has been reported for efficient corneal gene delivery in many species including human corneas ex vivo.[59,60] The route of administration is a single corneal intrastromal injection of a volume of 100 µL, which is consistent with the 50 –100 µL volume of voriconazole and amphotericin B injected into the cornea when treating fungal keratitis, and thus is an appropriate and clinically utilized volume for injection into the human cornea.[61-63] The drug formulation will rely on Andelyn Biosciences' proprietary buffer composition. Vector dosing will be based on ongoing in vivo dose-response studies and planned safety, toxicology and biodistribution studies.

The cellular target of AAV8-hSLC4A11 is the corneal endothelium, which consists of a monolayer of non-dividing endothelial cells, representing a suitable target for a primarily episomal AAV vector. SLC4A11, one of the highly expressed differentiation markers for corneal endothelium, is essential in facilitating energy producing glutaminolysis in the corneal endothelium by maintaining ammonia homeostasis, reducing glutaminolysis-associated oxidative stress, maintaining antioxidant signaling and preventing apoptosis.[21-25,27,28,34] Therefore, the restoration of SLC4A11 in the corneal endothelium via intrastromal delivery of AAV8-hSLC4A11 is expected to restore corneal endothelial cell function.

4.2 Scientific Rationale for the Use of AAV8-hSLC4A11 in the Rare Disease or Condition

A single injection of recombinant adeno-associated virus (rAAV) gene therapy vectors expressing wild type Slc4a11 in the anterior chambers of Slc4a11-deficient mice has been shown to provide significant restoration of corneal endothelial function and reversal of corneal edema. Our pre-clinical results of rAAV8 vector corneal intrastromal administration in large animal models, including rabbit[59], canine[60] and feline (unpublished), showed excellent safety profiles with limited to no ocular and non-ocular immunologic response, no vector-mediated transgene expression in non-corneal ocular tissues or in non-ocular tissues (liver, kidney, spleen, heart, and contralateral non-injected cornea) and no detectable neutralizing antibodies to the rAAV8 capsid in the aqueous, vitreous humor or serum. Additionally, our unpublished studies in human donor corneas demonstrated that a single deep intrastromal injection of rAAV8-GFP achieved

expression of GFP protein in more than 37% of corneal endothelial cells after 15 days, indicating the ability to deliver SLC4A11 protein from the stroma to the corneal endothelial cells that are affected in patients with CHED. These studies support the feasibility and safety of corneal intrastromal delivery of a rAAV8 vector expressing human SLC4A11 (AAV8-hSLC4A11) in infants and children with CHED. Further, our unpublished preclinical studies of bilateral sequential corneal intrastromal injection of rAAV8 at a 6 week interval support the feasibility of sequential treatment of both eyes using the same AAV8 vector, offering the possibility of treating both eyes of individuals with CHED using the same AAV8-hSLC4A11.

4.2.1 Clinical Efficacy of AAV8-hSLC4A11

To date, no clinical data have been collected using AAV8-hSLC4A11 in humans.

4.2.2 Nonclinical Efficacy of AAV8-hSLC4A11Clinical Efficacy of AAV8-hSLC4A11

Vector targeting

Intrastromal injection of AAV8-GFP transduces ex vivo human corneal endothelium

Intrastromal injection of rAAV8-CMV-GFP in donor human corneas at a dosage of 5 x 10^{10} vg per cornea showed the transduction efficiency in corneal endothelium is dependent on the depth and volume of the injection, independent of viral dosage (**Figure 5**). Viral vectors were diluted in Balanced Salt Solution sterile irrigating solution (BSS™, Alcon) to achieve the desired concentrations; BSS alone was used as the negative control. A single intrastromal injection was performed in each cornea using a 33G needle (**Figure 5A**). The following injection conditions were tested: 1) central mid-stromal injection of 50 µL rAAV8-GFP or 50 µL BSS; 2) central posterior stromal injection of 50 µL rAAV8-GFP or 50 µL BSS; 3) central posterior stromal injection of 100 µL rAAV8-GFP or 100 µL BSS; and 4) central deep stromal injection of 100 µL rAAV8-GFP or 100 µL BSS. Following injection, the corneas were replaced in OptiSol-GS corneal storage medium (Bausch & Lomb) at 37°C with 5% CO2 until processed for immunostaining. The Descemet membrane was peeled from the corneas immediately before immunostaining, *en face* visualization and quantification of GFP+ cells to estimate the transduction efficiency (GFP+ / total cells). In the cornea in which a central deep stromal injection of 100 µL rAAV8-GFP was performed, 37% of corneal endothelial cells were GFP+ (6640 GFP+ cells / 17886 DAPI+ nuclei) on post-injection day 15 (**Figure 5B, C**). The percentage of terminal deoxynucleotidyl transferase (dUTP) nick-end labeling (TUNEL) positive cells, indicative of apoptotic cells, was between 1 -2 % and was not significantly different between the rAAV8-GFP injected and BSS injected corneas.

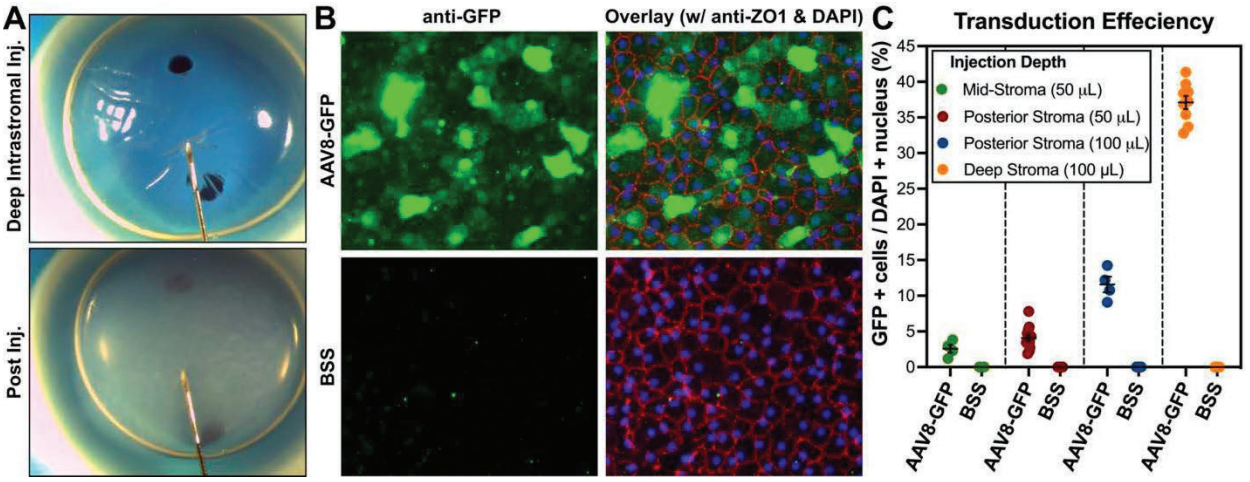


Figure 5. Intrastromal injection of AAV8-GFP in ex vivo donor human corneas transduces the corneal endothelium. A. Photographs of human cornea mounted on artificial anterior chamber prior to and after central deep stromal injection. Air in the anterior chamber facilitates visualization of folds in the Descemet membrane (DM) observed when the needle tip was advanced very close to the DM. B. Representative immunofluorescence staining images of peeled DM from AAV8-GFP or BSS injected human corneas labeled with anti-GFP and anti-ZO1 antibodies with DAPI nuclear staining. C. Scatter plot of the estimated percentage of transduced corneal endothelial cells in AAV8-GFP or BSS injected corneas in four testing conditions with varying injection depth and volume but constant 5e10 vg viral genome dosage.

Efficacy

Therapeutic candidate rAAV8- EF1α-hSLC4A11 produces mature SLC4A11 protein

The transduction efficiency of rAAV8-EF1α-GFP in an *SLC4A11*$^{-/-}$ human corneal endothelial cell line was only 0.2% - 1%, consistent with the recognized low transduction efficiency of AAV8 in immortalized cell lines,[64] and making *SLC4A11*$^{-/-}$ HCEnC-21T an unsuitable model for testing the efficacy of our therapeutic candidate rAAV8-EF1α-hSLC4A11. Therefore, we transduced HEK293 cells with rAAV8-EF1α -hSLC4A11 following cell transfection with plasmid containing the adenovirus gene E4ORF6, which increases AAV8 transduction in HEK293 cells by ~ 100-fold as shown previously.[65]

Mature SLC4A11 protein is dimerized and glycosylated when expressed on the plasma membrane.[39,66] The SLC4A11 monomer is 100 kDa in size and the glycosylated SLC4A11 monomer is ~130 kDa. rAAV8-EF1α -hSLC4A11 transduction of HEK293 cells resulted in the production of dimerized and glycosylated SLC4A11, as evidenced by Western Blot showing bands at 200 kDa and ~260 kDa when using an anti-SLC4A11 antibody (Invitrogen, PA5-101889, **Figure 6**). The pAAV-hSLC4A11 plasmid used to produce the AAV8-hSLC4A11 viral vector was transfected in HEK293 cells as a positive control and showed significant over-expression of mature SLC4A11 protein. *SLC4A11*$^{-/-}$ HCEnC-21T cells stably transduced with LV-hSLC4A11vBWT was also included as positive control.

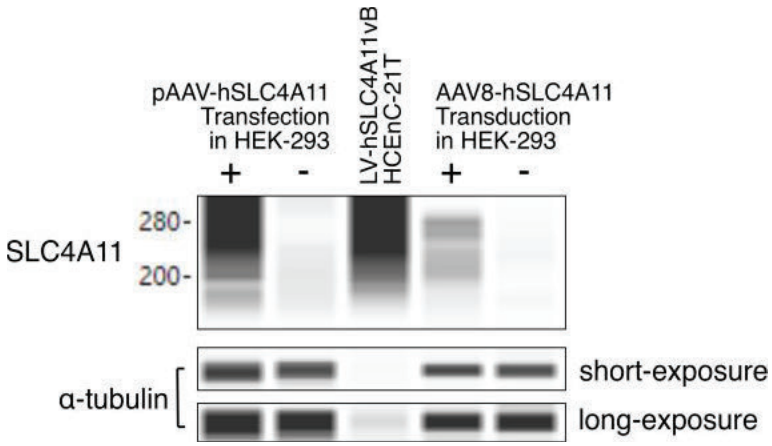


Figure 6. AAV8-hSLC4A11 transduction produces mature SLC4A11 protein. Western Blot results of HEK293 cell lysates after transduction of AAV8-hSLC4A11 blotted for SLC4A11 and α-tubulin proteins.

SLC4A11 replacement restores SLC4A11-mediated NH_3 and H^+ permeability in SLC4A11$^{-/-}$ human endothelial cells

Central corneal thickness is the functional measure of corneal endothelial "pump" activity *in vivo*. The corneal endothelium *in vivo* expresses SLC4A11 at a high level as evidenced by multiple publications[67-70] and our RNAseq data of *ex vivo* human cornea endothelial cells (evHCEnC) and *ex vivo* mouse corneal endothelial cells freshly dissected from *Slc4a11*$^{+/+}$ and *Slc4a11*$^{-/-}$ (exon 9-13 del) mice (*Slc4a11*$^{+/+}$ evMCEnC and *Slc4a11*$^{-/-}$*(exon 9-13 del)* evMCEnC).[71] For corneal endothelial cells *in vitro*, SLC4A11-mediated plasma membrane $NH_3/NH4^+$ and H^+ conductance measured by single-cell patch-clamp (voltage-clamp or current-clamp) recordings is a direct, sensitive and physiologically relevant parameter to evaluate mature SLC4A11 protein function. We observed consistent NH_4Cl-induced membrane depolarization with current-clamp in corneal endothelial cell lines expressing wild type human SLC4A11 or murine Slc4a11: primary human corneal endothelial cells (pHCEnC), immortalized human corneal endothelial cell lines (HCEnC-21T) and immortalized mouse corneal endothelial cell lines (*Slc4a11*$^{+/+}$ immorto-MCEnC) **(Figure 7B)**. In contrast, SLC4A11 knockout resulted in diminished NH_4Cl-induced membrane depolarization in human and mouse corneal endothelial cell-based models of CHED, including CRISPR mediated *SLC4A11* knock-out in HCEnC-21T (CRISPR *SLC4A11*-/- HCEnC-21T) and *Slc4a11*-/-*(exon 9-13 del)* evMCEnC **(Figure 7B)**. Additionally, transducing CRISPR *SLC4A11*-/- HCEnC-21T with lentiviral (LV) vector driving expression of wild-type SLC4A11 variant B or C (LV-SLC4A11varBWT or LV-SLC4A11varCWT) reinstated the SLC4A11-mediated plasma membrane NH_3/NH_4^+ and H^+ conductance, while transduction with lentiviral vector driving expression of SLC4A11 mutants showed a variable decrease in the amplitude of depolarization (**Figure 7C**).

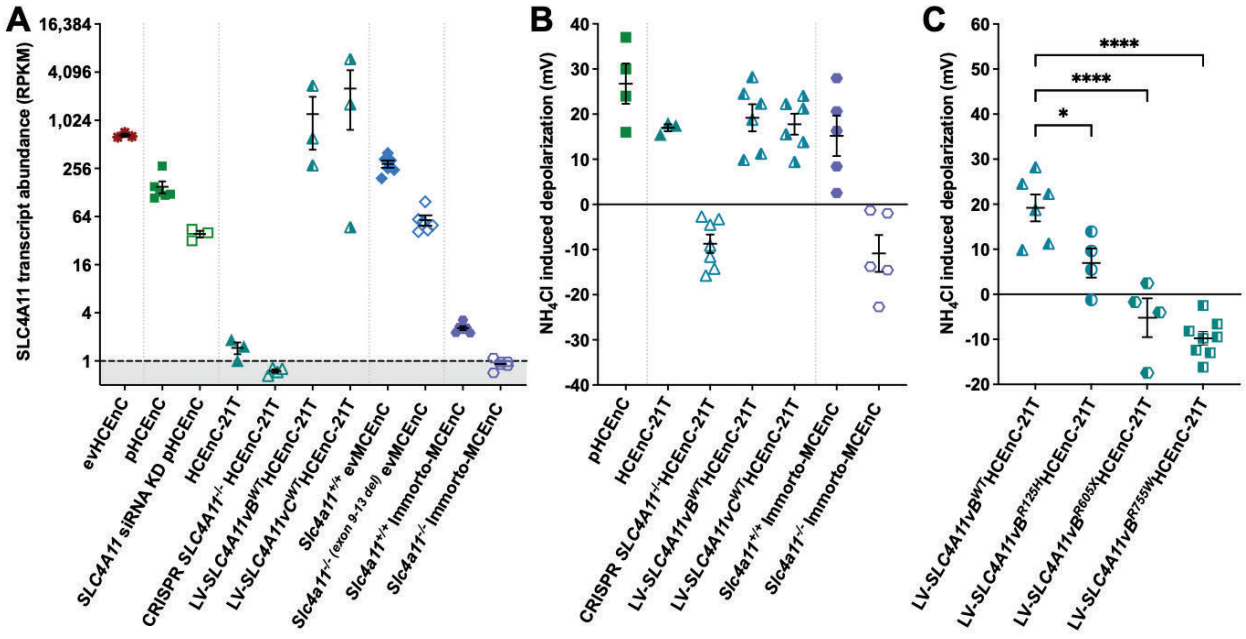


Figure 7. SLC4A11 mediated NH3/NH4+ and H+ conductance is a sensitive measure of mature SLC4A11 function. A. Scatter plot of SLC4A11/Slc4a11 mRNA transcript abundance from RNAseq data of corneal endothelial cells from various sources. RPKM, reads per kilobase of transcript, per million mapped reads; evHCEnC, ex vivo human corneal endothelial cells. RPKM less than 1 was considered no expression (region shaded grey). **B**. Scatter plot of NH4Cl (10 mM) induced membrane potential changes measured by single-cell current-clamp recordings of corneal endothelial cells from various sources. **C**. Scatter plot of NH4Cl (10 mM) induced membrane potential changes of CRISPR *SLC4A11*-/- HCEnC-21T transduced with lentiviral (LV) vectors driving expressing of SLC4A11vB wild type, R125H, R605X or R755W mutant, respectively. Mean ± SEM were plotted; *, p <0.05; ****, p <0.0001.

LV-SLC4A11 rescues NH_3:H^+ permeability and oxidative stress defense in SLC4A11$^{-/-}$ human endothelial cells

Stable transduction of an immortalized human corneal endothelial cell line in which *SLC4A11* expression was knocked out by CRISPR-Cas9 (CRISPR *SLC4A11*$^{-/-}$ HCEnC-21T) using LV vectors with the CMV promoter driving expression of wild type human SLC4A11 variant B (LV-CMV-hSLC4A11vB^{WT}) or variant C (LV-CMV-hSLC4A11vC^{WT}) restored both plasma membrane NH_3/NH_4^+ and H^+ conductance and antioxidative stress response with no significant difference observed between cells transduced with LV-hSLC4A11vB^{WT} and LV-hSLC4A11vC^{WT}(**Figure 8**).

Overexpression of human SLC4A11 in an immortalized human corneal endothelial cell line (HCEnC-21T) to ~3000 times its endogenous level, as measured by SLC4A11 mRNA transcript abundance, does not result in evidence of toxicity, and offers a protective effect against an oxidative stress insult (**Figure 8B**). Additionally, overexpression of human SLC4A11 does not over-correct SLC4A11 protein-mediated NH_3/NH_4 and H^+ conductance, as the amplitude of NH_4Cl-induced membrane depolarization following SLC4A11 overexpression in SLC4A11 knock-out HCEnC-21T is similar to that in HCEnC-21T expressing endogenous SLC4A11 (**Figure 8B**), possibly due to the role of ER-mediated SLC4A11 protein turnover, [39]and the roles of other ion transporters and channels in regulating corneal endothelial membrane potential.[72]

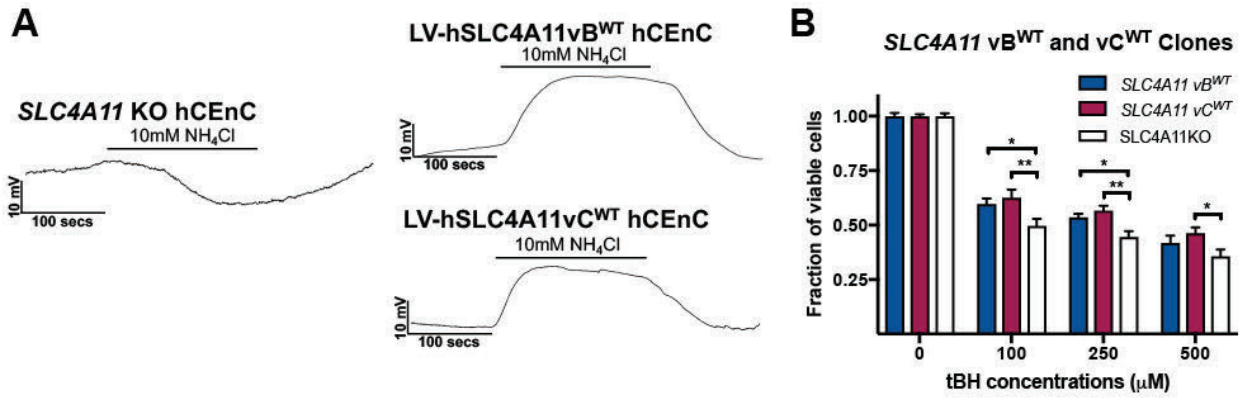


Figure 8. LV-hSLC4A11 transduction reinstated membrane NH_3/NH_4^+ and H^+ conductance, as well as cellular resistance to oxidative stress stimuli in SLC4A11-deficient HCEnC. A. Representative traces of membrane potential changes induced by 10 mM NH4Cl in CRISPR *SLC4A11*$^{-/-}$ hCEnC transduced with empty vector, LV-hSLC4A11vB^{WT} or LV-hSLC4A11vC^{WT}. B. Bar graph shows the fraction of viable cells after induction of acute oxidative stress by escalating concentrations of tBH. Stable transductions of CRISPR *SLC4A11*$^{-/-}$ hCEnC-21T with LV-hSLC4A11vB^{WT} or LV-hSLC4A11vC^{WT} exhibit increases in hCEnC viability during tBH-induced oxidative stress compared to empty vector. LV = Lentivirus; hCEnC = human corneal endothelial cells; tBH = tert-butylhydroperoxide; vB = SLC4A11 variant B; vC = SLC4A11 variant C.

Intracameral AAV9-mSlc4a11 rescues corneal edema in Slc4a11$^{-/-}$ *mice*

A *Slc4a11*$^{-/-}$ mouse model has been developed that demonstrates the progressive congenital corneal edema that characterizes CHED. This mouse model was generated in a mixed Bruce4/C57BL/6 background with a targeted deletion of exons 9-13 of the murine *Slc4a11* gene, in which the altered *Slc4a11* mRNA transcript, lacking the sequences of the transmembrane helices of the wild type protein, was a target for degradation by nonsense-mediated decay.[73,74] The initial in-depth characterization of this mouse model and subsequent observations from multiple laboratories have indicated that this C57BL/6 *Slc4a11*$^{exon9\text{-}13del}$ line accurately recapitulates the CHED disease phenotype, with conreal edema documented immediately after weaning at 3 weeks of age, and presumably present since birth, as noted in affected human newborns.[73-77]

The efficacy of rAAV vectors expressing wild type murine *Slc4a11* was demonstrated in *Slc4a11*$^{-/-}$ mice with an exon 9-13 deletion.[71] The estimated corneal endothelial cell transduction efficiency following intracameral injection of rAAV9-CAG-HA-mSlc4A11 in mice was 25% ± 12%.[71] A single intracameral injection of rAAV9 with chicken β-actin (CAG) promoter driving expression of murine *Slc4a11* with a N-terminal HA-tag (rAAV9-CAG-HA-mSlc4A11) at the dosage range of 5×10^{11} – 8×10^{11} vg in 5 and 11-week-old *Slc4a11*$^{-/-}$ mice demonstrated reversal or halting the progression of corneal edema. Gene therapy in 5-week-old *Slc4a11*$^{-/-}$ mice was able to reduce corneal edema in the rAAV9-mSlc4a11 injected eye compared to the non-injected contralateral eye **(Figure 9)**, whereas in 11-week-old *Slc4a11*$^{-/-}$ mice, rAAV-mSlc4a11 injection prevented further increase in corneal thickness, which was observed in the noninjected eye. rAAV9-mSlc4a11 injection also resulted in less endothelial cell loss in the treated eye compared with the non-injected contralateral eye in both 5 and 11-week-old *Slc4a11*$^{-/-}$ mice.[71]

No significant change in intraocular pressure and no ocular immune response was observed following intracameral injection of rAAV9-GFP or rAAV9-CAG-null in *Slc4a11*$^{+/+}$ mice and rAAV9-CAG-HA-mSlc4A11 in *Slc4a11*$^{-/-}$ mice.[71]

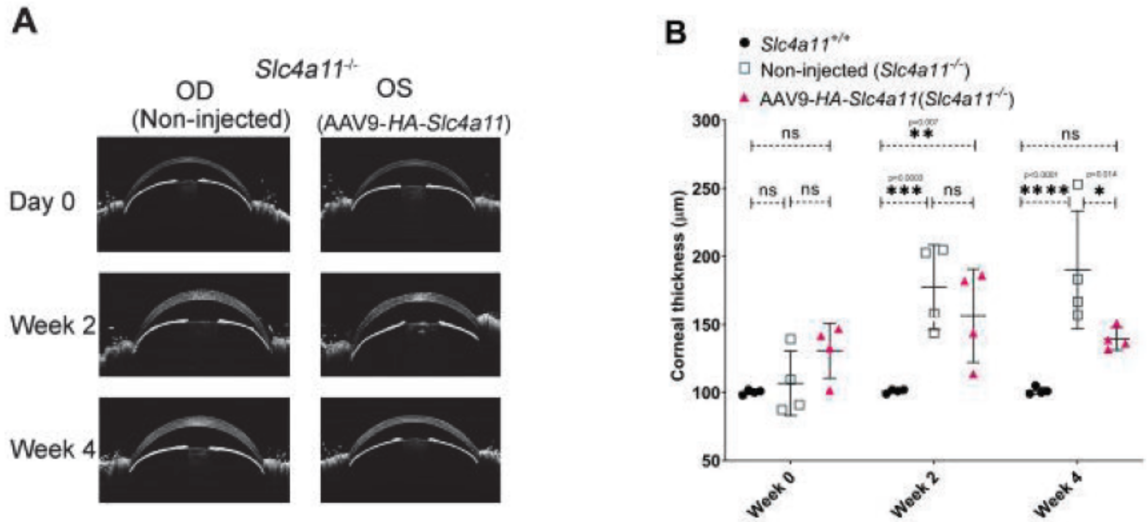
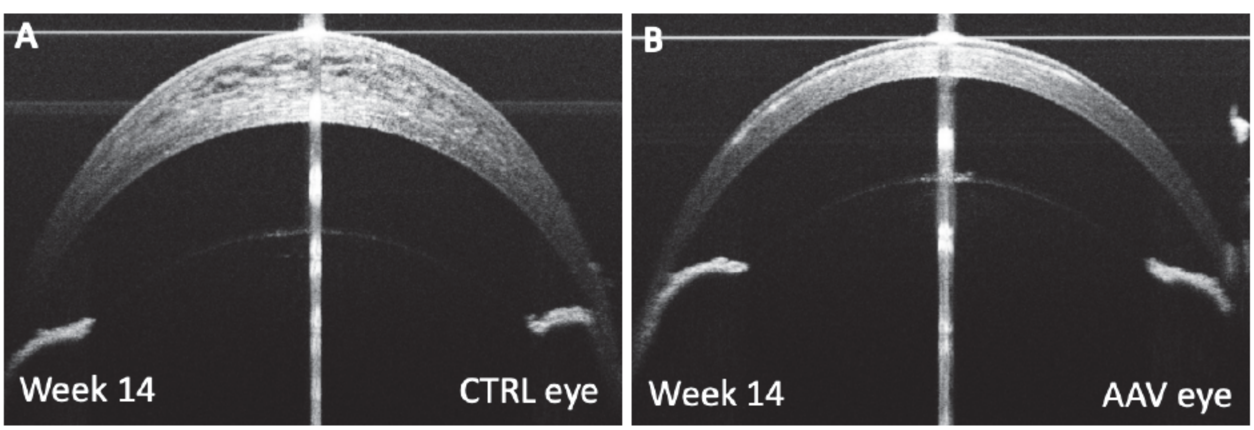


Figure 9. Corneal edema is reduced with AAV9-mSlc4a11. A. Representative OCT images of noninjected and AAV9-HA-*Slc4a11* injected eyes from 5-week-old *Slc4a11*$^{-/-}$ mice. B. Quantification of data from A. Mean ± SD. ns = not significant. OD = right eye; OS = left eye.

Intrastromal AAV8-mediated hSLC4A11 delivery rescues corneal edema in Slc4a11$^{-/-}$ *mice*

To evaluate the in vivo efficacy of corneal intrastromal delivery of a recombinant AAV8 vector encoding human SLC4A11 cDNA, we performed a single corneal intrastromal injection of 3 µL rAAV8-EF1α-hSLC4A11 vector in one eye of *Slc4a11*$^{-/-}$ mice at 4 weeks of age. Five dosages were tested: 1×10^9, 3.3×10^8, 1×10^8, 1×10^7 and 1×10^6 vg/eye. For each mouse, the eye receiving the AAV injection was randomized, and the contralateral eye served as the control. AAV-injected eyes showed a sustained reduction/stabilization of central corneal thickness (CCT) from baseline in a dose-dependent manner, whereas untreated contralateral eyes showed a progressive increase in CCT (Figures 10 and 11) A significant difference in the CCT of the treated eye compared with the control eye was observed at week 6 following the injection at each of the dosages tested, with the effect observed through the most recent



assessmen[illegible] weeks after the injection ([illegible]).

Figure 10. Intrastromal AAV8-hSLC4A11 injection rescues corneal edema in *Slc4a11* knock out mice. Anterior segment ocular coherence tomography (OCT) images of a Slc4a11-deficient mouse 14 weeks after corneal intrastromal injection of rAAV8-EF1α-hSLC4A11 vector (1×10^9 vg/eye) in one eye

(AAV eye). A significant difference in corneal thickness is seen when compared with the untreated eye (CTRL eye).

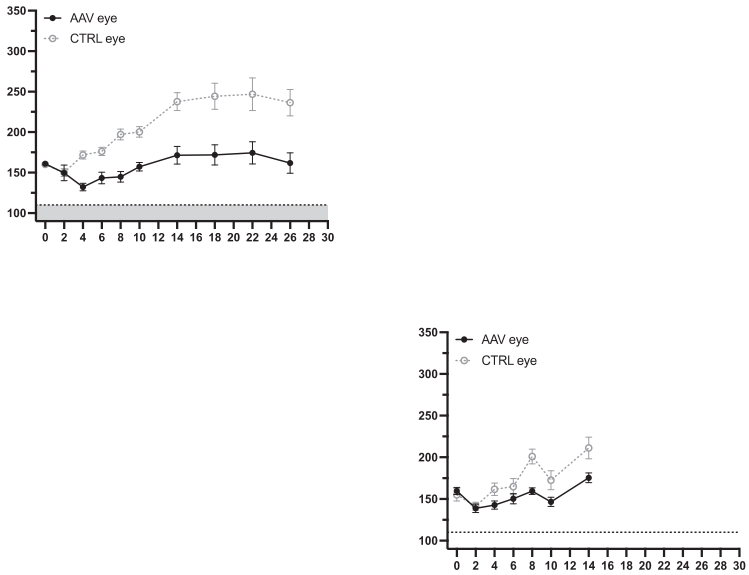


Figure 11. Intrastromal AAV8-hSLC4A11 injection rescues corneal edema in *Slc4a11* knock out mice. Central corneal thickness (CCT) measurements in *Slc4a11*$^{-/-}$ mice following AAV8-hSLC4A11 corneal intrastromal injection of four dosages of AAV8-hSLC4A11 vector in one eye (AAV eye) (1 x 10^9 (n = 14 mice), 3.3 x 10^8 (n = 10 mice), 1 x 10^8 (n = 10 mice), 1 x 10^7 (n = 11 mice), and 1 x 10^6 (n = 11 mice) vg/eye) and in the contralateral untreated eye (CTRL eye). Vg = viral genomes. Shaded area indicates CCT in *Slc4a11* wild type mice.

Vg/eye	AAV eyes vs CTRL eyes		AAV eyes		CTRL eyes	
$1x10^9$	Baseline	p = 0.62	Baseline vs		Baseline vs	
	W2	p = 0.97	W2	p = 0.181	W2	p = 0.016
	W4	p < 0.0001	W4	p < 0.0001	W4	p = 0.011
	W6	p = 0.001	W6	p = 0.048	W6	p = 0.011
	W8	p < 0.0001	W8	p = 0.025	W8	p < 0.0001
	W10	p = 0.0004	W10	p = 0.598	W10	p < 0.0001
	W14	p = 0.0015	W14	p = 0.406	W14	p < 0.0001
	W18	p = 0.0047	W18	p = 0.448	W18	p < 0.0001
	W22	p = 0.009	W22	p = 0.383	W22	p = 0.0006
	W26	P = 0.010	W26	P = 0.955	W26	P = 0.0003
	Overall AAV Treatment Effect (AAV eyes vs CTRL eyes) p = 0.004					
$3.3x10^8$	Baseline	p = 0.629	Baseline vs		Baseline vs	
	W2	p = 0.009	W2	p = 0.144	W2	p = 0.381
	W4	p = 0.359	W4	p = 0.707	W4	p = 0.937
	W6	p < 0.0001	W6	p = 0.562	W6	p = 0.0007
	W8	p <0.0001	W8	p = 0.045	W8	p = 0.013
	W10	p < 0.0001	W10	p = 0.052	W10	p = 0.002
	W14	p < 0.001	W14	p = 0.699	W14	p < 0.0001
	W18	P < 0.0001	W18	P = 0.259	W18	P < 0.0001
	Overall AAV Treatment Effect (AAV eyes vs CTRL eyes) p = 0.0002					
$1x10^8$	Baseline	p = 0.327	Baseline vs		Baseline vs	
	W2	p = 0.014	W2	p < 0.0001	W2	p = 0.640
	W4	p = 0.299	W4	p = 0.208	W4	p = 0.550
	W6	p = 0.021	W6	p = 0.034	W6	p = 0.724
	W8	p = 0.0006	W8	p = 0.001	W8	p = 0.132
	W10	p < 0.0001	W10	p = 0.511	W10	p = 0.0005
	W14	p = 0.088	W14	p = 0.167	W14	p = 0.004
	W18	p = 0.436	W18	p = 0.028	W18	p = 0.116
	W22	P = 0.863	W22	p = 0.019	W22	p = 0.060
	Overall AAV Treatment Effect (AAV eyes vs CTRL eyes) p = 0.115					
$1x10^7$	Baseline	p = 0.726	Baseline vs		Baseline vs	
	W2	p = 0.975	W2	p = 0.277	W2	p = 0.555

	W4	p = 0.254	W4	p = 0.968	W4	p = 0.371
	W6	p = 0.039	W6	p = 0.933	W6	p = 0.012
	W8	p = 0.0014	W8	p = 0.744	W8	p = 0.002
	W10	p = 0.0021	W10	p = 0.207	W10	p = 0.0006
	W14	p = 0.003	W14	p = 0.272	W14	p = 0.0005
	W18	p = 0.0007	W18	p = 0.111	W18	p = 0.0001
	W22	p = 0.0011	W22	p = 0.090	W22	p < 0.0001
	Overall AAV Treatment Effect (AAV eyes vs CTRL eyes) p = 0.0002					
$1x10^6$	Baseline	p = 0.528	Baseline vs		Baseline vs	
	W2	p = 0.598	W2	p < 0.0001	W2	p = 0.060
	W4	p = 0.023	W4	p = 0.007	W4	p = 0.433
	W6	p = 0.0.010	W6	p = 0.090	W6	p = 0.204
	W8	p = 0.0002	W8	p = 0.967	W8	p < 0.0001
	W10	p = 0.0055	W10	p = 0.039	W10	p = 0.121
	W14	p = 0.0019	W14	p = 0.489	W14	p = 0.001
	Overall AAV Treatment Effect (AAV eyes vs CTRL eyes) p = 0.0022					

Table 1. Intrastromal AAV8-hSLC4A11 injection rescues corneal edema in *Slc4a11* knock out mice. Mixed-effect analysis of central corneal thickness measurements Week 2 (W2) - Week 26 (W26) after corneal intrastromal injection of four dosages of AAV8-hSLC4A11 vector in one eye (AAV eye) (1×10^9 (n = 14 mice), 3.3×10^8 (n = 10 mice), 1×10^8 (n = 10 mice), 1×10^7 (n = 11 mice), and 1×10^6 (n = 11 mice) vg/eye) and in the contralateral untreated eye (CTRL eye).

5 ORPHAN DRUG STATUS

AAV8-hSLC4A11 is not designated as an orphan drug or product for the treatment of individuals with CHED resulting from biallelic mutations in *SLC4A11* or for any other indication in the United States or globally.

6 PATIENT SUBSET CONSIDERATIONS AND MEDICAL PLAUSIBILITY OF THE CHOSEN SUBSET

Fewer than 200,000 individuals in the United States have CHED. Therefore, subset considerations and medical plausibility of a subset are not applicable to the proposed indication of individuals with CHED resulting from biallelic mutations in *SLC4A11* in this request.

7 REGULATORY STATUS AND MARKETING HISTORY

Regulatory status: The sponsor has not had any interactions with the FDA in relation to AAV8-hSLC4A11.

Marketing history: AAV8-hSLC4A11 is not currently marketed or approved for use in any country, including the United States. In accordance with section 526(a)(1) of the Federal Food, Drug, and Cosmetic

Act [21 USC 360bb(a)(1)]; 21 CFR 316.20(b)(7); and 21 CFR 316.23(a), the sponsor has not previously submitted a marketing application to FDA for the same active moiety in AAV8-hSLC4A11 for the same rare disease or condition prior to submission of the AAV8-hSLC4A11 rare pediatric disease designation request.

8 DOCUMENTATION OF PATIENT POPULATION SIZE

CHED is a rare disease in the United States, and is an uncommon domestic indication for corneal transplantation. However, CHED is one of the most frequently encountered corneal dystrophies in countries where consanguineous marriages are more common (Middle East, South Asia and Southeast Asia), and is a well-recognized cause of congenital corneal opacification (CCO) due to the presence of corneal edema at birth. The incidence of CCO in the United States is estimated to be approximately 2.2 per 100,000 live births.[1] A determination of the percentage of the cases of CCO that are due to CHED can be performed by reviewing the published series of the causes of CCO (**Table 2**). The series from the US include between 14 and 56 infants. In none of these series was CHED identified as the cause of CCO in an infant. However, the percentage of eyes in each of these series in which the cause of the CCO was idiopathic (0%, 4%, 10%) or in which the cause was not specified (33%) was provided. Therefore, if one assumes that all of the infants with idiopathic CCO or in whom the cause was not specified had CHED, the birth incidence can be calculated by multiplying the incidence of CCO per live births in the US by the lowest and highest percentages of cases of CCO attributable to CHED in these series by the US population in the 2021 census: (2.2/100,000) x 0.04 x 330,218,929 to (2.2/100,000) x 0.33 x 330,218,929 = 291 to 2312. Given an average life expectancy of 76.4 years for both sexes in the US, the estimated number of individuals with CHED in the US would then be 22,232 – 176,637, below the 200,000 threshold to qualify for Orphan Disease designation.

Lead Author, Year of Publication	Country	Incidence of CCO (per 100, 000 live births)	Number of individuals with CCO	Percentage of individuals with CHED	Percentage of individuals with idiopathic CCO
Karadag, 2020[78]	US	Not determined	56	0	3.9%
Rezende, 2004[79]	US	Not determined	47	0	9.7%
Kurilec,2014[1]	US	2.2	36	Not specified	Not specified
Borik, 2023[80]	US	19.3	14	0	0
Bermejo, 1998[81]	Spain	3.11	35	Not specified	Not specified

Table 2. Summary of published series of the incidence and causes of congenital corneal opacification (CCO).

9 RARE PEDIATRIC DISEASE DESIGNATION JUSTIFICATION

Based on the evidence presented above, pursuant to Section 908 of FDASIA, and consistent with the Rare Pediatric Disease Priority Review Vouchers Guidance for Industry, Anthony J. Aldave, MD requests that

AAV8-hSLC4A11 be designated as a Rare Pediatric Drug Product for the treatment of Congenital hereditary endothelial dystrophy resulting from biallelic mutations in *SLC4A11*. We believe that AAV8-hSLC4A11 qualifies as a Rare Pediatric Drug Product for the treatment of CHED based on the fact that CHED is a rare pediatric disease, present at birth, with an estimated prevalence of fewer than 200,000 affected individuals in the US. CHED is a serious disease that causes significant visual impairment in both eyes present at birth, which results in depravation amblyopia and nystagmus, limiting vision even following corneal transplantation.[50-52] The majority of infants and children with CHED live in resource-limited settings and do not have access to either a corneal transplant surgeon or to donor corneal tissue. Thus, most affected individuals have no access to treatment at present. Even for those affected individuals who do, the long term prognosis following corneal transplantation for CHED is guarded, with a mean transplant survival of only approximately 10 years.[57] Thus, multiple corneal transplants are needed to maintain vision for individuals with CHED, with increasing rates of complications and decreasing duration of survival with each subsequent corneal transplant. Additionally, prolonged visual rehabilitation following even a successful corneal transplant means that amblyopia and nystagmus still commonly develop. Therefore, there is a significant need both in developed and developing countries for more effective treatments for CHED that do not require the use of donor corneal tissue.

It should be noted that AAV8-hSLC4A11 is intended as a gene therapy specifically for gene replacement due to mutations in *SLC4A11* and has no foreseeable use in the treatment of any other disease or a different adult indication. AAV8-hSLC4A11 is currently in pre-clinical testing, with a first-in-human clinical trial planned within the next few years. The likely initial patient population will be infants and children. We therefore request that AAV8-hSLC4A11 be designated as a Rare Pediatric Disease Product for the treatment of CHED associated with biallelic mutations in *SLC4A11*.

10 REFERENCES

1 Kurilec, J. M. & Zaidman, G. W. Incidence of Peters anomaly and congenital corneal opacities interfering with vision in the United States. *Cornea* **33**, 848-850 (2014). https://doi.org/10.1097/ico.0000000000000182

2 Mehta, N. *et al.* Updates on congenital hereditary endothelial dystrophy. *Taiwan J Ophthalmol* **13**, 405-416 (2023). https://doi.org/10.4103/tjo.TJO-D-23-00135

3 Desir, J. *et al.* Borate transporter SLC4A11 mutations cause both Harboyan syndrome and non-syndromic corneal endothelial dystrophy. *J Med Genet* **44**, 322-326 (2007). https://doi.org/10.1136/jmg.2006.046904

4 Siddiqui, S. *et al.* Congenital hereditary endothelial dystrophy caused by SLC4A11 mutations progresses to Harboyan syndrome. *Cornea* **33**, 247-251 (2014). https://doi.org/10.1097/ico.0000000000000041

5 Tananuvat, N. *et al.* Harboyan syndrome: novel SLC4A11 mutation, clinical manifestations, and outcome of corneal transplantation. *J Hum Genet* **66**, 193-203 (2021). https://doi.org/10.1038/s10038-020-00834-5

6 Jiao, X. *et al.* Autosomal recessive corneal endothelial dystrophy (CHED2) is associated with mutations in SLC4A11. *J Med Genet* **44**, 64-68 (2007). https://doi.org/10.1136/jmg.2006.044644

7 Vithana, E. N. *et al.* Mutations in sodium-borate cotransporter SLC4A11 cause recessive congenital hereditary endothelial dystrophy (CHED2). *Nat Genet* **38**, 755-757 (2006). https://doi.org/10.1038/ng1824

8 Sultana, A., Garg, P., Ramamurthy, B., Vemuganti, G. K. & Kannabiran, C. Mutational spectrum of the SLC4A11 gene in autosomal recessive congenital hereditary endothelial dystrophy. *Mol Vis* **13**, 1327-1332 (2007).

9 Kumar, A., Bhattacharjee, S., Prakash, D. R. & Sadanand, C. S. Genetic analysis of two Indian families affected with congenital hereditary endothelial dystrophy: two novel mutations in SLC4A11. *Mol Vis* **13**, 39-46 (2007).

10 Ramprasad, V. L. *et al.* Novel SLC4A11 mutations in patients with recessive congenital hereditary endothelial dystrophy (CHED2). Mutation in brief #958. Online. *Hum Mutat* **28**, 522-523 (2007). https://doi.org/10.1002/humu.9487

11 Aldave, A. J. *et al.* Autosomal recessive CHED associated with novel compound heterozygous mutations in SLC4A11. *Cornea* **26**, 896-900 (2007). https://doi.org/10.1097/ICO.0b013e318074bb01

12 Hemadevi, B. *et al.* Identification of mutations in the SLC4A11 gene in patients with recessive congenital hereditary endothelial dystrophy. *Arch Ophthalmol* **126**, 700-708 (2008). https://doi.org/10.1001/archopht.126.5.700

13 Shah, S. S. *et al.* Mutation in the SLC4A11 gene associated with autosomal recessive congenital hereditary endothelial dystrophy in a large Saudi family. *Ophthalmic Genet* **29**, 41-45 (2008). https://doi.org/10.1080/13816810701850033

14 Aldahmesh, M. A., Khan, A. O., Meyer, B. F. & Alkuraya, F. S. Mutational spectrum of SLC4A11 in autosomal recessive CHED in Saudi Arabia. *Invest Ophthalmol Vis Sci* **50**, 4142-4145 (2009). https://doi.org/10.1167/iovs.08-3006

15 Paliwal, P. *et al.* Congenital hereditary endothelial dystrophy - mutation analysis of SLC4A11 and genotype-phenotype correlation in a North Indian patient cohort. *Mol Vis* **16**, 2955-2963 (2010).

16 Kodaganur, S. G. *et al.* Mutation analysis of the SLC4A11 gene in Indian families with congenital hereditary endothelial dystrophy 2 and a review of the literature. *Mol Vis* **19**, 1694-1706 (2013).

17 Park, S. H., Jeong, H. J., Kim, M. & Kim, M. S. A novel nonsense mutation of the SLC4A11 gene in a Korean patient with autosomal recessive congenital hereditary endothelial dystrophy. *Cornea* **32**, e181-182 (2013). https://doi.org/10.1097/ICO.0b013e31828d9ffd

18 Kim, J. H., Ko, J. M. & Tchah, H. Fuchs Endothelial Corneal Dystrophy in a Heterozygous Carrier of Congenital Hereditary Endothelial Dystrophy Type 2 with a Novel Mutation in SLC4A11. *Ophthalmic Genet* **36**, 284-286 (2015). https://doi.org/10.3109/13816810.2014.881510

19 Moazzeni, H. *et al.* Observation of nine previously reported and 10 non-reported SLC4A11 mutations among 20 Iranian CHED probands and identification of an MPDZ mutation as possible cause of CHED and FECD in one family. *British Journal of Ophthalmology*, e314377 (2019). https://doi.org/10.1136/bjophthalmol-2019-314377

20 Brejchova, K. *et al.* IPSC-Derived Corneal Endothelial-like Cells Act as an Appropriate Model System to Assess the Impact of SLC4A11 Variants on Pre-mRNA SplicingCorneal Endothelial-like Cells Expressing SLC4A11. *Investigative Ophthalmology & Visual Science* **60**, 3084-3090 (2019). https://doi.org/10.1167/iovs.19-26930

21 Frausto, R. F., Wang, C. & Aldave, A. J. Transcriptome analysis of the human corneal endothelium. *Invest Ophthalmol Vis Sci* **55**, 7821-7830 (2014). https://doi.org/10.1167/iovs.14-15021

22 Chng, Z. *et al.* High throughput gene expression analysis identifies reliable expression markers of human corneal endothelial cells. *PLoS One* **8**, e67546 (2013). https://doi.org/10.1371/journal.pone.0067546

23 Chen, Y. *et al.* Identification of novel molecular markers through transcriptomic analysis in human fetal and adult corneal endothelial cells. *Human Molecular Genetics* **22**, 1271-1279 (2012). https://doi.org/10.1093/hmg/dds527

24 Guha, S., Chaurasia, S., Ramachandran, C. & Roy, S. SLC4A11 depletion impairs NRF2 mediated antioxidant signaling and increases reactive oxygen species in human corneal endothelial cells during oxidative stress. *Sci Rep* **7**, 4074 (2017). https://doi.org/10.1038/s41598-017-03654-4

25 Liu, J. *et al.* Depletion of SLC4A11 causes cell death by apoptosis in an immortalized human corneal endothelial cell line. *Invest Ophthalmol Vis Sci* **53**, 3270-3279 (2012). https://doi.org/10.1167/iovs.11-8724

26 Zhang, W. *et al.* Glutaminolysis is Essential for Energy Production and Ion Transport in Human Corneal Endothelium. *EBioMedicine* (2017). https://doi.org/http://dx.doi.org/10.1016/j.ebiom.2017.01.004

27 Zhang, W. *et al.* Conditionally Immortal Slc4a11-/- Mouse Corneal Endothelial Cell Line Recapitulates Disrupted Glutaminolysis Seen in Slc4a11-/- Mouse Model. *Invest Ophthalmol Vis Sci* **58**, 3723-3731 (2017). https://doi.org/10.1167/iovs.17-21781

28 Ogando, D. G., Choi, M., Shyam, R., Li, S. & Bonanno, J. A. Ammonia sensitive SLC4A11 mitochondrial uncoupling reduces glutamine induced oxidative stress. *Redox Biology* **26**, 101260 (2019). https://doi.org/https://doi.org/10.1016/j.redox.2019.101260

29 Edelhauser, H. F. The balance between corneal transparency and edema: the Proctor Lecture. *Invest Ophthalmol Vis Sci* **47**, 1754-1767 (2006). https://doi.org/10.1167/iovs.05-1139

30 Bonanno, J. A. Molecular mechanisms underlying the corneal endothelial pump. *Experimental eye research* **95**, 2-7 (2012). https://doi.org/10.1016/j.exer.2011.06.004

31 Srinivas, S. P. Dynamic regulation of barrier integrity of the corneal endothelium. *Optom Vis Sci* **87**, E239-254 (2010). https://doi.org/10.1097/OPX.0b013e3181d39464

32 Riley, M. V., Winkler, B. S., Peters, M. I. & Czajkowski, C. A. Relationship between fluid transport and in situ inhibition of Na(+)-K+ adenosine triphosphatase in corneal endothelium. *Invest Ophthalmol Vis Sci* **35**, 560-567 (1994).

33 Zhang, W. *et al.* Energy Shortage in Human and Mouse Models of SLC4A11-Associated Corneal Endothelial Dystrophies. *Invest Ophthalmol Vis Sci* **61**, 39 (2020). https://doi.org/10.1167/iovs.61.8.39

34 Zhang, W. *et al.* Glutaminolysis is Essential for Energy Production and Ion Transport in Human Corneal Endothelium. *EBioMedicine* **16**, 292-301 (2017). https://doi.org/10.1016/j.ebiom.2017.01.004
35 Ehlers, N., Modis, L. & Moller-Pedersen, T. A morphological and functional study of Congenital Hereditary Endothelial Dystrophy. *Acta Ophthalmol Scand* **76**, 314-318 (1998). https://doi.org/10.1034/j.1600-0420.1998.760312.x
36 Ogando, D. G. *et al.* Inducible Slc4a11 Knockout Triggers Corneal Edema Through Perturbation of Corneal Endothelial Pump. *Invest Ophthalmol Vis Sci* **62**, 28 (2021). https://doi.org/10.1167/iovs.62.7.28
37 Loganathan, S. K., Schneider, H. P., Morgan, P. E., Deitmer, J. W. & Casey, J. R. Functional assessment of SLC4A11, an integral membrane protein mutated in corneal dystrophies. *Am J Physiol Cell Physiol* **311**, C735-C748 (2016). https://doi.org/10.1152/ajpcell.00078.2016
38 Li, S. *et al.* R125H, W240S, C386R, and V507I SLC4A11 mutations associated with corneal endothelial dystrophy affect the transporter function but not trafficking in PS120 cells. *Exp Eye Res* **180**, 86-91 (2019). https://doi.org/10.1016/j.exer.2018.12.003
39 Hara, S., Tsujikawa, M., Kawasaki, S. & Nishida, K. Homeostasis of SLC4A11 protein is mediated by endoplasmic reticulum-associated degradation. *Exp Eye Res* **188**, 107782 (2019). https://doi.org/10.1016/j.exer.2019.107782
40 Mittal, V., Sehdev, N. & Mittal, R. Descemet Membrane Endothelial Keratoplasty in Congenital Hereditary Endothelial Dystrophy: Initial Experiences. *Cornea* **40**, 972-976 (2021). https://doi.org/10.1097/ICO.0000000000002701
41 Panahi-Bazaz, M., Sharifipour, F. & Malekahmadi, M. Modified Descemet's Stripping Automated Endothelial Keratoplasty for Congenital Hereditary Endothelial Dystrophy. *J Ophthalmic Vis Res* **9**, 522-525 (2014). https://doi.org/10.4103/2008-322X.150836
42 Cunnusamy, K. *et al.* Congenital Corneal Endothelial Dystrophies Resulting From Novel De Novo Mutations. *Cornea* **35**, 281-285 (2016). https://doi.org/10.1097/ICO.0000000000000670
43 Bellucci, R., Chierego, C. & Bellucci, C. Endothelial keratoplasty in a newborn baby with CHED. *Cornea* **30**, 1488-1490 (2011). https://doi.org/10.1097/ICO.0b013e318221c2f3
44 Saad, A., Ghazzal, W., Keaik, M., Indumathy, T. R. & Fogla, R. Outcomes of Descemet's membrane endothelial keratoplasty for congenital hereditary endothelial dystrophy. *J AAPOS* **24**, 358 e351-358 e356 (2020). https://doi.org/10.1016/j.jaapos.2020.07.018
45 Fogla, R. & Srinivasan, B. Corneal Folds After Descemet Membrane Endothelial Keratoplasty in Congenital Hereditary Endotheial Dystrophy. *Cornea* **40**, 715-719 (2021). https://doi.org/10.1097/ICO.0000000000002471
46 Maumenee, A. E. Congenital hereditary corneal dystrophy. *Am J Ophthalmol* **50**, 1114-1124 (1960). https://doi.org/10.1016/0002-9394(60)90998-3
47 Mullaney, P. B., Risco, J. M., Teichmann, K. & Millar, L. Congenital hereditary endothelial dystrophy associated with glaucoma. *Ophthalmology* **102**, 186-192 (1995). https://doi.org/10.1016/s0161-6420(95)31037-8
48 Mehta, N. & Ramappa, M. Novel Proposed Algorithm in Congenital Hereditary Endothelial Dystrophy. *Semin Ophthalmol* **38**, 108-115 (2023). https://doi.org/10.1080/08820538.2022.2094713
49 Busin, M., Beltz, J. & Scorcia, V. Descemet-stripping automated endothelial keratoplasty for congenital hereditary endothelial dystrophy. *Arch Ophthalmol* **129**, 1140-1146 (2011). https://doi.org/10.1001/archophthalmol.2011.114
50 Kirkness, C. M., McCartney, A., Rice, N. S., Garner, A. & Steele, A. D. Congenital hereditary corneal oedema of Maumenee: its clinical features, management, and pathology. *Br J Ophthalmol* **71**, 130-144 (1987). https://doi.org/10.1136/bjo.71.2.130
51 Sajjadi, H. *et al.* Results of penetrating keratoplasty in CHED. Congenital hereditary endothelial dystrophy. *Cornea* **14**, 18-25 (1995).

52 al-Rajhi, A. A. & Wagoner, M. D. Penetrating keratoplasty in congenital hereditary endothelial dystrophy. *Ophthalmology* **104**, 956-961 (1997). https://doi.org/10.1016/s0161-6420(97)30200-0
53 Al-Ghamdi, A., Al-Rajhi, A. & Wagoner, M. D. Primary pediatric keratoplasty: indications, graft survival, and visual outcome. *J AAPOS* **11**, 41-47 (2007). https://doi.org/10.1016/j.jaapos.2006.09.012
54 Pearce, W. G., Tripathi, R. C. & Morgan, G. Congenital endothelial corneal dystrophy. Clinical, pathological, and genetic study. *Br J Ophthalmol* **53**, 577-591 (1969).
55 Javadi, M. A. *et al.* Penetrating keratoplasty in young children with congenital hereditary endothelial dystrophy. *Cornea* **22**, 420-423 (2003). https://doi.org/10.1097/00003226-200307000-00006
56 Shyam, R. *et al.* Rescue of the Congenital Hereditary Endothelial Dystrophy Mouse Model by Adeno-Associated Virus–Mediated Slc4a11 Replacement. *Ophthalmology Science* **2**, 100084 (2022). https://doi.org/https://doi.org/10.1016/j.xops.2021.100084
57 Ramappa, M. *et al.* Descemet Stripping Automated Endothelial Keratoplasty in Pediatric Age Group: A Decade of Our Experience. *Cornea* **40**, 1571-1580 (2021). https://doi.org/10.1097/ico.0000000000002811
58 Malhotra, D., Loganathan, S. K., Chiu, A. M., Lukowski, C. M. & Casey, J. R. Human Corneal Expression of SLC4A11, a Gene Mutated in Endothelial Corneal Dystrophies. *Sci Rep* **9**, 9681 (2019). https://doi.org/10.1038/s41598-019-46094-y
59 Song, L. *et al.* Ocular Tolerability and Immune Response to Corneal Intrastromal AAV-IDUA Gene Therapy in New Zealand White Rabbits. *Mol Ther Methods Clin Dev* **18**, 24-32 (2020). https://doi.org/10.1016/j.omtm.2020.05.014
60 Miyadera, K. *et al.* Intrastromal Gene Therapy Prevents and Reverses Advanced Corneal Clouding in a Canine Model of Mucopolysaccharidosis I. *Mol Ther* **28**, 1455-1463 (2020). https://doi.org/10.1016/j.ymthe.2020.04.004
61 Prakash, G., Sharma, N., Goel, M., Titiyal, J. S. & Vajpayee, R. B. Evaluation of intrastromal injection of voriconazole as a therapeutic adjunctive for the management of deep recalcitrant fungal keratitis. *Am J Ophthalmol* **146**, 56-59 (2008). https://doi.org/10.1016/j.ajo.2008.02.023
62 Kalaiselvi, G., Narayana, S., Krishnan, T. & Sengupta, S. Intrastromal voriconazole for deep recalcitrant fungal keratitis: a case series. *Br J Ophthalmol* **99**, 195-198 (2015). https://doi.org/10.1136/bjophthalmol-2014-305412
63 Sharma, N. *et al.* Evaluation of intrastromal voriconazole injection in recalcitrant deep fungal keratitis: case series. *Br J Ophthalmol* **95**, 1735-1737 (2011). https://doi.org/10.1136/bjo.2010.192815
64 Ellis, B. L. *et al.* A survey of ex vivo/in vitro transduction efficiency of mammalian primary cells and cell lines with Nine natural adeno-associated virus (AAV1-9) and one engineered adeno-associated virus serotype. *Virol J* **10**, 74 (2013). https://doi.org/10.1186/1743-422x-10-74
65 Song, L., Samulski, R. J. & Hirsch, M. L. Adeno-Associated Virus Vector Mobilization, Risk Versus Reality. *Hum Gene Ther* **31**, 1054-1067 (2020). https://doi.org/10.1089/hum.2020.118
66 Vilas, G. L. *et al.* Oligomerization of SLC4A11 protein and the severity of FECD and CHED2 corneal dystrophies caused by SLC4A11 mutations. *Hum Mutat* **33**, 419-428 (2012). https://doi.org/10.1002/humu.21655
67 Vilas, G. L., Morgan, P. E., Loganathan, S. K., Quon, A. & Casey, J. R. A Biochemical Framework for SLC4A11, the Plasma Membrane Protein Defective in Corneal Dystrophies. *Biochemistry* **50**, 2157-2169 (2011). https://doi.org/10.1021/bi101887z
68 Bonanno, J. A., Shyam, R., Choi, M. & Ogando, D. G. The H(+) Transporter SLC4A11: Roles in Metabolism, Oxidative Stress and Mitochondrial Uncoupling. *Cells* **11** (2022). https://doi.org/10.3390/cells11020197
69 Ogando, D. G. *et al.* Inducible Slc4a11 Knockout Triggers Corneal Edema Through Perturbation of Corneal Endothelial Pump. *Investigative Ophthalmology & Visual Science* **62**, 28-28 (2021). https://doi.org/10.1167/iovs.62.7.28

70 Gröger, N. *et al.* SLC4A11 Prevents Osmotic Imbalance Leading to Corneal Endothelial Dystrophy, Deafness, and Polyuria *. *Journal of Biological Chemistry* **285**, 14467-14474 (2010). https://doi.org/10.1074/jbc.M109.094680

71 Shyam, R. *et al.* Rescue of the Congenital Hereditary Endothelial Dystrophy Mouse Model by Adeno-Associated Viruse-Mediated Slc4a11 Replacement. *Ophthalmol Sci* **2** (2022). https://doi.org/10.1016/j.xops.2021.100084

72 Bonanno, J. A. Identity and regulation of ion transport mechanisms in the corneal endothelium. *Prog Retin Eye Res* **22**, 69-94 (2003). https://doi.org/10.1016/s1350-9462(02)00059-9

73 Vilas, G. L. *et al.* Transmembrane water-flux through SLC4A11: a route defective in genetic corneal diseases. *Hum Mol Genet* **22**, 4579-4590 (2013). https://doi.org/10.1093/hmg/ddt307

74 Han, S. B. *et al.* Mice with a targeted disruption of Slc4a11 model the progressive corneal changes of congenital hereditary endothelial dystrophy. *Invest Ophthalmol Vis Sci* **54**, 6179-6189 (2013). https://doi.org/10.1167/iovs.13-12089

75 Zhang, W. L., Ogando, D. G., Li, S. M., Liu, C. Y. & Bonanno, J. A. Slc4a11 knock-out model of Endothelial Corneal Dystrophy reveals a phenotype consistent with ammonia toxicity. *Investigative ophthalmology & visual science* **57**, 5303-5303 (2016).

76 Zhang, W. L., Ogando, D. G. & Bonanno, J. A. Glutamine is an essential contributor to the human corneal endothelial ATP pool. *Investigative ophthalmology & visual science* **56**, 2578-2578 (2015).

77 Zhang, W. *et al.* Sex Difference in Congenital Hereditary Endothelial Dystrophy and a Slc4a11-/- mouse model. *Investigative Ophthalmology & Visual Science* **63**, 2283-2283 (2022).

78 Karadag, R., Rapuano, C. J., Hammersmith, K. M. & Nagra, P. K. Causes of congenital corneal opacities and their management in a tertiary care center. *Arq Bras Oftalmol* **83**, 98-102 (2020). https://doi.org/10.5935/0004-2749.20200023

79 Rezende, R. A. *et al.* Congenital corneal opacities in a cornea referral practice. *Cornea* **23**, 565-570 (2004). https://doi.org/10.1097/01.ico.0000126317.90271.d8

80 Borik, K., Mohney, B. G., Hodge, D. & Reynolds, M. M. Birth prevalence and characteristics of congenital corneal opacities. *Eur J Ophthalmol*, 11206721231202900 (2023). https://doi.org/10.1177/11206721231202900

81 Bermejo, E. & Martínez-Frías, M. L. Congenital eye malformations: clinical-epidemiological analysis of 1,124,654 consecutive births in Spain. *Am J Med Genet* **75**, 497-504 (1998).

APPENDIX A

Nucleotide Sequence of AAV8-hSLC4A11. The complete sequence of AAV8-hSLC4A11 drug product, including the codon optimized human *SLC4A11* gene (in bold) and the different AAV8 cassette elements with the corresponding ITRs and promoter.

CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACC
TTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGG
GTTCCTTCTAGACAACTTTGTATAGAAAAGTTGGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATC
GCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGC
GGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTA
TATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTG
CCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCA
CCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCT
TGCGCTTAAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGCGT
GCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTG
ATGACCTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAGATCTGCACACTGG
TATTTCGGTTTTTGGGGCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGC
GGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGT
GCCTGGTCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCACCAGTTG
CGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAAATGGAGGACGCGGCGCTC
GGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCAT
GTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTCG
TCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGT
TAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATT
CTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCAGGTGTCGTGACAAGTTTGTACAAAA
AAGCAGGCTGCCACC**ATGAGCCAGGTCGGGGGGCGGGGAGACAGGTGCACACAGGAGGTCCAG**
GGCTTGGTCCATGGGGCTGGTGACCTTTCTGCTTCCCTTGCAGAAAACTCTCCCACCATGTCGC
AGAATGGATACTTCGAGGATTCAAGCTACTACAAGTGTGACACAGATGACACCTTCGAAGCCCG
AGAGGAGATCCTGGGGGATGAGGCCTTCGACACTGCCAACTCCTCCATCGTGTCTGGCGAGAGT
ATCCGTTTTTTTGTCAATGTCAACCTTGAGATGCAGGCCACCAACACTGAGAATGAAGCGACTTC
CGGTGGCTGTGTGCTCCTGCACACCTCCCGAAAGTACCTGAAGTTAAAGAACTTCAAGGAAGAG
ATCCGTGCGCACCGCGACCTAGATGGCTTCCTGGCGCAGGCCAGCATCGTCCTGAACGAGACGG
CCACCTCCCTGGATAACGTGCTGCGGACCATGCTTCGCCGCTTCGCCAGGGACCCTGACAACAA
TGAGCCCAACTGCAACCTGGACCTGCTCATGGCCATGCTCTTCACCGATGCCGGGGCACCCATG
CGGGGTAAAGTCCACCTGCTGTCAGATACCATCCAAGGGGTCACCGCCACAGTGACAGGGGTGC
GGTACCAGCAGTCGTGGCTCTGCATCATCTGTACCATGAAGGCCCTACAGAAGCGGCACGTGTG
CATCAGCCGCCTGGTTCGCCCACAGAACTGGGGGGAGAATTCCTGTGAGGTTCGGTTCGTCATC
CTGGTGCTGGCCCCACCCAAGATGAAAAGCACTAAGACTGCGATGGAGGTGGCGCGCACGTTTG
CCACCATGTTCTCGGATATCGCCTTCCGCCAGAAGCTCCTGGAGACCCGCACAGAGGAGGAATT
CAAGGAGGCCTTGGTGCATCAGAGACAGCTGCTCACCATGGTGAGCCACGGTCCAGTGGCGCC
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AAGGACTTTGTCCCTTTTGGGAAGGGCATCCGGGAGGACATCGCACGCAGGTTCCCCTTGTACC
CCTTGGACTTCACTGATGGCATTATTGGGAAAAACAAGGCTGTGGGCAAATACATCACCACCAC
CCTGTTCCTCTACTTCGCCTGCCTCCTGCCCACCATCGCTTTCGGGTCTCTCAATGACGAGAACA
CAGACGGGGCCATCGACGTGCAGAAGACCATAGCCGGGCAGAGCATCGGGGGCCTGCTCTACG
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GGTGATTCGTGTCATCTGTGATGACTATGACCTGGACTTCAACTCCTTCTACGCATGGACGGGC
CTGTGGAATAGTTTCTTCCTTGCGCTTTATGCCTTTTTCAACCTCAGCCTGGTCATGAGTCTCTT
CAAGAGGTCGACGGAGGAGATCATCGCCCTCTTCATTTCCATCACGTTTGTGCTGGATGCCGTC
AAGGGCACGGTTAAAATCTTCTGGAAGTACTACTATGGGCATTACTTGGACGACTATCACACAA
AAAGGACTTCATCCCTTGTCAGCCTGTCAGGCCTCGGCGCCAGCCTCAACGCCAGCCTCCACAC
TGCCCTCAACGCCAGCTTCCTCGCCAGCCCCACGGAGCTGCCCTCGGCCACACACTCAGGCCAG
GCGACCGCCGTGCTCAGCCTCCTCATCATGCTGGGCACGCTCTGGCTGGGCTACACCCTCTACC
AATTCAAGAAGAGCCCCTACCTGCACCCCTGCGTGCGAGAGATCCTGTCCGACTGCGCCCTGCC
CATCGCGGTGCTCGCCTTCTCCCTCATCAGCTCCCATGGCTTCCGGGAAATCGAGATGAGCAAG
TTCCGCTACAACCCCAGCGAGAGCCCCTTTGCGATGGCGCAGATCCAGTCGCTGTCCCTGAGGG

**CCGTCAGCGGTGCCATGGGCCTCGGCTTCCTGCTGTCCATGCTCTTCTTCATCGAGCAGAACTT
GGTGGCCGCCTTGGTGAATGCACCGGAGAACAGGCTGGTGAAGGGCACTGCCTACCACTGGGA
CCTCCTGCTCCTCGCCATCATCAACACAGGGCTGTCTCTGTTTGGGCTGCCTTGGATCCATGCCG
CCTACCCCCACTCCCCGCTGCACGTGCGAGCCCTGGCCTTAGTGGAGGAGCGTGTGGAGAACG
GACACATCTATGACACGATTGTGAACGTGAAGGAGACGCGGCTGACCTCGCTGGGCGCCAGCGT
CCTGGTGGGCCTGTCCCTGTTGCTGCTGCCGGTCCCGCTTCAGTGGATCCCCAAGCCCGTGCTC
TATGGCCTCTTCCTCTACATCGCGCTCACCTCCCTCGATGGCAACCAGCTCGTCCAGCGCGTGG
CCCTGCTGCTCAAGGAGCAGACTGCGTACCCCCCGACACACTACATCCGGAGGGTGCCCCAGAG
GAAGATCCACTACTTCACGGGCCTGCAGGTGCTTCAGCTGCTGCTGCTGTGTGCCTTCGGCATG
AGCTCCCTGCCCTACATGAAGATGATCTTTCCCCTCATCATGATCGCCATGATCCCCATCCGCTA
TATCCTGCTGCCCCGAATCATTGAAGCCAAGTACTTGGATGTCATGGACGCTGAGCACAGGCCT
TGAACCCAGCTTTCTTGTACAAAGTGGGAATTCCTAGAGCTCGCTGATCAGCCTCGACTGTGCCT
TCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCAC
TCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTA
TTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGAGAATAGCAGGCAT
GCTGGGGAGGGCCGCAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTC
GCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGT
GAGCGAGCGAGCGCGCAGCTGCCTGCAGGGGCGCCTGATGCGGTATTTTCTCCTTACGCATCTG
TGCGGTATTTCACACCGCATACGTCAAAGCAACCATAGTACGCGCCCTGTAGCGGCGCATTAAG
CGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCTTAGCGCCCGC
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CGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGT
GTCAGAGGTTTTCACCGTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATT
TTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCCTGGCCCGTGTCTCAAAATCTCT
GATGTTACATTGCACAAGATAAAAATATATCATCATGAACAATAAAACTGTCTGCTTACATAAAC
AGTAATACAAGGGGTGTTATGAGCCATATTCAACGGGAAACGTCGAGGCCGCGATTAAATTCCA
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AATCTATCGCTTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGC
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CAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCT
ACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTAC
CGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTC
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TGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTC
GGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTC
GGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTAT
GGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATG
T**