**SUPPLEMENTAL MATERIALS**

*Primers and PCR conditions used for CHST6 screening*

PCR reactions were performed using GoTaq® Green Master Mix (Promega) with the following conditions: initialization at 95°C for 3 minutes; 40 cycles of denaturing (95°C for 30 seconds), annealing (60 - 65°C for 30 seconds (see **Supplemental Table 1** for primer set-specific annealing temperatures)), and extension (72° for 35 seconds); and final elongation at 72°C for 5 minutes. Prior to sequencing, each amplicon was purified by treatment with Exonuclease I and Shrimp Alkaline Phosphatase (USB Corp.), followed by incubation at 37°C for 15 minutes and inactivation at 80°C for 15 minutes. Sanger sequencing of the purified PCR template was then performed (Laragen Inc., Culver City, CA). Replacement and deletion mutations upstream of *CHST6* were detected by PCR followed by gel electrophoresis using primer combinations F1-R1, F1-R1M, F2-R2 and F2M-R2, as described previously.[16](#_ENREF_16), [29](#_ENREF_29)

*GATK variant calling*

Aligned sequences were marked with duplication and underwent base quality score recalibration (BQSR). Variant calling of base quality score recalibrated sequences was performed with GATK HaplotypeCaller in GVCF mode, followed by variant quality score recalibration (VQSR). Genotype posterior was calculated and low-quality variants (Genotype Quality (GQ) < 20) were labeled. Any possible *de novo* variant in family trios was marked. Variants were labeled with dbSNP ID, and variants that were present only in unaffected individuals and not present in any affected individuals were removed. Common variants (MAF > 0.01) were removed based on global MAF in gnomAD, 1000Genome, TOPMED, ExAC and UK10K databases. Common variants in the South Asian population were also removed, based on South Asian MAF in gnomeAD r3.0 and 1000Genome databases. All computations were performed on the UCLA Hoffman2 Cluster.

*WES variant filtration*

All enrolled individuals from Families A, B, and C underwent WES. However, Family C was used as the index family as it contained the largest number of recruited individuals among the three families that underwent WES. Variants were first filtered to retain only novel or rare non-synonymous coding variants and splice region variants. Retained candidate variants were then further filtered under the following two scenarios: assuming autosomal recessive inheritance in Family C, homozygous or compound heterozygous variants in the same gene that segregated with the affected status in Family C; and assuming autosomal dominant inheritance in Family C, heterozygous variants that segregated with the affected status in Family C. Genes containing homozygous or compound heterozygous variants that segregated with the affected status in Family C were then screened in affected and unaffected members of Families A and B to determine segregation with the affected phenotype. Similarly, genes containing heterozygous variants that segregated with the affected status in Family C were then screened in affected and unaffected members of Families A and B to determine segregation with the affected phenotype.

*Human corneal endothelial cell line cell culture*

A telomerase immortalized human corneal endothelial cell line (HCEnC) was cultured in a 1:1 mixture of F12-Ham’s medium and M199 medium, supplemented with 5% fetal bovine serum (Corning), 20 μg/mL human recombinant insulin (Thermo Fisher Scientific), 20 μg/mL ascorbic acid (Sigma-Aldrich), 10 ng/mL recombinant human fibroblast growth factor (basic), 100 μg/mL penicillin (Thermo Fisher Scientific), and 100 μg/mL streptomycin (Thermo Fisher Scientific). The HCEnC line was maintained in a humidified incubator containing 5% CO2 at 37°C.

*Human corneal keratocyte cell culture*

A telomerase immortalized human corneal keratocyte cell line (HK) was cultured in DMEM supplemented with 10% fetal bovine serum (Corning), 100 μg/mL penicillin (Thermo Fisher Scientific), and 100 μg/mL streptomycin (Thermo Fisher Scientific). The HK line was maintained in a humidified incubator containing 5% CO2 at 37°C.

A close-up of a few images of a human body

Description automatically generated

**Supplemental Figure 1. Anterior segment ocular coherence tomography (AS-OCT) images of individuals with Peripheral macular endothelial dystrophy (PMED).** (A) Posterior corneal stromal opacities are observed in the right and left eyes of the proband in Family A. (B) Diffuse stromal edema is present in the right and left eyes of the proband in Family E. (C) Epithelial bullae, subepithelial fibrosis and diffuse stromal edema are present in the right and left eyes of the affected younger sister of the proband in Family E.

A screenshot of a video game

Description automatically generated

**Supplemental Figure 2. Functional assay of *CHST6* mutants including previously reported case.** Bar graph summary of 5D4+ KS relative fold change in human corneal endothelial cells (HCEnC) or human keratocytes (HK) transfected with mutant CGn6STwPro expression vectors compared to wild-type CHST6 expression construct (WT/WT). Previously reported compound heterozygous *CHST6* mutations c.-26C>A / c.803A>G, p.(Tyr268Cys) shown as c.- 26C>A/Y268C.[22](#_ENREF_22)