Genetics of Corneal Dystrophies

Program Number: 5648 Poster Board Number: B0151
Presentation Time: 11:30 AM–1:15 PM

Title: Transcriptomic Profiling of Posterior Polymorphous Corneal Dystrophy
Authors: Doug D. Chung, Ricardo F. Frausto, Benjamin Lin, E. Maryam M. Hanser, Anthony J. Aldave
Institution: Ophthalmology, Jules Stein Eye Institute, UCLA, Los Angeles, CA.
Purpose: To investigate the molecular basis of posterior polymorphous corneal dystrophy (PPCD) by examining the transcriptome in affected individuals and the effect of decreased ZEB1 expression on corneal endothelial gene expression.
Methods: RNA-seq analysis was performed on corneal endothelium from individuals with PPCD, age-matched controls, and primary corneal endothelial cell cultures (pHCEnC) transfected with siRNA targeting ZEB1. Transcriptomic analyses were performed, including differential gene expression, gene ontology and pathway. The expression of selected differentially expressed genes was validated by qPCR and/or assessed by in situ hybridization in the corneal endothelium of independent cases of PPCD.
Results: Corneal endothelium from two individuals with PPCD expressed (RPKM > 0.1) 83.7% (87/104) and 53.8% (56/104) of the 104 protein coding genes specific to ex vivo corneal endothelial cells. Comparing gene expression in the corneal endothelium of these affected individuals, one with and one without a truncating ZEB1 mutation, to controls revealed 5049 and 5952 differentially expressed (fold-change ≥ 2 and RPKM values ≥ 0.1) protein coding genes. Thirty-two genes associated with ZEB1 and three genes (BMP4, CCND1, ZEB1) associated with OVOL2 were differentially expressed in the same direction in both individuals with PPCD. Corroborating our RNAseq results, in situ hybridization in corneas from affected individuals demonstrated increased transcript levels of BMP4 and CCND1 and lower ZEB1 levels compared to a control cornea.
RNA-seq analysis and immunohistochemical staining demonstrated variable expression of each type IV collagen in corneas from affected individuals. Decreasing ZEB1 expression in pHCEnC resulted in altered expression (fold-changes ≥ 1.25, p-values <0.05, and RPKM values ≥ 0.1) of 711 protein coding genes that are associated with canonical pathways involved in cell proliferation, migration, adhesion, and morphology.
Conclusions: Identification of the altered transcriptome in PPCD and in a cell-based ZEB1 knockdown model of PPCD provides insights into the molecular alterations that characterize PPCD. Further study of the differentially expressed protein coding genes that are known to interact with ZEB1 and OVOL2 is expected to identify other candidate genes for PPCD that may harbor mutations in affected individuals without a ZEB1 or OVOL2 mutation.

Commercial Relationships: Doug D. Chung, None; Ricardo F. Frausto, None; Benjamin Lin, None; E. Maryam M. Hanser, None; Anthony J. Aldave, None
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Mitochondrial DNA Point Mutation

Program Number: 5649 Poster Board Number: B0152
Presentation Time: 11:30 AM–1:15 PM

Title: Endothelial Corneal Dystrophy Associated with A3243G Mitochondrial DNA Point Mutation
Authors: Mathieu F. Bakhoum1,2, Eugenia C. White1, Wei-Pu Wu1, Jesse D. Sengillo1, Gregory D. Kramer2, Henry D. Perry1,3, Stephen H. Tsang1
Institution: Ophthalmology, Columbia University Medical Center, Glen Oaks, NY; Ophthalmology, Nassau University Medical Center, East Meadow, NY; Ophthalmic Consultants of Long Island, Rockville Center, NY.
Purpose: The A3243G mitochondrial DNA (mtDNA) point mutation leads to a spectrum of syndromes ranging from MIDD (maternally inherited diabetes and deafness) to MELAS (mitochondrial encephalopathy with lactic acidosis and stroke-like episodes). Given the role of mitochondria in the metabolism of corneal endothelial cells in vitro, we tested the hypothesis that corneal endothelial dystrophy is present in patients carrying the A3243G mtDNA point mutation.
Methods: We performed a cross sectional, observational clinical study to identify endothelial corneal abnormalities in patients diagnosed with MIDD or MELAS. Slit-lamp corneal examination and specular microscopy were performed. Patients who were diagnosed based on clinical examination were genetically tested for the mitochondrial point mutation A3243G with pyrosequencing, a sensitive method that detects low rates of heteroplasmy.
Results: A3243G mtDNA point mutation was confirmed in all patients tested using pyrosequencing. Corneal endothelial changes were observed using slit-lamp examination, primarily guttata and beaten-bronze appearance. One patient had a retrocorneal opacity. None of the patients had signs or symptoms of corneal edema. The average corneal thickness was 536 μm. Specular microscopy showed mainly polymegathism along with guttata. Given that mitochondrial DNA mutation load can be variable in each eye due to heteroplasmy, measurements of individual eyes were analyzed separately, n=6. The average endothelial cell area was 402 μm² vs 358 μm² in controls, P = 0.04. The average coefficient of variation of cell size was 39.5% vs 33.3% in controls, P = 0.002. The endothelial cell count was 2519 cells per mm² vs 2796 cells per mm² in controls, P = 0.04. When compared to the average population, the average coefficient of variation was significantly higher than predicted for the patients’ age.
Conclusions: In MIDD, endothelial corneal dystrophy is a highly penetrant phenotype. Patients with the A3243G mtDNA point mutation exhibit signs of endothelial corneal dystrophy. This previously uncharacterized corneal dystrophy is mainly associated with polymegathism along with guttata, while maintaining appropriate corneal thickness. The prevalence of MIDD in the diabetic population ranges from 0.5% to 2.8%. This observation will help further unravel the role of mitochondria in the pathogenesis of endothelial corneal dystrophies.

Commercial Relationships: Mathieu F. Bakhoum, Eugenia C. White, None; Wei-Pu Wu, None; Jesse D. Sengillo, None; Gregory D. Kramer, None; Henry D. Perry, None; Stephen H. Tsang, None

Program Number: 5650 Poster Board Number: B0153
Presentation Time: 11:30 AM–1:15 PM

Title: Investigating the pathogenicity of VSX1 P247R and its role in corneal dystrophies
Authors: Anastasia M. Litke, Robert L. Chow
Institution: Biology, University of Victoria, Victoria, BC, Canada.
Purpose: Despite the association of missense mutations in the transcription factor-encoding gene, Visual System Homeobox 1 (VSX1), and two corneal dystrophies, posterior polymorphous...
dystrophy (PPD) and keratoconus, the role of VSX1 in these diseases is controversial. To address this issue, we utilized in vitro and in vivo approaches to determine the pathogenicity of VSX1 P247R, a change that is found in disease populations and is located in the highly conserved CVC domain.

**Methods:** Wild type and P247R VSX1 expression constructs were transfected in HEK 293T cells and transcriptional activity, protein stability and localization were examined. Transcriptional activity was investigated using a luciferase reporter assay that utilized a reporter construct carrying Gal4 upstream activator sites and VSX1 binding sites. Co-transfection of the reporter, VSX1 and HSFl-1 or VP16-Gal4 fusion activators allowed for VSX1-dependent transcriptional activity to be measured. Protein stability and localization were addressed with western blotting and immunohistochemistry respectively. To investigate the pathogenicity of the VSX1 P247R mutation in vivo, CRISPR/Cas9 gene editing was carried out on C57BL/6 mouse embryos at the one cell stage to generate stable mouse lines. Immunolabeling and histological analyses on VSX1 P247R mice were performed on retinal and corneal tissue and compared to wild type and Fxx1-null mouse controls.

**Results:** The in vitro reporter assay supported previous findings that wild-type VSX1 can function as a transcriptional repressor. VSX1 P247R, showed a 50% increase in repression when compared to wild-type VSX1 (P < 0.001, n = 9). No significant differences were observed with respect to protein stability and subcellular localization. Two independent mouse lines with the VSX1 P247R mutation were generated. Each line was confirmed by sequencing for accurate integration of the VSX1 P247R mutation and absence of off-target mutations.

**Conclusions:** Our results are consistent with previous findings that VSX1 can function as a transcriptional repressor and also showed that VSX1 P247R leads to increased transcriptional repression. This indicates a previously uncharacterized role for the CVC domain in regulating transcriptional activity. Although this supports the idea that VSX1 P247R is pathogenic, a comprehensive analysis of VSX1 P247R mice for retinal and corneal phenotypes is still in progress to support these findings.

**Commercial Relationships:** Anastasia M. Litke, None; Robert L. Chow, None

**Support:** CIHR Grant MOP 137 101

**Program Number:** 5651 **Poster Board Number:** B0154

**Presentation Time:** 11:30 AM–1:15 PM

**Gene Mutation and Genotype-Phenotype Description in Corneal Dystrophy Associated with TGFBI Genes**

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**Purpose:** To investigate the genetic mutation characteristics as well as the genotype-phenotype relations in patients with TGFBI gene-associated corneal dystrophy.

**Methods:** All patients received detailed clinical examination, including determining the best-corrected visual acuity (BCVA) with the Binocular International Standard Visual Acuity Chart (snellen visual acuity), and examining the anterior segment with the slit-lamp microscope; and some patients received corneal confocal laser scanning microscopy (HRT). TGFBI gene amplification and direct sequencing were conducted on the genome DNA of 73 cases of patients with corneal dystrophy through the polymerase chain reaction (PCR) technique.

**Results:** Nine kinds of TGFBI gene heterozygosis missense mutations were detected in 54 patients, including 8 reported pathogenic mutations: c.371G>A(p.R124H), c.370C>T(p.R124C), c.371G>T(p.R124L), c.1663C>T(p.R555W), c.1664G>A(p.R555Q), c.1514T>A(p.V505D), c.1859C>A(p.A620D), c.1877A>G(p.H656R) and the novel mutation first discovered in this research, the c.1694T>A(p.L565H). The mutations were mainly distributed in the exon 4, with the mutation frequency of 41%; of them, patients with Avellino corneal dystrophy who carried the c.371G>A(p.R124H) mutation accounted for the greatest proportion (32/54). The average onset age of the 54 positive patients was 29.1±17.0 years (range 2-61 years); Deposits could be seen from the corneal epithelium to the stroma layer in bilateral eye through the slit-lamp microscopy, and various mutations led to varied morphology of the corneal precipitates in patients.

**Conclusions:** Our finding expand the spectrum of TGFBI mutation, a novel pathogenic mutation is discovered in this research, and it is re-confirmed that c.371G>A (p.R124H) is the most common mutation form in Asian patients with TGFBI genetic mutation-associated corneal dystrophy. Patients with corneal dystrophy show phenotypic heterogeneity, the corneal precipitate features are distinctly related to the genetic mutation that it carries, and the corneal dystrophy classification method based on the molecular genetics can improve the accuracy of clinical diagnosis.

**Commercial Relationships:** Xu Ke, None

**Program Number:** 5652 **Poster Board Number:** B0155

**Presentation Time:** 11:30 AM–1:15 PM

**The functional impact of CRISPR-Cas9-mediated ZEB1 deficiency in human corneal endothelial cells**

Yue Li, Ricardo F. Frausto, Doug D. Chung, E. Maryam M. Hansen, Austin Kassels, Marina Zakharevich, Anthony J. Aldave. The Jules Stein Eye Institute, Monterey Park, CA.

**Purpose:** To investigate the impact of ZEB1 deficiency on corneal endothelial cell (CEnC) proliferation, migration and barrier function in a CEnC culture model of posterior polymorphous corneal dystrophy 3 (PPCD3).

**Methods:** ZEB1−/−, ZEB1−/+ and ZEB1−/− HCEnc-21T cell lines, generated using CRISPR-Cas9, were used to test the functional impact of ZEB1 deficiency on various cellular processes (cell proliferation, cell migration and cell barrier). To measure cell proliferation, each HCEnc-21T line was seeded at 10% confluence. No statistical difference in cell proliferation was observed between ZEB1−/− and ZEB1−/+ at any of the time points tested. Cell migration assays demonstrated significantly slower migration for ZEB1−/− compared with ZEB1−/+. The ZEB1−/− cells demonstrated significantly greater cell barrier function compared with both ZEB1−/− and ZEB1−/+ beginning at approximately 60 hours post-seeding. By 120 hours, ZEB1−/− established a significantly increased cell barrier function compared with ZEB1−/−, but significantly reduced compared with ZEB1−/+.

**Conclusions:** We have established a cell-based model of PPCD3 using CRISPR-Cas9. Herein, we provide the first experimental evidence that human corneal endothelial cells deficient in ZEB1 manifest altered cellular proliferation, migration and barrier function. However, additional investigation is necessary to clarify the relevance of these alterations to the development of PPCD3.
Generation of ZEB1-deficient human corneal endothelial cells using CRISPR-Cas9 as a model for posterior polymorphous corneal dystrophy

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1University of California Los Angeles, Santa Monica, CA; 2Jules Stein Eye Institute, Los Angeles, CA.

Purpose: To generate a human corneal endothelial cell (HCEnc) culture model of posterior polymorphous corneal dystrophy 3 (PPCD3), which is characterized by ZEB1 haploinsufficiency.

Methods: Editing of ZEB1 in an immortalized HCEnc line (HCEnc-21T) was accomplished using CRISPR-Cas9. To ensure effective knockout of all known ZEB1 alternative transcript variants, we targeted the Cas9 nuclease using a guide RNA (gRNA) to exon 4, which is shared by all known transcript variants. Optimal guide sequence and predicted off-target sites were obtained using the CRISPR Design web tool, which scores (0-100%) gRNA quality by accounting for off-target hits. HCEnc-21T were transfected with the pSpCas9(BB)-2A-Puro construct containing the Cas9 nuclease gene, gRNA and the puromycin selection marker. Subsequently, puromycin selection and limited dilution were performed to generate single cell clones. Clones were expanded and characterized for ZEB1 editing by Sanger sequencing and CRISP-IP, and for ZEB1 protein production by Western blotting (WB). Allele-specific sequencing was performed using PCR and TA cloning. Potential sites of off-target Cas9-mediated double-stranded DNA breaks were examined by Sanger sequencing.

Results: Submission of exon 4 sequence to the CRISPR Design web tool resulted in 12 gRNA sequences. The highest scoring (72%) gRNA was predicted to potentially cleave at 182 off-target sites, 12 of which were in coding regions. Of the 77 clones generated and screened using CRISP-1D, 11 were selected for ZEB1 protein analysis. WB showed that 5 clones lacked the wild type ZEB1 protein and 3 clones expressed moderate ZEB1 protein levels compared with 3 clones that expressed higher ZEB1 levels. Three clones representing each of the three genotypes (ZEB1+/+, ZEB1−/+ and ZEB1−/−) were selected for allele-specific sequencing and off-target screening. Allele-specific sequencing demonstrated that each clone possessed allele sequences consistent with the results obtained by WB. None of the three clones demonstrated indels at any of the top 9 off-target sites.

Conclusions: We demonstrate the utility of the CRISPR-Cas9 editing tool to generate HCEnc deficient in ZEB1 as a model for PPCD3.

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pH that follows the concurrent loss of the renal isof orm of NBCe1. To study the importance of NBCe1 to corneal health, in the absence of complications from systemic pH imbalance, we have generated a novel strain of conditional NBCe1-knockout mice. These mice have been engineered to retain renal expression of NBCe1, but to lack NBCe1 expression elsewhere. We hypothesized that these mice should exhibit corneal edema.

Methods: We studied male and female wild-type (WT) and knockout (KO) mice at 6-7 weeks of age. We measured blood pH using a handheld blood-analysis system. We assessed the central corneal thickness (CCT) of live mice using an ultrasonic pachymeter. We performed immunohistochemical analysis on sections of mouse eye and kidney to examine the presence or absence of NBCe1 and other membrane proteins important for maintaining corneal hydration.

Results: In KOs, renal NBCe1 expression and blood pH are normal. As expected, NBCe1 is absent from KO EECs. In WT s, CCT is 109 ± 4 μm (mean ± SEM, n=4), in KOs CCT is significantly greater 138 ± 4 μm (n=7; P=0.002, t-test), an observation consistent with our hypothesis. Unexpectedly, we find the expression of two other determinants of endothelial function to be disturbed in KOs: the H+/lactate cotransporter MCT1 is absent from KO EECs and the usually basolateral Na+/K+ - ATPase appears to be mislocalized to the apical membrane.

Conclusions: NBCe1 loss results in corneal edema in the anatomical presence of corneal endothelial cells. However, disturbed expression of other endothelial transporters in KOs indicates that, beyond the intrinsic transport activity of NBCe1, the protein plays an additional role in coordinating the expression of other components of the corneal hydration mechanism. Our model also suggests that mutations in NBCe1 that specifically affect non-renal NBCe1 could underlie rare instances of endothelial dystrophy for which the molecular basis is not known.

Commercial Relationships: Mark D. Parker, None; Sangita P. Patel, None; Ani ko Marshall, None; Cheikh Mballo, None; Emily E. Salerno, None

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Presentation Time: 11:30 AM–1:15 PM

SLC4A11 variant is associated with susceptibility to Fuchs endothelial corneal dystrophy patients in Korean Choun-Ki Joo1,2, Jung Seon Seo2, Jeevon Mok1. 1Catholic Institutes of Visual Science, Catholic Univ of Korea, Seoul, Korea (the Republic of); 2Seoul St. Mary’s hospital Eye Institute (SSEI), Seoul, Korea (the Republic of).

Purpose: To determine the possibility of solute carrier family 4, sodium borate transporter, member 11 (SLC4A11), as potential susceptibility candidate gene for Korean Fuchs’ cornea dystrophy patients, we investigated whether SNPs of SLC4A11 are associated with FECD patients.

Methods: Genomic DNA was extracted from blood samples of 8 families included 33 affected individuals and 69 sporadic patients with FECD, visited the Eye Center of Seoul St. Mary’s Hospital. To screen genetic variations in SLC4A11, we investigated using polymerase chain reaction and direct sequencing. Control individuals were selected from the general population without FECD.

Results: In this study, we detected 5 SNPs in promoter region, 7 SNPs and one deletion in intervening region, and 7 silence mutations in SLC4A11. In IVS8-15 a>c, the frequencies of the *a/*a, *a/*c and *c/*c genotypes were 48.6%, 41.7% and 9.7% in FECD patients and were 17.1%, 59.2% and 23.7% in control subjects, respectively. The *a/*a genotype (p=0.001, OR = 4.58) was significantly more prevalent in 6 affected patients of 6 families and 28 sporadic patients with FECD than among control subjects. FECD patients had significantly higher *a allele frequency than controls (p=0.001, OR = 2.59). The genotype distributions of all polymorphisms of SLC4A11 among the control subjects and the affected individuals were in Hardy-Weinberg equilibrium.

Conclusions: This is the first report of genetic variation screening of SLC4A11 in Korean FECD patients and our results suggested that a genetic variant, IVS8-15 a>c, seem to be associated with FECD predisposition in a Korean.

Commercial Relationships: Choun-Ki Joo, None; Jung Seon Seo, None; Jeevon Mok, None

Support: This research was supported by Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Education (2016R1A6A1A03010528)

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Presentation Time: 11:30 AM–1:15 PM

Genome-wide association study of Fuchs endothelial corneal dystrophy in a Japanese population

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Purpose: Fuchs endothelial corneal dystrophy (FECD, MIM: 136800) is an inherited, progressive degenerative disease that affects the corneal endothelium. In the United States, FECD is regarded as a common disease, i.e., ~5% of the population over 40 years of age suffer from the disease, whereas the prevalence of FECD is less amongst the Japanese population. A genome-wide association study (GWAS) revealed that an intronic variant (rs613872) in transcription factor 4 (TCF4) is a significant risk factor of late-onset FECD in Caucasians, and the expansion of the cystosine-thymine-guanine (CTG) trinucleotide repeat in the third intron of TCF4 was found to be associated with FECD in those patients. We also found that the CTG repeat in TCF4 was significantly expanded in Japanese FECD patients compared to the controls, although the frequency of patients possessing expanded repeats was less than that in Caucasian patients. These results suggested that additional undiscovered variants are likely involved in the pathogenesis of FECD in Japanese.

Methods: In this study, we performed a GWAS to identify novel susceptible variants in Japanese FECD patients. This study involved 55 Japanese FECD patients (17 males and 38 females, mean age: 68.7 years) and 445 Japanese controls (150 males and 295 females, mean age: 67.3 years). Genomic DNA from the subjects was genotyped on an Infinium HumanCoreExome BeadChip (illumina®, Inc.) with 538,448 variants according to the manufacturer’s protocol. After removing samples and variants that failed to pass the quality control testing, the genotype data were applied to a GWAS analyzing 278,032 autosomal variants.

Results: We were unable to obtain any significant variants within 1 Mb of the TCF4 locus. Especially, rs613872 in TCF4 turned out to be monomorphic in our population, which was supported by the Japanese data based on NCBI dbSNP. By contrast, we succeeded in obtaining some suggestive loci other than the TCF4 locus, which seemed to be associated with Japanese FECD patients. In particular, a genome-wide significant variant (P < 5.0 × 10-8) was identified at 6q15.

Conclusions: Our results suggest the existence of novel FECD-associated variants in a Japanese population, and should provide a

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Ocular manifestations of Fabry disease are well documented but a few publications addressed their longitudinal evolution over time. This observational clinical study aims to answer this question, with a specific interest in the corneal, conjunctival and lenticular manifestations.

Methods: This was an observational study conducted from 2010 to 2016. Subjects diagnosed with Fabry disease in Montreal area were investigated using polymerase chain reaction and direct sequencing. Subjects were recruited from 12 different families. CRISPR/Cas9 technology was used to generate unrelated 200 Keratoconus patients, visited the Eye Center of Seoul St. Mary’s hospital Eye Institute, Seoul, Korea (the Republic of).

Purpose: To determine the possibility of cornea remodeling related gene, aldehyde dehydrogenase 3A1 (ALDH3A1), as potential susceptibility candidate gene for Korean Keratoconus patients, we investigated whether SNPs of ALDH3A1 are associated with the Keratoconus patients.

Methods: Genomic DNA was extracted from blood samples of unrelated 200 Keratoconus patients, visited the Eye Center of Seoul St. Mary’s Hospital. To screen genetic variations in ALDH3A1, we investigated whether SNPs of ALDH3A1 are associated with the Keratoconus patients.

Results: In this study, we detected 5 SNPs (IVS3-193g>a, IVS3-170c>t, IVS3-62c>t, IVS3-43g>t, S134A) in exon 4, 4 SNPs (G309E, R314C, IVS7+33 c>t, IVS7+104 g>ct) in exon 7, 3 SNPs (IVS7-41 g>t, IVS7-29 g>a, P329A;rs2228100) in exon 8 and 1 SNP (Y413Y) in exon 10. Among them, the *g*a genotype of IVS3-193g>a (p=0.03, OR = 1.9) was significantly different between patient and control groups. In exon 8, the *C*/C genotype (p=0.01, OR = 2.1) and *C* allele (p=0.03, OR = 1.6) of rs2228100 (P329A) were strongly associated with the risk of Keratoconus in Korean population.

Conclusions: This is the first report of genetic variation screening of cornea remodeling related gene, ALDH3A1, in Korean keratoconus patients and our results suggested that genetic variations, ALDH3A1 gene seem to be associated with keratoconus predisposition in a Korean.

Commercial Relationships: Jeewon Mok, None; Choun-Ki Joo, None

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Early Ocular Manifestations of Fabry Disease in α-galactosidase A-deficient Rats

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Purpose: Fabry disease is an X-linked disorder caused by deficiency of lysosomal α-galactosidase A (α-Gal A) activity from mutation of the GLA gene. α-galactosyl glycolipids, notably globotriaosylceramide (Gb3) and globotriaosylsphingosine (lyso-Gb3), accumulate within lysosomes, leading to cellular dysfunction. Cornea verticillata, cataracts, and vascular tortuosity are common and useful diagnostic signs. Current mouse knockout models do not report the ocular phenotype of Fabry disease. We developed rats with α-Gal A deficiency and determined if they would express any of the ocular phenotypes seen in humans.

Methods: CRISPR/Cas9 technology was used to generate α-Gal A deficient (Gla KO) rats. At 3 months of age, serum glycolipids were quantified using mass spectrometry in 3 wild type (WT) and 3 KO male rats. Mean glycolipid levels were compared between WT and KO rats using an unpaired, two-tailed t-test. Three-month-old rats were examined and photographed with slit lamp biomicroscopy for anterior segment findings of Fabry disease. Corneal and lenticular opacities were scored by a masked examiner, and mean scores were compared using a Mann-Whitney test for males (WT vs KO) and a Kruskal-Wallis test for females (WT vs heterozygous vs KO).

Results: Gla KO rats have dramatic increases in the established biomarkers for patients with Fabry disease. Mean serum Gb3 levels increased over time (18%) vs baseline (4%). Fabry’s posterior cataracts was found in 38% of the subjects and retinal vessels tortuosity in 28%. Other significant findings include higher corneal hysteresis in Fabry’s group compared to a matched group of normal patients (X2 ; p=0.018). Threshold visual field was found abnormal in 42% of the cases, despite a normal OCT of the optic nerve.

Conclusions: Data collected over five years showed that cornea verticillata was the most common ocular sign, followed by conjunctival vessels tortuositities. New findings include upper lid vessels tortuosity. They also include the presence of microaneurysm elsewhere (inner canthus, lower palpebral conjunctiva) in some individuals. Corneal pigmentation did not vary significantly over time, but its presence is strongly correlated with the severity of the disease. To the contrary, blood vessels tortuositues seem to evolve as the disease progresses. Enzyme therapy does not seem to influence these outcomes.
concentration is 364 ± 59 nM in KO rats, which is elevated >100-fold above WT (P < 0.001). Mean serum lyso-Gb3 concentration is 151 ± 10 nM in KO rats, which is elevated >30-fold above WT (P < 0.001). There is no difference in serum non-α-galactosyl glycolipids, such as ganglioside GM3, in Gla KO and WT rat serum (P = 0.49). At three months, Gla KO (hemizygous males and homozygous females) rats develop significantly more anterior corneal opacities ranging from anterior deposits in a fine honeycomb pattern to a confluent plaque (P < 0.01). Lenticular opacities were also more common and ranged from multiple small punctate lenticular opacities to a denser central cataract. Histopathology revealed Oil Red O staining of the corneal epithelium and evidence of retinal vascular occlusion in KO rats.

Conclusions: The ocular phenotypes observed in Gla KO rats indicate that this rat model recapitulates early disease stages of Fabry disease. This genetic animal model may be used to test novel treatments and to study corneal and lenticular opacity development in Fabry disease.

Commercial Relationships: Iris S. Kassem; James J. Miller, None; Kazuhiro Aoki, None; Carly A. Murphy, None; Jonathon Young, None; Michael Tiemeyer, None; Nancy M. Dahms, None

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Presentation Time: 11:30 AM–1:15 PM

Corneal Findings in Arterial Tortuosity Syndrome
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1Ophthalmology, University of Arkansas for Medical Sciences, Little Rock, AR; 2Section of Genetics and Metabolism, University of Arkansas for Medical Sciences, Little Rock, AR; 3Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium.

Purpose: Arterial tortuosity syndrome (ATS) is a rare autosomal recessive disease clinically hallmarked by tortuosity, stenosis, and aneurysm development of large and medium sized arteries. Patients carry mutations in the SLC2A10 gene, encoding a facilitative glucose transporter, which causes severe disruption of tissue elastin. Several case reports have noted associated ophthalmic manifestations such as keratoconus, keratoglobus, and myopia without detailed descriptions or standardized examinations. We report the first dedicated case series describing the ophthalmic findings in patients with molecularly confirmed ATS and obligate carriers. We discuss the potential pathophysiology basis for ocular manifestations in ATS.

Methods: Five ATS patients and two carriers (6 children and 1 adult) presented for an ATS specialty clinic at the Arkansas Children’s Hospital in Little Rock, AR. Patients underwent full eye examinations (including corneal pachymetry, topography, and OCT / photos when indicated). One additional carrier (an adult and parent of an ATS patient) submitted eye examination information later, which we have included for completeness.

Results: All five patients with ATS had myopia (Avg. Spherical Equivalent -2.21) and thin corneas (Avg. Central Corneal Thickness 426.8 μm), four had evidence of corneal ectasia, two had early keratoconus, one had keratoglobus with deep stromal corneal opacities, one had bilateral high irregular astigmatism, and one had unilateral high regular astigmatism. Both assessed carriers had myopia and one of them had corneal thinning. The adult carrier, whose exam information was obtained later, developed keratectasia in one eye many years after LASIK eye surgery despite normal post-operative corneal thickness.

Conclusions: We document a spectrum of ophthalmic manifestations of ATS with universal findings of myopia, corneal thinning, and a strong propensity for corneal ectasia leading to keratoconus or keratoglobus. Our data warrants regular eye examinations for all ATS patients with follow-up tailored to clinical findings. Literature review affirms elastin as an important corneal constituent and indicates that lysyl oxidase could bridge the gap between the pathophysiology underlying ATS and its effects on the cornea.

Commercial Relationships: Joshua S. Hardin, None; Yuri Zarate, None; Bert Callewaert, None; David Warner, None