Corneal Endothelial Cell Density in Ametropic Children and Adolescents

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**Purpose:** Endothelial cell density (ECD) is known to decrease with age in adulthood. Less is known about ECD in childhood, particularly in children with substantial ametropia and amblyopia.

**Methods:** ECD was measured (n = 244 eyes) by automated and manual counting methods from noncontact scans obtained using a Nidek CS4 Confocal Microscope. Children and adolescents age 4-20 years (mean 10.2 years) were imaged before pediatric refractive excimer laser or phakic IOL surgery performed for ametropic amblyopia.

**Results:** Refractive error of myopes was an average -8.04 D (range -1 to -22.5) and of hyperopes +3.97 D (range +1.25 to 6.75) with mean cylinder 1.57 D. ECD was 3495 ± 244 cells/mm² in 4yo’s and 3020 ± 220 in 20yo’s. ECD decreased an average 28 cells/mm² per year of age (r² = 0.76; p < .01). Substantial differences were not found in ECD between hyperopic and myopic children (p = .24). ECD was ~ 6% greater than that reported in normal children age 5-14 years using 1990 era technology.

**Conclusions:** Highly ametropic and amblyopic children have ECD comparable to that reported previously in a population of normal children. ECD decreases in quasi-linear fashion throughout the first two decades of life.

**Commercial Relationships:** Nicholas Faron, None; James Hoekel, None; Lawrence Tykrens, None

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**Presentation Time:** 3:15 PM–5:00 PM

Endothelial Cell microRNA Expression in a Mouse Model of Fuchs Dystrophy

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**Purpose:** MicroRNAs (miRNAs) are a group of small non-coding RNAs, and regulate gene expression at the posttranscriptional level. We generated a corneal endothelial miRNA expression profile of a transgenic Col8a22ASiK:QH5K mouse model of early-onset Fuchs endothelial corneal dystrophy (FEDC) to analyze the role of differential miRNA expression in FEDC pathogenesis.

**Methods:** Clinical confocal microscopy was performed in Col8a22ASiK:QH5K mutant (MUT) and wild-type (WT) mice. Endothelial miRNA expression was analyzed in three respective groups of ten-month-old old MUT and WT mice using Taqman Array Rodent MicroRNA A+B cards v3.0 (Applied Biosystems) and the ExpressionSuite version 1.0 software package (Applied Biosystems). Differential expression of individual miRNAs was validated using stem-loop RT qPCR.

**Results:** Clinical confocal microscopy confirmed the FEDC and normal phenotype of MUT and WT mice. Taqman array analysis demonstrated differential expression of at least 55 miRNAs in MUT compared to WT mice applying 1.5-fold change and p<0.05 as cut-off criteria. Validation experiments using individual stem-loop RT qPCR assays (mmu-miR-362-5p, mmu-miR-10a, mmu-miR-34a, mmu-miR-532-5p, mmu-miR-34c, mmu-miR-322, mmu-miR-320 and mmu-miR-218-1#) showed good consistency with the initial array experiment.

**Conclusions:** The present study provides the first miRNA profile in a mouse model of FEDC and may serve as a helpful resource for further studies of the disease. Overlap and differences to a previously generated endothelial miRNA expression profile in human FEDC are discussed.

**Commercial Relationships:** Mario Matthaei, None; Laura Kallay, None; Claus Cursiefen, None; Albert S. Jun, None

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Commercial Relationships: Esben Nielsen, None; Jesper Hjortdal, None; Anders Ivarsen, None

Program Number: 994 Poster Board Number: A0383
Presentation Time: 3:15 PM–5:00 PM
Evaluation of the central and peripheral corneal endothelial cells in patients with Fuchs’ Endothelial Corneal Dystrophy
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Purpose: To analyze the clinical characteristics and the central and peripheral corneal endothelial cells of Japanese patients suffering from Fuchs’ Endothelial Corneal Dystrophy (FECD).
Methods: This study involved 96 eyes of 48 Japanese FECD patients. Patient age, sex, history of keratoplasty, severity of the disease, corneal endothelial cell density (ECD), and corneal thickness were analyzed. Severity was graded on the Krachmer Grading Scale (graded 1-5). Noncontact specular microscopy (SP-3000; TOMEY Corp., Nagoa, Japan) was used to evaluate corneal endothelium at the central region, and wide-field contact specular microscopy (prototype model; Konan Medical, Inc., Nishinomiya, Japan) was used to evaluate the peripheral corneal regions.

Results: Of the total 48 patients, 17 (35%) were male and 31 (65%) were female (mean age: 69 years). Of those, 30 eyes of 25 patients had undergone keratoplasty (penetrating keratoplasty: 5 eyes, Descemet’s Striping Automated Endothelial Keratoplasty: 25 eyes). Severity grading results revealed 3 (3%) Grade-1 eyes (>12 central guttae in either eye), 3 (3%) Grade-2 eyes (confluent central guttae 1-2mm), 9 (9%) Grade-3 eyes (confluent central guttae 2-5mm), 37 (39%) Grade-4 eyes (confluent central guttae >5mm), and 44 (46%) Grade-5 eyes (Grade 4+ edema). The mean ECD in the central region was 1385±655 cells/mm2 (15 eyes) (excluding the 30 keratoplasty eyes and 51 eyes in which ECD was unmeasurable). The mean ECD in the peripheral region (horizontal peripheral area at approximately 8mm) was 1540±713 cells/mm2 (29 eyes). The mean corneal thickness was 595±59μm. A negative correlation was found between peripheral ECD and corneal thickness (p=0.02).

Conclusions: The findings of this study show that analysis of the distribution of guttae in the central and peripheral corneal ECD is important for evaluating the severity and for elucidating the pathology of FECD.

Commercial Relationships: Hiroko Nakagawa, None; Tsutomu Inatomi, None; Shigeru Kinoshita, None

Program Number: 995 Poster Board Number: A0384
Presentation Time: 3:15 PM–5:00 PM
Corneal thickness and Intraocular pressure After DSAEK: comparison of Dynamic Contour tonometry with Goldmann Application tonometry
Barbara Borges, André Vicente, Rita Anjos, Luisa Vieira, Arnaldo D. Santos, Joana Ferreira, Vitor Maduro, Nuno Alves, João Feijão.
Centro Hospitalar Lisboa Central, Lisbon, Portugal.
Purpose: To compare intraocular pressure (IOP) measurements with dynamic contour tonometry (DCT) and Goldmann application tonometry (GAT) after Descemet’s stripping automated keratoplasty (DSAEK).
Methods: Transverse observational study of 22 eyes of 19 patients with successful DSAEK. IOP was measured with DCT and GAT and central corneal thickness (CCT) was determined using anterior chamber OCT. IOP measurements were compared and the CCT influence was evaluated. A control group with 22 eyes of 17 patients was also created.

Results: Mean IOP in pressure in patients with DSAEK evaluated by GAT was 13.9±3.7 and 17.4±3.3 with DCT. Mean CCT in these patients was 601.8±58.9. In the control group mean IOP evaluated by GAT was 13.5±2.4 and by DCT was 16.5±2.6. In this group mean CCT was 531.1±39.1. The IOP values obtained with DCT were in average higher than those obtained with GAT in both samples. The difference between the two IOP evaluating methods was similar in both groups. In the group of patients with DSAEK, no statistically significant correlation was found between CCT and the different IOP evaluating methods.

Conclusions: The IOP difference between GAT and TCD was not influenced in a statistically significant way by the CCT that was artificially increased in the patients with DSAEK. Therefore, both GAT and CCT can be used safely and reliably in these patients.

Commercial Relationships: Barbara Borges, None; André Vicente, None; Rita Anjos, None; Luisa Vieira, None; Arnaldo D. Santos, None; Joana Ferreira, None; Vitor Maduro, None; Nuno Alves, None; João Feijão, None

Program Number: 996 Poster Board Number: A0385
Presentation Time: 3:15 PM–5:00 PM
Selecting Fuchs Patients for Drug Trials Involving Endothelial Cell Migration
Atsuko Fujii1,2, Winston Chamberlain2, Mitsuyoshi Azuma1,3, Thomas R. Shearer1. 1Laboratory of Ocular Sciences, Senju Pharmaceutical Co, Ltd, Portland, OR; 2Department of Ophthalmology, Oregon Health & Science University, Portland, OR; 3Department of Integrative Biosciences, Oregon Health & Science University, Portland, OR.
Purpose: Patients with Fuchs endothelial dystrophy (FED) comprise a large pool of patients seeking corneal endothelial keratoplasties (EK). Pathological features of FED include corneal opacity and edema, decreased endothelial cell density, and endothelial cell polymegathism and pleomorphism. Since FED is usually late onset, patients may exhibit other geriatric eye conditions such as glaucoma and cataracts. Recent studies indicate that drug treatment has the potential for improving migration of endothelial cells in FED. Hence, FED might be managed by drug treatment before becoming severe enough to require EK. For a clinical trial of such a drug, we hypothesize that selecting an adequate number of FED with only moderately compromised cell densities will be challenging. Thus, the purpose of the present study was to determine the frequency and base line values of FED patients with moderately decreased cell densities.

Methods: A retrospective data mining study was performed on patient charts presenting at a large US NY academic health center by searching for diagnosis ICD9 code 371.57 and Fuchs corneal dystrophies, including those with prior cataract surgeries and/or existing glaucoma. Prior corneal transplants were excluded. Non-contact specular photomicroscopic data (Topcon-2000) were obtained from the central region whenever possible, and individual eyes were grouped according to cell density (cells/mm2): Severe (<800), Moderate (800-1500), and Mild (>1500).

Results: The values for 66 eyes from the FED patients were (mean ± SD): thickness 0.563 ± 0.058 mm, area 575 ± 310 μm2/cell, CV 22 ± 6, and density 1989 ± 700 cells/mm2. The Moderate subgroup with cell density values of 1228 ± 191 (14) comprised 21% of the total FED patient pool. Corneal thickness did not correlate with cell density in the overall FED pool or within the Moderate subgroup (Pearson correlation test, r2 = 0.01).

Conclusions: In an initial screening of a population, we found approximately 1/5 of the patients fell into the Moderate subgroup. When testing for enhanced endothelial cell migration by topical drugs, clinical trials will need to initially screen a large number of...
FED patients to obtain an adequate number of those with moderately compromised cell densities. Drs. Shearer and Chamberlain receive a research contract and/or consulting fees from, and Dr. Azuma and Ms. Fujii are employees of, Senju Pharmaceutical Co., Ltd.

Commercial Relationships: Atsuko Fujii, Senju pharmaceutical Co., Ltd. (E); Winston Chamberlain, Senju Pharmaceutical Co., Ltd. (C); Mitsuyoshi Azuma, Senju Pharmaceutical Co., Ltd. (E); Thomas R. Shearer, Senju Pharmaceutical Co., Ltd. (C)

Program Number: 997 Poster Board Number: A0386
Presentation Time: 3:15 PM–5:00 PM

Oxidative DNA damage in Fuchs endothelial corneal dystrophy

Adna Halilovic, Thore Schmedt, Ula Jurkunas. Ophthalmology, Scheepens Eye Research Institute, Mass Eye and Ear Infirmary, Harvard Medical School, BOSTON, MA.

Purpose: The corneal endothelium is a highly metabolically active tissue and is known for its high abundance of mitochondria. Fuchs endothelial corneal dystrophy (FED) is a progressive disease in which there is a loss of corneal endothelial cells leading to corneal edema and eventually corneal blindness. The goal of our study is to examine the effect of oxidative stress on DNA damage in FED.

Methods: Long-amplicon quantitative PCR (LA-QPCR) was performed to assess DNA damage using small (250bp) and large (8.9kb) mtDNA primers. Normal immortalized human corneal endothelial (HCEnCi) and Fuchs (FECi) cells were exposed to 25µM menadione (MN). DNA damage was quantified using a PicoGreen dsDNA quantitation assay. The PCR signal from a treated sample was compared to that from an untreated sample, which was expressed as lesion frequencies per 10kb DNA.

Results: In this study, amplification of a small mitochondrial target is a relative measurement of mtDNA copy number changes. We found a 3.8-fold decrease in mtDNA copy number of a 250bp-fragment of mtDNA in FECi compared to normal HCEnCi. A 5h exposure to 25µM MN resulted in 2.3-fold increase of mtDNA copy number in FECi compared to normal HCECi cells. In addition, 25µM MN treatment increased lesion frequency from basal condition by 12-fold in FECi cells compared to normal HCECi cells.

Conclusions: Our studies indicate that FECi cells are preferentially damaged by MN treatment compared to normal HCEnCi cells. Decrease in small mtDNA fragment in FED at basal state may signify a decrease in mitochondrial number and altered cellular dysfunction which may result in impaired energy metabolism yielding clinical manifestation of FED. Hence, a decrease in amplified mtDNA target fragment resulted in an increase in DNA lesions in FED as compared to normal endothelium suggesting a potential susceptibility of mtDNA damage in FED pathogenesis.

Commercial Relationships: Adna Halilovic, None; Thore Schmedt, None; Ula Jurkunas, None
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Presentation Time: 3:15 PM–5:00 PM

Bullous keratopathy after anterior chamber phakic intraocular lens: description and descemet membrane endothelial keratoplasty (DMEK) management

Alexandre Portmann, Julie Gueudry; Marc Muraine. Ophthalmology, CHU, Rouen, France.

Purpose: To describe features of bullous keratopathy after phakic anterior chamber intraocular lens implantation and their management with descemet membrane endothelial keratoplasty.

Methods: A retrospective case series of 19 patients implanted with anterior chamber intraocular lens, presenting with at least a unilateral bullous keratopathy, between 2008 and 2012 in a tertiary referral center. All patients had a complete ophthalmologic examination including best corrected visual acuity (BCVA), endothelial cell count, . The main outcome was the description of bullous keratopathy after phakic intraocular lens implantation. Secondary outcome measure was prognosis of surgical management with DMEK (Descemet membrane endothelial keratoplasty) in this indication.

Results: Thirty-seven eyes of this 19 patients had phakic lens intraocular implantation. 26 eyes (70.3%) presented bullous keratopathy. Seven patients (37%) had bilateral bullous keratopathy. Mean age of affected patients was 57 years. Phakic angle-supported intraocular lenses was responsible of bullous keratopathy in 24 eyes (92.3%) and iris-claw intraocular lens in two eyes (7.7%). Mean time between implantation and bullous keratopathy was 7.3 years. Twenty eyes (77%) had endothelial keratoplasty : before surgery, mean best corrected visual acuity was 1.07±0.68 LogMAR, after 6 months follow up, mean BCVA was 0.39±0.53 LogMAR. Mean endothelial cell density was.

Mean endothelial cell density decreased from 2800±298 cells/mm2 before surgery to 1613±501 cells/mm2 two months after surgery, and 1195±430 cells/mm2 6 months after surgery.

Conclusions: This study shows high incidence of bullous keratopathy after angle-supported intraocular lens implantation. Affected patients were young with high rate of bilateral decompensation (37%). Despite endothelial keratoplasty, low endothelial cell counts suggested different mecanisms to explain the difference observed with endothelial keratoplasty in Fuch’s dystrophy. Life monitoring and specially corneal periphery observation should be advised in this patients.

Commercial Relationships: Alexandre Portmann, None; Julie Gueudry, None; Marc Muraine, None

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Presentation Time: 3:15 PM–5:00 PM

Comparison of Non-contact Specular and Confocal Microscopy for the Evaluation of the Corneal Endothelium

Jianyan Huang, Jyotsna Maram, Cristina Modak, Srinivas R. Sadda, Vikas Chopra, Olivia L. Lee. Doheny Image Reading Center, Doheny Eye Institute, Los Angeles, CA.

Purpose: To compare endothelial cell assessments obtained by non-contact specular and confocal microscopy using the Konan NSP-9900 and Nidek ConfoScan4 systems.

Methods: Sixty healthy eyes and 40 diseased eyes (15 eyes with Fuchs’ Endothelial Dystrophy and 26 with glaucoma) were examined with the Nidek ConfoScan4 confocal microscope and the Konan NP-9900 specular microscope. Certificated anterior segment graders at the Doheny image reading center compared the images from both instruments side by side for image quality assessment. Endothelial cell density (ECD) measurements were calculated with each instrument's machine-generated automated (Konan Auto and Nidek Auto) and manual (Konan Center and Nidek Manual) methods, and compared to each other.

Results: All normal and glaucomatous eyes had gradable images. However, in corneas with Fuchs’ Endothelial Dystrophy, poor image quality precluded ECD grading in 6.7% (1/15) and 46.7% (7/15) of the eyes, as obtained by confocal and specular microscopy respectively. ECD values were significantly higher in glaucomatous eyes compared to control eyes, irrespective of the counting method (Table 1). Regardless of whether the eye was normal or glaucomatous, ECD values obtained manually from either device were not statistically significantly different (P>0.05).

In contrast, machine-generated ECD values were significantly...
different from manual results, measuring greater in all cases with specular microscopy. Machine-generated ECD values from confocal microscopy also differed significantly from manual determinations, but not in a consistent direction.

Table 1 ECD values obtained by 4 methods of either instrument. (Mean±SD cells/mm2)

<table>
<thead>
<tr>
<th>Method</th>
<th>Konan Auto</th>
<th>Konan Center Nidek</th>
<th>Auto Nidek</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2865±272</td>
<td>2690±299</td>
<td>2552±231</td>
</tr>
<tr>
<td>Glaucoma</td>
<td>2184±762</td>
<td>2013±736</td>
<td>1998±736</td>
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<tr>
<td>Glaucoma</td>
<td>2185±456</td>
<td>1998±736</td>
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</tbody>
</table>

**Conclusions:** Automatic machine-generated ECD measurements differed significantly from manual assessments, suggesting that the automated results should be used with caution. On the other hand, ECD values derived from the Konan Center method were comparable to Nidek’s Manual method in both normal and glaucomatous eyes, suggesting that, with manual grading, the two instruments can be used interchangeably for reliable ECD measurements. Because of a higher proportion of gradable images, confocal microscopy might be superior to specular microscopy for ECD measurements in Fuch’s corneal dystrophy.

**Commercial Relationships:** Jianyan Huang, None; Jyotsna Maram, None; Cristina Modak, None; Srinivas R. Sadda, Allergan (C), Allergan (F), Carl Zeiss Meditec (C), Carl Zeiss Meditec (F), Genentech (C), Genentech (F), Optos (C), Optos (F), Regeneron (C); Vikas Chopra, Allergan (C); Olivia L. Lee, Allergan (C), Allergan (F)

**Program Number:** 1000 Poster Board Number: A0389

**Presentation Time:** 3:15 PM–5:00 PM

**TGFBI, CHST6 and Gelsolin gene analysis in mexican patients with stromal corneal dystrophies**

Johanna Gonzalez1, 2, Arturo J. Ramirez-Miranda2, Sergio Hernandez-da Mota1, Juan C. Zenteno1.

**Purpose:** To present the results of clinical and genetic analysis in a group of Mexican patients with stromal corneal dystrophies.

**Methods:** Diagnosis of corneal dystrophy was confirmed by specialized ophthalmologic clinical examination. Molecular analysis included PCR amplification and automated direct sequencing of the complete coding region of TGFBI, CHST6 and Gelsolin genes.

**Results:** A total of 20 Mexican patients (13 females/7 males) ranging from 26 to 75 years of age, pertaining to 9 different families were included. Ten patients from 5 distinct pedigrees were clinically diagnosed with lattice corneal dystrophy, 3 patients from 2 different pedigrees with granular corneal dystrophy type 2 (or Avellino), 2 unrelated probands were classified as Finnish type corneal amyloidosis (or lattice type 2), and 1 subject was clinically diagnosed with macular corneal dystrophy.

Seven affected subjects from 4 out of 5 families diagnosed with lattice corneal dystrophy were shown to carry a heterozygous c.1877A>G mutation in exon 14 of the TGFBI gene, predicting a histidine to arginine change at codon 626 (p.H626R). Three affected subjects from the remaining lattice dystrophy family were shown to carry a heterozygous c.370C>T mutation in TGFBI exon 4, predicting a p.R124C mutation. Two subjects from a family with granular type 2 corneal dystrophy showed a heterozygous p.H626R mutation. Affected subjects from another granular type 2 corneal dystrophy pedigree carried a heterozygous c.1856T>G mutation in exon 14 of TGFBI, predicting a p.M619K substitution. Molecular analysis of the GSN gene in 2 unrelated probands with Finnish type corneal amyloidosis revealed a heterozygous c.654G>A transition in one of them, which predicts a p.D187N substitution. No GSN mutations were identified in the second unrelated proband with this diagnosis. Molecular analysis of the CHST6 gene in 1 patient clinically diagnosed with macular corneal dystrophy showed a homozygous c.328T>C transition, predicting a p.Y110C change.

**Conclusions:** Three genes, TGFBI, CHST6, and GSN, previously implicated in the disease, were screened in a group of Mexican patients. A total of 5 different mutations were demonstrated in these 3 genes, broadening our current knowledge of the mutational spectrum associated with stromal corneal dystrophies in our country.

**Commercial Relationships:** Johanna Gonzalez, None; Arturo J. Ramirez-Miranda, None; Sergio Hernandez-da Mota, None; Juan C. Zenteno, None

**Program Number:** 1001 Poster Board Number: A0390

**Presentation Time:** 3:15 PM–5:00 PM

**Metabolic defects and oxidative stress in keratoconus**

Dimitrios Karamichos1, Jesper Hjortdal2, Audrey E. Hutcheon3, John M. Asara4, James D. Zieske5, 6, Ophthamology, Dean McGee Eye Institute, University of Oklahoma Health Sciences Center, Oklahoma City, OK; 6Ophthamology, Schepens Eye Research Institute/ Massachusetts Eye and Ear and Harvard Medical School, Boston, MA; 6Division of Signal Transduction/Mass Spectrometry Core, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; 6Ophthamology, Aarhus University Hospital, Aarhus C, Denmark.

**Purpose:** Corneal bulging, stromal thinning and scarring are three of the main characteristics of the Keratoconus (KC) disorder.

KC in most cases appears in early adulthood and can progress rapidly resulting in severe vision loss. Its prevalence ranges from 4-600/100,000 people worldwide. While efforts have been made over the years, the exact cause of the disease remains unknown. The aim of this study was to identify alterations in endogenous metabolites in the tears of KC patients depending on treatment.

**Methods:** 16 patients were included in this study and monitored using Pentacam tomography. Human tears were collected from healthy individuals and KC patients with different treatments. Three groups were tested: 1) Age-matched controls with no eye disease, 2) KC – No Correction, and 3) KC – Rigid Gas permeable lenses. All samples were processed for metabolomic analysis using LC/MS/MS.

**Results:** We evaluated 16 patients, 12 of which have been diagnosed with KC. Ages ranged between 20-53 y/o with maximum keratomic (Kmax) values of 44.9 - 63.7. We identified a total of 296 different metabolites of which >30 were significantly regulated between groups. The citric acid cycle included some of the affected metabolites that had notable changes, such as Fumarate, which had a ~4-fold upregulation in Group 2 and a 2-fold downregulation in Group 3 when compared to Healthy-No KC (Group 1). Also, Oxaaloacetate had an ~2-fold downregulation in both Groups 2 and 3 when compared to Group 1. The oxidation state (redox) was also severely affected. Group 2 was under severe oxidative stress causing multiple metabolites to become upregulated when compared to the other Groups. Lactate and Arginine are just two of the significantly regulated metabolites related to redox. Lactate and Arginine levels were elevated 7 and 4 fold, respectively, in Group 2 compared to the others. Glutathione exists in both reduced (GSH) and oxidized (GSSG) states. A decreased GSH-to-GSSG ratio is considered indicative of oxidative stress; Group 2 showed 4-fold downregulation when compared to others, indicating significant oxidative stress levels.

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Conclusions: Overall, our data supports the growing consensus that levels of metabolites are predictive for particular processes and conditions. These in vivo data showed clear links with our in vitro findings as well as indicating metabolism alterations in the tears of KC patients dependent on treatment.

Commercial Relationships: Dimitrios Karamichos, None; Jesper Hjortdal, None; Audrey E. Hutcheon, None; John M. Asara, None; James D. Zieske, None

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Program Number: 1002 Poster Board Number: A0391
Presentation Time: 3:15 PM–5:00 PM

Association of CHRD1 Mutations with X-linked Megalocornea and Megalocornea-Mental Retardation (MMR) Syndrome

Sek Shir Cheong1, Alice Davidson1, Vincent Plagnol2, Jonathan B. Ruddle1, Hala Ali1, Jessica C. Gardner1, Jens M. Hertz1, Daniela T. Pilz1, Stephen J. Tuft1, Alison J. Hardcastle1. 1Institute of Ophthalmology, University College London, London, United Kingdom; 2Genetics Institute, University College London, London, United Kingdom; 3Department of Ophthalmology, Centre for Eye Research, University of Melbourne, Victoria, VIC, Australia; 4Moorefields Eye Hospital, London, United Kingdom; 5Department of Clinical Genetics, Odense University Hospital, Odense, Denmark; 6Institute of Medical Genetics, University Hospital of Wales, Cardiff, United Kingdom.

Purpose: CHRD1 mutations have previously been reported to cause X-linked megalocornea (MGC1). In this study, eight MGC1 families were ascertained and screened for CHRD1 mutations. Megalocornea is also a key pathognomonic feature of Megalocornea-Mental Retardation (MMR) syndrome. MMR is a rare, phenotypically heterogeneous condition and the underlying genetic cause(s) are unknown. We therefore performed whole exome sequencing (WES) to identify the causative gene(s) in a male patient diagnosed with MMR.

Methods: After informed consent was obtained genomic DNA was isolated from whole blood. All coding exons and splice sites of CHRD1 were amplified by PCR, followed by Sanger sequencing. WES was performed for the MMR patient. The WES dataset was filtered for rare variants with a minor allele frequency ≤ 0.01 and cross-referenced with genes that have been reported to cause intellectual disability, hypotonia or seizures that are features of MMR. This dataset was generated with reference to the KEGG disease database. Candidate variants were then validated by PCR and Sanger sequencing.

Results: In each MGC1 family, a novel CHRD1 mutation was identified which included nonsense mutations (p.Cys80X and p.Cys99X), missense mutations (p.Cys289Arg and p.Cys291Tyr), a frameshift mutation (p.Glu101Glyfs*42), a splice site mutation (c.1247_1247-1del(GG)) and an entire CHRD1 gene deletion. Interestingly, analysis of the WES dataset for the MMR patient resulted in identification of a novel missense mutation in CHRD1 (p.Cys155Tyr) that was subsequently validated. The mother of the MMR proband was found to be a carrier of the CHRD1 mutation. Although his ocular phenotype could therefore be attributed to CHRD1, his non-ocular phenotypes (including intellectual disability and seizures) are unlikely to be caused by CHRD1. The WES dataset was also interrogated for rare variants in genes known to cause these conditions, but no likely causative mutations were identified.

Conclusions: All MGC1 families investigated were found to have a mutation in CHRD1. The missense mutations identified are located at conserved cysteine residues within von Willebrand factor C domains. We describe the first mutation causing the ocular component of MMR syndrome. CHRD1 screening in other male MMR patients will reveal the contribution of mutations in this gene.

Commercial Relationships: Sek Shir Cheong, None; Alice Davidson, None; Vincent Plagnol, None; Jonathan B. Ruddle, None; Hala Ali, None; Jessica C. Gardner, None; Jens M. Hertz, None; Daniela T. Pilz, None; Stephen J. Tuft, None; Alison J. Hardcastle, None

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Program Number: 1003 Poster Board Number: A0392
Presentation Time: 3:15 PM–5:00 PM

Comprehensive Assessment of Genetic Variants within TCF4 in Fuchs Corneal Dystrophy

Keith H. Baratz1, Ross A. Aleff2, Jean-Pierre A. Kocher3, Bruce W. Eckloff4, Elizabeth J. Atkinson5, Saurabh Baheti1, Sumit Middha1, Sanjay V. Patel6, Eric D. Wieben7, 1Ophthalmology, Mayo Clinic, Rochester, MN; 2Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN; 3Health Sciences Research - Biomedical Statistics and Informatics, Mayo Clinic, Rochester, MN; 4Medical Genome Facility, Mayo Clinic, Rochester, MN.

Purpose: A genome-wide association study identified the single nucleotide variant (SNV), rs613872, in the transcription factor 4 (TCF4) gene to be strongly associated (p = 6 x 10^-26) with Fuchs endothelial corneal dystrophy (FECD). Subsequently, an unstable expansion of the repeating trinucleotides, TGC, in a non-coding portion of the gene was found to be even more predictive of the disease. We performed comprehensive sequencing of the entire TCF4 gene region in order to identify the best genetic marker for FECD within TCF4 and to identify other novel variants that may be involved in disease pathogenesis.

Methods: Leukocyte DNA was isolated from 78 unrelated subjects with FECD (modified Krachmer grade 2 through 6 in the more affected eye) and 16 unaffected (grade 0) individuals. An Agilent custom capture panel was used to isolate the entire region surrounding the two previously-validated genomic markers of FECD. Sequencing of the captured region, spanning 465 kilobases and including the entire TCF4 coding region, introns and flanking sequence was performed at >200x average coverage using the Illumina HiSeq 2000.

Results: Trinucleotide expansion (>50 TGC repeats) was present in 53 (68%) FECD-affected and 1 (6%) normal subject and absent in the remaining 25 affected and 15 normal subjects. A total of 1651 variants, including 1493 single nucleotide variants (SNVs), were identified in this sample set, of which only 2 previously reported SNVs resided in the coding region of TCF4. Neither of these coding SNVs segregated with disease status. No variant, including TGC expansion, correlated perfectly with disease status. Trinucleotide repeat expansion was a better predictor of disease than any other variant. A total of 366 SNVs found in these samples were previously unreported in either dbSNP or the 1000 genome samples.

Conclusions: Complete sequencing of the genomic region identified by the previous genome-wide association study revealed no single causative variant for FECD. The intronic, trinucleotide repeat expansion within TCF4 was more strongly associated with FECD than any other variant and, therefore, is likely a pathogenic contributor to the disease.

Commercial Relationships: Keith H. Baratz, , , , Ross A. Aleff, , , , Jean-Pierre A. Kocher, None; Bruce W.
A severity score was created for each patient using the sum of the grades from both eyes. Association of the expanded TCF4 CTG 18.1 allele with the severity of FECD and the likelihood of requiring keratoplasty were evaluated.

**Results:** 240 subjects with FECD were recruited. Over two thirds of patients were females (N=165, 68.8%) and the majority were Caucasians (N=218, 90.8%). Patients with expansion negative FECD (N=158, 65.8%) had a mean age of 68.32 years ± 13.88 (mean ± SD), 63.9% were females (N=101) and 98.1% were Caucasians (N=155). Patients with expansion positive FECD (N=82, 34.2%) had a mean age of 63.45 years ± 13.18 (mean ± SD), 78.0% were females (N=64) and 76.8% were Caucasians (N=63). Compared with the expansion negative FECD subgroup, patients with the expanded CTG18.1 allele had increased clinical severity of FECD (P<0.0001) and were more likely to undergo keratoplasty (p=0.0004). No difference was found in the prevalence of the expanded allele among familial vs. presumed sporadic cases (p=0.1704).

**Conclusions:** The expanded TCF4 CTG 18.1 allele confers significant risk for increased clinical severity of FECD and the likelihood of requiring keratoplasty for visual rehabilitation. The presence of the expanded CTG 18.1 allele was not significantly different among familial and presumed sporadic cases in this cohort.

**Commercial Relationships:** Ahmed Z. Soliman, None; Xin Gong, None; Imran Hussain, None; Chao Xing, None; Vinod V. Mootha, 1\textsuperscript{,} 3

**Support:** R01 EY022161, NEI Grant P30EY020799, National Institutes of Health, Bethesda, MD and an unrestricted grant from Research to Prevent Blindness, New York
Purpose: Posterior amorphous corneal dystrophy (PACD) is a rare, autosomal dominant disorder linked to a 3.5 Mb region on chromosome 12q21.33. We sought to identify the genetic basis of PACD in the family linked to this region (family 1) as well as in two other affected families.

Methods: Whole exome sequencing (WES) was performed using DNA from 5 affected and 1 unaffected members of family 1. Copy number variant (CNV) analysis was performed by cyogenetic array using DNA from 9 members of family 1, 3 members of a second (unreported) family and one member of a third (previously reported) family with PACD. Copy number analysis by qPCR was performed for 43 (12 affected and 31 unaffected) individuals from family 1, 7 (6 affected and 1 unaffected) individuals from family 2 and in 3 (2 affected and 1 unaffected) individuals from family 3. qPCR was used to determine keratocyte transcript levels for the small leucine-rich proteoglycans (SLRP) encoded by genes in the PACD locus.

Results: WES failed to identify a novel, heterozygous, non-synonymous coding region mutation in the PACD locus that segregated with the affected phenotype. CNV analysis by cyogenetic array detected a 701kb heterozygous deletion in PACD locus containing the four SLRP genes (EPYC, KERA, LUM, and DCN) and a fifth protein-coding gene, CCER1, in family 1. The deletion was confirmed to segregate in the other family members using copy number analysis by qPCR. In family 2, a 1.318Mb heterozygous deletion that involved only the SLRP genes and CCER1 was detected in the PACD locus in affected individuals only. In family 3, the same 701kb deletion present in family 1 segregated with the affected phenotype, although haplotype analysis demonstrated that the families were not related. Evaluation of corneal expression of the SLRP genes demonstrated that KERA exhibited significantly higher expression than DCN, which showed a higher level of expression than EPYC and CCER1.

Conclusions: PACD is caused by a heterozygous deletion of a region on chromosome 12q21.33 that contains the four SLRPs, KERA, LUM, DCN and EPYC, as evidenced by the segregation of the deletion with the affected phenotype in an unreported and 2 previously reported families. The only other protein-coding gene contained within the deleted region, CCER1, is unlikely to play a role in the pathogenesis of PACD given its negligible expression in the cornea.

Commercial Relationships: Anthony J. Aldave, None; Michelle Kim, None; Ricardo F. Frausto, None; George Rosenwasser, None; Edwin M. Stone, None

Support: NIH Grant R01 EY022082

Program Number: 1008 Poster Board Number: A0397
Presentation Time: 3:15 PM–5:00 PM

Inhibitory effect of tranilast on transforming growth factor beta expression in corneal fibroblasts derived from granular corneal dystrophy type II

Ae Young Kwak1, 2, Han Lee1, 2, Kyoungh Young Yoo3, 2, Hyung Keun Lee1, 2, Eung Kweon Kim1, 2, Tae-in Kim1, 2.

The Institute of Vision Research, Department of Ophthalmology, Yonsei University College of Medicine, Seoul, Republic of Korea; "Cornea Research Institute, Yonsei University College of Medicine, Seoul, Republic of Korea.

Purpose: To investigate the effects of tranilast on expression of the transforming growth factor beta (TGFβ) receptor type II (TβR II) and the TGFβ receptor type I (TβRI) in corneal fibroblasts derived from patients with granular corneal dystrophy type II (GCD 2) compared with normal control fibroblasts.

Methods: Corneal fibroblasts were incubated with tranilast (0.01, 0.025, 0.05, 0.1, 0.25, 0.5 or 1.0 mM), after which cell proliferation and cytotoxicity were measured. Changes in expression of TGFβRI and TGFβRII mRNA after application of tranilast in WT and HO GCD 2 corneal fibroblasts co-treated with TGF-β (5.0 ng/mL) were examined by Western blot analysis and real-time polymerase chain reaction, respectively. Furthermore, effects of tranilast on type I collagen expression were measured. The expression of type I collagen was quantified using Western blot analysis and real-time polymerase chain reaction.

Results: Tranilast decreased the number of viable corneal fibroblasts, but did not induce cytotoxicity. TGF-β increased expression of TGFβRII and TGFβRI mRNA in WT and HO GCD 2 corneal fibroblasts. Application of tranilast reduced levels of TGFβRI and TGFβRII compared with untreated control fibroblasts. Application of tranilast reduced levels of TGF-β but did not induce cytotoxicity in WT and HO GCD 2 corneal fibroblasts. Application of tranilast reduced levels of TGFβRI and TGFβRII compared with untreated control fibroblasts.

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TGF-β, and pSmad3 in HO corneal fibroblasts. Tranilast delayed wound healing by inhibiting expression of α-SMA and integrins.

**Conclusions:** Tranilast can be used for delaying or prevention of recurrence of corneal opacity in TGFBI-linked corneal dystrophies by inhibiting TGF-β signaling pathway.

**Commercial Relationships:** Ae Young Kwak, None; Hun Lee, None; Kyoung Yul Seo, None; Hyung Keun Lee, None; Eung Kweon Kim, None

**Program Number:** 1009 **Poster Board Number:** A0398

**Presentation Time:** 3:15 PM–5:00 PM

**Phenotype of a Potential New Corneal Endothelial Dystrophy in the Middle East**

Ashley Behrens1, 2, Hind Alkatan1. 1King Khaled Eye Specialist Hospital, Riyadh, Saudi Arabia; 2Ophthalmology, Johns Hopkins Wilmer Eye Inst, Baltimore, MD.

**Purpose:** To describe and compare the clinical findings/histopathology features of a series of patients with a potentially newly identified corneal endothelial cell dystrophy with distinct features.

**Methods:** Corneal specimens were obtained from penetrating keratoplasty/Descemet’s membrane samples with clinical diagnosis of primary corneal decompensation (n=9). Cases with previous surgery or a clinical diagnosis of eye disease (corneal dysgenesis or ICE) were excluded. Histopathologic findings of specimens were compared to patients with documented clinical diagnosis of pseudoexfoliation keratopathy (PEX) (n=4).

**Results:** 13 specimens with corneal decompensation were reviewed, none showed histopathologic features of Fuchs (no guttata). 4 cases had history of PEX and showed findings matching stage 1 PEX as described by Naumann et al. in year 2000. However, 9 cases shared a unique appearance with a thin fibrillar layer between Descemet and endothelium, along with significantly low endothelial cell counts. Endothelial cells were enlarged, presenting flattened nuclei with or without pigment deposits. Polymegathism, pleomorphism and low cell counts were identified at specular microscopy. In this group, left eye was more affected in 2/3, male to female was 5:4, and age of onset was 55-82 with a mean of 67. In the PEX cases, both eyes were equally affected, male to female was 3:1 and the age of onset was 66-77 with a mean of 73.

**Conclusions:** This appears to be a new primary endothelial dystrophy with distinct clinical and histopathological features different from Fuchs dystrophy or PEX keratopathy. Affected patients have gradual endothelial cell loss without guttata in the absence of previous surgery or concomitant eye disease. Pedigree and genotype studies are underway in the families affected by this condition.

**Expression studies of keratoconus corneal buttons reveal abnormalities in the regulation of extracellular matrix and adhesion molecules**

Yelena Bykhovskaya1, 3, Helen P. Makarenkova2, Yaron S. Rabinowitz1, 3. 1Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA; 2Department of Cell and Molecular Biology, The Scripps Research Institute, La Jolla, CA; 3Cornea Genetic Eye Institute, Beverly Hills, CA.

**Purpose:** Keratoconus (KC) is a non-inflammatory corneal disorder of complex genetic inheritance characterized by progressive corneal thinning. Genes encoding proteins of extracellular matrix (ECM), including collagens, and adhesion molecules have been proposed to contribute to normal variation of central corneal thickness and a number of corneal diseases, including KC. In this study we performed
expression study to identify changes in the expression of these genes in KC patients, patients with corneal opacities, and controls.

**Methods:** We analyzed 20 corneal tissue samples from 13 KC patients, 2 patients with corneal opacities and 5 controls. We used Human Extracellular Matrix & Adhesion Molecules Profiler PCR Array (SABiosciences) to measure expression levels of 84 genes using total RNA extracted from the corneal tissue. To identify statistically significant changes in gene expression, Ct values were analyzed using the Data Analysis software v.3.5 (SABiosciences).

**Results:** Unsupervised clustering analysis revealed several distinct expression signatures between KC patients, patients with corneal opacities, and normal controls. Comparison of KC and control corneas with thresholds of fold change of 1.5 or greater and p-value of 0.05 or lower, revealed 25 differentially expressed genes, 17 genes were significantly downregulated and 8 upregulated. Among transcripts downregulated in KC patients we identified several collagens, integrins, metalloproteinases, as well as tissue inhibitors of metalloproteinases, THBS1, and FN1. Among upregulated genes, we identified TGFBI (p=0.0009), which plays a role in cell-collagen interactions, and was previously identified in the KC library constructed by our group. TGFBI mutations have been linked to several corneal dystrophies and its overexpression in the transgenic mice results in the abnormal corneas.

**Conclusions:** Based on the results of our study we find that change of the expression of multiple collagens and related proteins (i.e. TGFBI) may be largely responsible for the thinning of the corneal stroma whereas decrease in FN1 and THBS1 may potentially affect corneal repair. These expression results provide further support to the potential deregulation of ECM and adhesion proteins as contributors to the development of KC.

**Commercial Relationships:** Yelena Bykhovskaya, None; Helen P. Makarenkova, None; Yaron S. Rabinowitz, None

**Support:** EY09052; EY012383, None; None

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**Program Number:** 1011 **Poster Board Number:** A0400

**Presentation Time:** 3:15 PM–5:00 PM

**Identifying the role of matrix metalloproteinases in the pathomechanism of TGFBI Arg124Cys related Lattice Corneal Dystrophy Type I**

Johnny E. Moore1, David G. Courtney1, Sarah D. Atkinson1,2, Eleonora Maurizi1,4, Andrew M. Nesbit1, Graziella Pellegrini4, Dimitri T. Azar4, Irwin W. McLean2, Tara C. Moore1, 2

1School of Biomedical Sciences, University of Ulster, Coleraine, United Kingdom; 2Department of Genetic Medicine, University of Dundee, Dundee, United Kingdom; 3Centre for Regenerative Medicine, University of Modena and Reggio Emilia, Modena, Italy; 4Department of Ophthalmology and Visual Sciences, University of Illinois College of Medicine at Chicago, Chicago, IL.

**Purpose:** This study was designed to investigate whether matrix metalloproteinases (MMPs) play a pivotal role in the development of TGFBI-Arg124Cys lattice corneal dystrophy type I (LCDI) using an ex vivo model of LCDI corneal epithelial cell cultures and corneal tissue excised from an LCDI patient.

**Methods:** To initially determine whether the dissolution of Bowman’s layer is apparent in an LCDI affected cornea an H&E stain was carried out on sections of a corneal button excised from a patient suffering from TGFBI-Arg124Cys LCDI. Immunohistochemistry (IHC) was also carried out to determine whether there is a localisation of MMPs around Bowman’s layer. To confirm the induction of MMPs due to the presence of the TGFBI-Arg124Cys mutant allele qRT-PCR was carried out on LCDI corneal epithelial cultures and expression levels were compared to those observed in wild type corneal epithelial cultures.

**Results:** H&E staining confirmed dissolution of Bowman’s layer in an LCDI affected cornea while IHC staining revealed a localisation of MMPs around Bowman’s layer. MMP expression levels were also found to be significantly increased at the mRNA level in the LCDI corneal epithelial cultures.

**Conclusions:** The induction of MMP expression observed in the LCDI corneal epithelial cultures coupled with the H&E and IHC MMP staining indicates that MMPs may play a pivotal role in the development of TGFBI-Arg124Cys LCDI. Further investigation into silencing the TGFBI-Arg124Cys allele in LCDI corneal epithelial cultures and determining whether this has an inhibitory effect on MMP expression would further substantiate the concept that MMPs play a pivotal role in the onset of TGFBI-Arg124Cys LCDI.

**Commercial Relationships:** Johnny E. Moore, None; David G. Courtney, None; Sarah D. Atkinson, None; Eleonora Maurizi, None; Andrew M. Nesbit, None; Graziella Pellegrini, None; Dimitri T. Azar, None; Irwin W. McLean, None; Tara C. Moore, None

**Program Number:** 1012 **Poster Board Number:** A0401

**Presentation Time:** 3:15 PM–5:00 PM

**Identification of Candidate Genes for a Corneal Dystrophy of Bowman Layer Not Associated with a TGFBI Mutation**

Derek J. Le, Ricardo F. Frausto, Anthony J. Aldave. Stein Eye Institute, Los Angeles, CA.

**Purpose:** To identify the genetic basis of a corneal dystrophy of Bowman layer (CDB) not associated with a TGFBI gene mutation using whole exome sequencing.

**Methods:** Slit-lamp examination was performed for affected members of a family with 7 individuals in 3 consecutive generations demonstrating Bowman layer opacities consistent with a CDB. Genomic DNA was isolated from peripheral blood of 2 affected and 1 unaffected individuals, and next-generation whole exome sequencing (WES) was performed. Heterozygous variants segregating with the affected phenotype were filtered against the SNP databases dbSNP137 and the 1000 Genomes Project. Variants identified in genes that did not show corneal expression were then filtered out, with the remaining variants validated by Sanger sequencing. The validated variants were then screened by Sanger sequencing in 2 additional affected individuals who did not undergo WES, and variants not present in both were filtered out. The predicted impact of the remaining missense variants on the function of the encoded protein was assessed using a variety of commonly used tools (PolyPhen2, SIFT, Provean, Condel, and MutationAssessor).

**Results:** WES identified 27 novel heterozygous variants, each in a unique gene expressed in the cornea, in both affected individuals and not in the unaffected individual or in the SNP databases. Screening for these variants in the 2 additional affected family members who did not undergo WES revealed that 10 of the 27 variants were present in both individuals. Two of these variants, located in BAZA2 and PAPLN, were predicted to have deleterious effects on the encoded protein by ≥ 4 of the 6 prediction tools.

**Conclusions:** Using whole exome sequencing and corneal gene expression data, we have identified 10 candidate genes for a CDB not associated with a TGFBI gene mutation. Screening of these candidate genes in other reported families with CDB not related to a TGFBI gene mutation will likely lead to the identification of the genetic basis of the disorder in these families.

**Commercial Relationships:** Derek J. Le, None; Ricardo F. Frausto, None; Anthony J. Aldave, None

**Support:** NIH Grants: 1R01 EY022082 (AJA) and Core Grant P30 EY000331. Unrestricted grant from Research to Prevent Blindness.
Program Number: 1013 Poster Board Number: A0402

Presentation Time: 3:15 PM–5:00 PM

Novel ZEB1 mutations and associated posterior polymorphous corneal dystrophy phenotypes

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1. Laboratory of the Biology and Pathology of the Eye, Institute of Inherited Metabolic Diseases; First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Prague, Czech Republic; 2. Department of Ophthalmology, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Prague, Czech Republic; 3. UCL Institute of Ophthalmology, London, United Kingdom; 4. Moorfields Eye Hospital NHS Foundation Trust, London, United Kingdom.

Purpose: To identify disease-causing mutations in ZEB1 and to determine genotype-phenotype correlation in patients with posterior polymorphous corneal dystrophy 3 (PPCD3).

Methods: Clinical examination and direct sequencing of ZEB1 coding region in six Czech and two British probands with PPCD was performed.

Results: Three novel mutations, predicted to result in haploinsufficiency, were identified; c.1749_1750del[p.(Pro584*)] and c.1717_1718del[p.(Val573Phefs*12)] in two Czech families and c.1176dup[p.(Ala393Serfs*19)] in one British family. Ocular examination of six molecularly confirmed individuals with PPCD3 revealed that one proband had congenital onset and developed nystagmus later in life. Another affected male presented with corneal edema before the age of 2 years and subsequently underwent repeated keratoplasties in each eye. Abnormal corneal steepening (keratometry readings, flat 46.0 - 50.6 D and steep 45.7 - 54.0 D at 3.0 mm) was present in nine out of ten eyes examined.

Conclusions: PPCD3 may present with corneal edema in early childhood. High corneal steepening is a common feature of PPCD3.

Commercial Relationships: Petra Liskova, None; Lubica Dudakova, None; Alice E. Davidson, None; Sarka Kalasova, None; Alison J. Hardcastle, None; Stephen J. Tuft, None

Support: GACR P301/12/P591

Program Number: 1014 Poster Board Number: A0403

Presentation Time: 3:15 PM–5:00 PM

Expression and characterization of the proline mutants in the 4th FAS1 domains of TGFβIP associated stromal corneal dystrophies

Elavazhagan Murugan1, None; Rajamani Lakshminarayanan1, None; Anandalakshmi Venkatraman1, None; Victoria Mouvet1, Roger W. Beuerman1, None; Jodhbir S. Mehta, None

1. Department of Ophthalmology and Vision Sciences, Hospital for Sick Children, Toronto, ON, Canada.

Purpose: To investigate the clinical course and outcome of penetrating keratoplasty in children with Peters’ anomaly

Program Number: 1015 Poster Board Number: A0404

Presentation Time: 3:15 PM–5:00 PM

Clinical course and outcome of penetrating keratoplasty in children with Peters’ anomaly


Purpose: To study the clinical course and outcome of penetrating keratoplasty in children with Peters anomaly

Methods: This retrospective chart review study included 70 eyes from 48 patients diagnosed with Peters anomaly at the Hospital for Sick Children from 2000 to 2012. Clinical data was collected through the examination of patient records, clinical images and ultrasound.

Results: Twenty-three patients (47.9%) had bilateral involvement, 20 (41.6%) had unilateral involvement and five (10.5%) had one eye with Peters anomaly and other anomalies in fellow eye (such as anophthalmos). Type I phenotype (no lenticular involvement) was found in 77% of eyes and the remainder had type II anomaly (lens involved). Penetrating keratoplasty (PKP) was performed in 44 eyes of 31 patients (63% of all eyes). The median age at first keratoplasty was 3.6 months. The majority of eyes received one graft, however 12% required two or more grafts (range 2-5). The rate of failure was 15% for the first graft and increased to greater than 60% for subsequent PKPs during an average follow up of 56 months. Type II Peters anomaly resulted in higher need for PKP (72% vs 58%) and graft failure requiring regrafting (30% vs 13%) than Peters anomaly type I. The graft was clear at last follow-up in 75% of all eyes (84% of type I and 64% of type II). One third of eyes developed glaucoma with mean age of onset of 19.7 months. More than 75% of eyes which had corneal transplant developed glaucoma after corneal.

The genes encoding the mutant 4th FAS1 domains were cloned, expressed and purified. The proteins were examined using CD spectropolarimetry.

Results: The proline mutants exhibited positive ln(vmut/vwt) values suggesting that proline substitution accelerated amyloid formation. The ln(vmut/vwt) values for the GCDII mutations (L509P 2.32, L550P 2.32) were lower than the LCD mutations (L518P 3.02, T538P 2.54, L558P 2.75, H626P 2.5). The GCDII L509P mutation was at the end of an α-helix and GCDII L550P was in a coil suggesting lesser implications of the proline substitutions on the 2° structural elements. However, in the LCD mutations the residues were either positioned inside an α-helix (L518P, L558P) or a β-sheet (T538P, H626P), thereby having a direct effect on the 2° structure and hence the folding of the domains. We optimized the expression conditions to enhance the solubility of the recombinant proteins. The LCD mutants showed relatively lower levels of expression compared to the GCDII mutants. While the GCDII mutants displayed the characteristic CD spectra corresponding to native TGFβIP, the LCD mutations revealed minor perturbations in their 2° structure. The GCDII and LCD mutants also exhibited differences in their stabilities showing that the proline substitution has direct implications on the proper folding of the domains.

Conclusions: The proline mutants exhibit a clear propensity to form amyloids. It is possible that proline substitution in the 4th FAS1 domain could increase the amyloid aggregation propensity by destabilizing the 2° structure. Initial studies on the mutants showed their distinct biophysical and biochemical properties invitro which could explain their different modes of aggregation.

Clinical Relationships: Elavazhagan Murugan, None; Rajamani Lakshminarayanan, None; Anandalakshmi Venkatraman, None; Victoria Mouvet, None; Roger W. Beuerman, None; Jodhbir S. Mehta, None

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transplant. Glaucoma incidence and age of onset were the same in both subtypes. Other treatments performed were optical iridectomy in 10%, rotational autograft in 3% and pharmacologic mydriasis in 3%.

**Conclusions:** Children with Peters anomaly type II have a higher rate of PKP and graft rejections than those with a type I phenotype. Glaucoma was more likely to develop after PKP, suggesting that the etiology was either steroid-induced or related to chronic angle closure, further compromising any pre-existing goniodysgenesis.

**Commercial Relationships:** Hermina Strungaru, None; Kamiar Mireskandari, None; Asim Ali, None

**Program Number:** 1016 Poster Board Number: A0405

**Presentation Time:** 3:15 PM–5:00 PM

**Hereditary Benign Intraepithelial Dykeratosis in a Native American Tribe**

Terri L. Young, Stuart W. Tompson, Kristina N. Whisenhunt, Quintin DeGroot, Krystina Quow, Xiaoyan Luo, Natalie A. Afshari.

**Purpose:** Autosomal dominant hereditary benign intraepithelial dyskeratosis (HBID) is a rare syndrome of variable ocular surface irritation with overgrowth and leukoplaikia of the conjunctiva and cornea, and occasional accompanying oral mucosal keratosis. Reported cases are primarily from a large tri-ethnic North Carolina Haliwa-Saponi Native American tribe isolate, although a novel gene- NLRP1- has been identified in a Caucasian French family. The chromosome 4q35 disease locus was determined by microsatellite linkage analysis in 2 families within the tribal population. Two of the linked markers displayed tri-allellism in all 25 affected individuals of 55 subjects studied, suggesting that a duplication of this genomic region was causal. We independently ascertained HBID families from the same isolate, and performed repeat linkage analysis with denser coverage to confirm linkage, and to delimit the genomic interval in which the true causal variant might reside.

**Methods:** Three families (9 affected/ 4 unaffected) were ascertained, and participant DNA was used for study. We designed and analyzed 25 FAM tagged fluorescent microsatellite markers from the telomeric 4q35 locus to the centromere for over 122Mb. After standard PCR and fragment analysis, the samples were interpreted by Peak Scanner (ABI). Sanger sequencing of tribal member DNA samples was performed of the coding and intron-exon boundary regions of the NLRP1 gene.

**Results:** We confirmed the presence of tri-allellism at markers D4S1652 and D4S2390 in 6 affected individuals. This was also identified in 3 unaffected individuals, thereby excluding this genomic variant as disease causing. No pathogenic variants of NLRP1 were determined for affected tribal members.

**Conclusions:** Our analysis did not identify a haplotype common to all affected individuals, effectively excluding the q-arm of chromosome 4 as the locus for HBID. The HBID phenotype displays genetic heterogeneity. To delineate the causal genotype/variant, exome sequencing of tribal members is underway.

**Commercial Relationships:** Terri L. Young, None; Stuart W. Tompson, None; Kristina N. Whisenhunt, None; Quintin DeGroot, None; Krystina Quow, None; Xiaoyan Luo, None; Natalie A. Afshari, None

**Support:** NIH Grant EY014685

**Program Number:** 1017 Poster Board Number: A0406

**Presentation Time:** 3:15 PM–5:00 PM

**Loss of Ion Transport along with Unfolded Protein Response in Late Onset FECD**

Supriya Jalimarada, Diego G. Ogando, Clark L. Springs, Robert D. Deitch, Joseph A. Bonanno.

**Purpose:** Late onset Fuchs endothelial corneal dystrophy (FECD), affecting 5% of the US population, is a major cause for corneal transplantation. Mutations of a variety of unrelated genes: SLC4A11, COL8A2, TCF8 and LOXHD1, are associated with FECD. Current pathological hypotheses include deficiency of the CE (corneal endothelium) pump function and/or induction of the unfolded protein response (UPR). This study aims to determine the contribution of the above two mechanisms by assessing the expression levels of: (1) Endothelial ion-transporters known to regulate stromal hydration and (2) UPR related genes, in CE obtained from FECD patients compared to that of normal controls.

**Methods:** Fuchs Corneal Endothelium was collected immediately post- Keratoplasty surgery (at Midwest eye clinic, Indianapolis) and transferred to RNA stabilizing agent and refrigerated. Normal specimens were similarly collected at Lion’s eye bank, Indianapolis. Total RNA from six CE specimens from each category i.e. Fuchs and normal control were individually extracted using RNeasy Micro Kit (Qiagen). Expression levels of ion transporters and UPR genes were tested in each of the RNA samples using quantitative Real Time PCR and UPR specific PCR array, respectively. Primers specific to human SLC4A11, Na+/HCO3- co-transporter, Na+/K+/ATPase pump, Monocarboxylate Transporters (MCT -1, 2 and 4) and Na+/H+ exchanger 1 were designed and standardized for efficiency. β-actin was included as a normalizing control. First strand cDNA synthesis (Applied Biosystems) and RT2 amplification for qRT-PCR (SB Biosciences) were used to prepare samples for qRT-PCR and UPR PCR array (SB Biosciences), respectively; Stratgene Mx3000 was employed to run PCR.

**Results:** The PCR array tested 84 UPR related genes. The PCR array data analysis showed upregulation of 41 genes and downregulation of 3 genes i.e. ~52% of the tested genes had their expression altered in FECD samples. 13 genes that were altered were significant with p-value < 0.05. These genes were validated by qRT-PCR. Among the ion-transporters tested, Na+/K+/ ATPase and Mono Carboxylate Transporters 1 & 4 were significantly downregulated in FECD samples (p-value < 0.05).

**Conclusions:** FECD samples had evident UPR with significant alteration of genes associated with the UPR pathways along with significant down regulation of ion transporters indicating simultaneous compromised CE pump function under dystrophic condition.

**Commercial Relationships:** Supriya Jalimarada, None; Diego G. Ogando, None; Clark L. Springs, None; Robert D. Deitch, None; Joseph A. Bonanno, None

**Support:** NIH EY008834
Purpose: Macular corneal dystrophy (MIM #217800), an autosomal recessive disorder primarily affecting the corneal stroma, is characterized by abnormal accumulation of glycosaminoglycans. Our goal was to analyse the expression level of protein aggregation regulatory molecules in human macular dystrophy corneas and in cultured human corneal epithelial cells (HCE-2) under proteasomal inhibition in vitro.

Methods: Four cases of macular dystrophy and 4 normal human corneal buttons collected during corneal transplantation were examined for their expression patterns of Heat shock protein 70 (Hsp70), SQSTM1/p62 and ubiquitin protein conjugates by fluorescent immunohistochemistry. Expression levels in response to different concentrations of proteasomal inhibitor treatment (MG-132) were also analysed in HCE-2 cells by western blotting and transmission electron microscopy.

Results: In macular dystrophy samples, strongly upregulated Hsp70, SQSTM1/p62 and ubiquitin protein conjugates were observed in basal epithelial cells. Weak Hsp70 labelling, moderate ubiquitin and SQSTM1/p62 positivity were present in stromal keratocytes in macular dystrophy. All the studied proteins were also highly elevated under proteasomal inhibition in HCE-2 cells in vitro.

Conclusions: This study first demonstrates the upregulation of Hsp70, SQSTM1/p62 and ubiquitin protein conjugates in the basal epithelial cells and stromal keratocytes in macular dystrophy. Our data may support the role of impaired ubiquitin/proteasomal protein degradation and abnormal protein homeostasis in the pathogenesis of macular corneal dystrophy.

Commercial Relationships: Eszter Szalai, None; Kai Kaarniranta, None; Laszlo Modis Jr., None; Andras Berta, None; Adrian Smedowski, None; Johanna Viiri, None; Bogumil Wowra, None; Dariusz Dobrowolski, None; Edward Wylegala, None; Szabolcs Felszeghy, None

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in VIP and CGRP content were observed in corneas with leukemia when compared to controls.

A high variability in SP values was detected in all the samples evaluated leading to no significant differences between groups.

**Conclusions:** This study is one of the first to quantify SP, CGRP and VIP in the human cornea, and to indicate a possible correlation with pathological conditions. Alterations in the local levels of VIP and CGRP in keratoconus, but not in corneas with a leukemia, may reflect an alteration of corneal innervation and trophism and could represent an additional pathogenic mechanism involved in keratoconus progression.

**Commercial Relationships:** Marta Sacchetti, None; Vincenzo Scocia, None; Flavio Mantelli, None; Augusto Pocobelli, None; Alessandro Lambiase, Dompé © (C); Stefano Bonini, None

**Program Number:** 1021 **Poster Board Number:** A0410
**Presentation Time:** 3:15 PM–5:00 PM
**En face spectral domain optical coherence tomography (SD-OCT) for corneal dystrophies**

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**Purpose:** To evaluate the usefulness of en face spectral-domain optical coherence tomography (SD-OCT) for the analysis of corneal dystrophies.

**Methods:** Thirty-five eyes of 20 patients with various corneal dystrophies (epithelial basement membrane dystrophy, Salzmann nodular degeneration, Reis-Bucklers corneal dystrophy, corneal macular dystrophy, Groenouw type 1 corneal dystrophy, crocodile shagreen dystrophy and Fuchs endothelial dystrophy) were included in the present study. All subjects underwent detailed ophthalmic examination, were photographed and were then evaluated using en face anterior segment SD-OCT.

**Results:** En face anterior segment SD-OCT provided additional information to conventional cross-sectional OCT imaging, allowing the description of the size, depth, shape, and location of corneal lesions in various corneal dystrophies. En face OCT Images were well correlated with previously reported conventional B-scans OCT, in vivo confocal microscopy (IVCM) and histopathologic findings. This new technique was capable of identifying corneal microstructural changes related to corneal dystrophies in patients, with no corneal contact. The C-scan images provided for instance clear visualization of subepithelial multilaminar, linear, and curvilinear hyperreflective lines in epithelial basement membrane dystrophy. Despite a lower resolution than IVCM, en face SD-OCT appeared to be a non-invasive and reproducible tool that allowed the analysis of larger corneal areas.

**Conclusions:** En face SD-OCT is an innovative technique that provides non invasively interesting features in corneal dystrophies. It will probably become an important imaging device for the assessment of these anterior segment conditions.

**Support:** For Prof. Christophe Baudouin: Grants from Alcon pharmaceuticals, Santen pharmaceuticals, Thea pharmaceuticals (F)

**Commercial Relationships:** Wajdene Ghouali, None; Rachid Tahiri Joutei Hassani, None; Hong Liang, None; Emmanuelle Brasnu, None; Antoine Labbe, None; Christophe Baudouin, Alcon Pharmaceuticals (F), Santen pharmaceuticals (F), Thea pharmaceuticals (F)

**Program Number:** 1022 **Poster Board Number:** A0411
**Presentation Time:** 3:15 PM–5:00 PM
**Corneal confocal microscopy following conventional, transepithelial by iontophoresis, and accelerated corneal collagen cross-linking procedures for keratoconus**

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**Purpose:** To compare early corneal healing following conventional, transepithelial by iontophoresis, and accelerated corneal collagen cross-linking (CXL) protocols.

**Methods:** Forty five patients with progressive keratoconus were divided into three groups to receive conventional, transepithelial by iontophoresis, or accelerated CXL. In vivo corneal confocal microscopy was performed on each patient preoperatively and at 1, 3, and 6 months postoperatively.

Density of corneal sub-basal nerves, anterior and posterior keratocytes, and corneal endothelium were assessed.

**Results:** The subbasal nerve plexus was essentially obliterated immediately following conventional and accelerated CXL. The anterior stroma showed significant changes after CXL: complete obliteration of keratocytes, increased tissue reflectivity, a honey-comb
like appearance, and circular lacunae. These changes were more pronounced following conventional and accelerated CXL. There were no apparent changes to the posterior stroma or endothelium.

**Conclusions:** In vivo corneal confocal microscopy analysis of the postoperative impact of CXL on the cornea revealed clear differences among conventional, accelerated, and transepithelial by iontophoresis CXL protocols. Conventional CXL had a greater impact on the sub-basal nerveplexus and loss of anterior stromal keratocytes in the early postoperative period. The posterior stroma and corneal endothelium were unaffected.

**Commercial Relationships:** Nacim Bouheraoua, None; Lea Jouve, None; Mohamed El Sanharawi, None; Otman Sandali, None; Patrick Loriaut, None; Cyril Temstet, None; Elena Basli, None; Vincent Borderie, None; Laurent Laroche, None

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**Program Number:** 1023 **Poster Board Number:** A0412  
**Presentation Time:** 3:15 PM–5:00 PM

**High-throughput NGS of 60 Genes Involved in Inherited Corneal Disorders**

Xinjing Wang1, Danyao Nie1, Angela Turner1, Keith Wetherby1, Kerry Gotez1, Alexandra Garafalo1, Rebecca Parrish1, Santa J. Tumminia1, Kory Johnson1, Yang Fann1.  
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**Purpose:** To develop a next-gen sequencing (NGS) based platform to detect coding sequence alterations in causative and disease-related genes for inherited corneal disorders (CDs).

**Methods:** An assay to detect sequence alterations in genes involved in inherited CD has been developed. In this assay, a primer library which targeted 1830 amplicons from 853 exons of 60 genes involved in corneal function and/or retinal development was designed to enrich the target genomic region. The RainDance PCR enrichment method was used to produce enriched targets and the PCR products were sequenced using NGS by the Illumina MiSeq. The NGS data were processed using CLC Bio software. A series of custom filters was developed and applied to screen for sequence variations in patient samples. The identified variations were independently validated by Sanger sequencing. A number of bioinformatic tools such as PolyPhen-2 and SIFT were used to further evaluate the potential pathogenic variations.

**Results:** In this pilot study, we analyzed 18 samples from patients with CDs. Preliminary bioinformatics analysis indicated that this procedure was able to cover 99% of target sequence. Data mining identified previously reported mutations and novel pathogenic-likely variants in a variety of genes responsible for corneal dystrophies in 15 of the 18 patients.

**Conclusions:** These results indicate that this assay is capable of screening mutations in CD patients, especially in simplex cases with high sensitivity and efficiency, and has powerful potential in clinical applications.

**Commercial Relationships:** Xinjing Wang, None; Danyao Nie, None; Angela Turner, None; Keith Wetherby, None; Kerry Gotez, None; Alexandra Garafalo, None; Rebecca Parrish, None; Santa J. Tumminia, None; Kory Johnson, None; Yang Fann, None

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**Program Number:** 1024 **Poster Board Number:** A0413  
**Presentation Time:** 3:15 PM–5:00 PM

**Analysis of Superficial Deposit Depth in Granular Corneal Dystrophy Type 2 Using Spectral-Domain Optical Coherence Tomography**

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**Purpose:** The appearance of amyloid and hyaline deposits at various depths in the corneal stroma is well known in cases of granular corneal dystrophy type 2. Although the analysis of deposit depth in the stroma is essential for phototherapeutic keratectomy (PTK), only a few detailed analyses have been reported in the literature. In the present study, we aimed to assess the deposit depth by using spectral-domain optical coherence tomography (SD-OCT) at the anterior eye.

**Methods:** We included 95 eyes from 51 patients (18 males and 33 females; average age, 67.6 years) in this study. We measured the deposit depth from the surface of cornea in patients with granular corneal dystrophy type 2 and without a history of corneal operation, at Osaka University Hospital from February 2008 to August 2013. Using SD-OCT (RTVue-100; Optovue, Inc.), we analyzed the frequency of protrusion from surface of corneal stroma and the depth from the epithelium to the apex of the hyaline granule deposit in 2 slices, in the horizontal and vertical directions. Furthermore, we compared the deposit depth between patients aged <65 years and those aged ≥65 years.

**Results:** Protrusion of the deposit from the surface of the corneal stroma was detected in 78.9% of cases (75/95 eyes). The average depth from the surface of the cornea to the apex of the deposit was 30.9 ± 10.8 μm. This average depth was 30.1 μm among the patients aged <65 years (30 eyes) and 31.5 μm among the patients aged ≥65 years; no significant difference was noted in this value between the 2 groups (p = 0.598).

**Conclusions:** Considering that protrusion of the deposit from the surface of the corneal stroma occurs in approximately 79% of eyes of the patients with granular corneal dystrophy type 2, we recommend that PTK should be carefully performed. Moreover, we did not identify any relationship between aging and the depth from the surface of the cornea to the apex of the deposit.

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**Presentation Time:** 3:15 PM–5:00 PM

**Triplet repeat primed PCR assay to genotype the CTG18.1 trinucleotide repeat polymorphism in TCF4**

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**Purpose:** To genotype the CTG18.1 trinucleotide repeat polymorphism using a combination of short tandem repeat (STR) analysis and triplet repeat primed polymerase chain reaction (TP-PCR) assay and validate our genotyping approach with Southern blot analysis. Fuchs' dystrophy is strongly associated with the intronic expanded CTG18.1 trinucleotide repeat polymorphism in TCF4. The TP-PCR assay was originally described as a general method for the detection of CAG repeat expansion in myotonic dystrophy.

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Methods: The CTG18.1 polymorphism was genotyped using genomic DNA from over 515 subjects using a combination of STR analysis and TP-PCR assay. On samples where STR analysis detected only one allele or failed to detect any alleles, TP-PCR was performed to confirm the presence of an expanded allele(s).

Our TP-PCR assay utilized P1, a fluorescent primer designed to a region upstream from the CTG18.1 allele. The companion reverse primer P4 on the complementary strand was comprised of 5 units of the CTG repeat and a 5′ tail to serve as an anchor for a second reverse primer P3, which prevents progressive shortening of the PCR products during subsequent cycles. The 5′ tail of primer P4 and the “common” flag primer P3 share no homology with human sequence.

PCR was performed with an initial denaturation of 9 min at 95°C, followed by 10 cycles of 95°C for 30 s, 62°C for 30 s, and 72°C for 4 min, and then 30 cycles of 95°C for 45 s, 62°C for 45 s, and 72°C for 4 min with a 15 s extension at each cycle. The final extension step was 72°C for 10 min. The TP-PCR products were analyzed on the ABI 3730XL DNA analyzer.

Southern blot analysis was performed on 10 DNA samples.

Results: The TP-PCR assay was able to resolve zygosity of the CTG18.1 allele on samples where the STR analysis revealed only one allele or no alleles. Characteristic tracing patterns of the CTG repeat primed electropherograms allowed us to distinguish samples that were truly homozygous for a stable CTG18.1 allele from those that harbored an expanded CTG18.1 allele that was undetectable by STR analysis. TP-PCR was also able to confirm the presence of two expanded CTG18.1 alleles in samples where STR analysis failed to detect any allele. Southern blot analysis on 10 samples validated our genotyping results obtained by STR and TP-PCR assays.

Conclusions: The combination of STR and TP-PCR assays is a simple and efficient method to genotype the CTG18.1 polymorphism.

Commercial Relationships: Imran Hussain, National Eye Institute (F), National Institutes of Health (F), Research to Prevent Blindness (F); Xin Gong, National Eye Institute (F), National Institutes of Health (F), Research to Prevent Blindness (F); Vinod V. Mootha, National Eye Institute (F), National Institutes of Health (F), Research to Prevent Blindness (F)

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