438 Corneal Genetics and Myopia

Wednesday, May 04, 2016 11:00 AM–12:45 PM

Exhibit/Poster Hall Poster Session

Program #/Board # Range: 4820–4840/A0036–A0056

Organizing Section: Genetics Group

Contributing Section(s): Anatomy/Pathology, Clinical/Epidemiologic Research

Program Number: 4820 Poster Board Number: A0036
Presentation Time: 11:00 AM–12:45 PM

SLC4A11 Three-Dimensional Model Explains Structural Basis for Endothelial Corneal Dystrophy-CAssociating Mutations

Kumari Alka, Katherine E. Badior, Joseph R. Casey. Biochemistry, University of Alberta, Edmonton, AB, Canada.

Purpose: SLC4A11 is an integral membrane protein abundant in corneal endothelium. Point mutations of SLC4A11 protein cause genetic endothelial corneal dystrophies manifesting very early in life (congenital hereditary endothelial corneal dystrophy (CHED) and Harboyan syndrome (HS)) or in the fifth to sixth decade of life (Fuchs endothelial corneal dystrophy (FECD)). The molecular defect associated with these mutations include mis-folding of the protein, leading to its retention in endoplasmic reticulum (ER) and a loss of SLC4A11 water flux function. We aim to elucidate the effect of disease-causing SLC4A11 mutations on protein structure. This is a first attempt to classify SLC4A11 mutations into structural sub-categories, to provide a molecular explanation for SLC4A11-associated disease.

Methods: A 3D homology model of the human SLC4A11 protein membrane domain was created on the basis of the crystal structure of human SLC4A1 structure at 3.5 Å. The homology modelling was performed, using the SWISS-MODEL server. To test the validity of the SLC4A11 homology model, mutations were made at positions corresponding to the protein’s predicted catalytic site. Functional activity of these mutants was assessed by whole cell swelling assay.

Results: Amongst 29 disease-causing mutations of SLC4A11 membrane domain, 17 altered helix packing and two were in the dimeric interface. The other mutations mapped to the SLC4A11 transport catalytic site. Consistent with the homology model, protein-packing mutants were associated with protein mis-folding and ER retention. Catalytic site mutants affected SLC4A11 water flux activity.

Conclusions: Missense mutants of SLC4A11 were categorized as disrupting 1. Helix packing, 2. Catalytic activity, or 3. Dimerization. The 3D SLC4A11 model provides the ability to predict pathogenicity of variants of this protein identified in the future.

Commercial Relationships: Kumari Alka, None; Katherine E. Badior, None; Joseph R. Casey, None

Program Number: 4821 Poster Board Number: A0037
Presentation Time: 11:00 AM–12:45 PM

Autosomal dominant corneal endothelial dystrophies CHED1 and PPCD1 are allelic disorders caused by non-coding mutations in the promoter of OVOL2

Alice E. Davidson1, Petra Liskova2, Cerys J. Evans3, Lubica Dudakova3, Lenka Noskova3, Nikolas Pontikos4, Vincent Plagnol5, Martin Filipce5, Stanislav Knoch1, Stephen J. Tuft6, Alison J. Hardcastle1. 1UCL Institute of Ophthalmology, London, United Kingdom; 2Department of Ophthalmology, First Faculty of Medicine, Charles University in Prague and General Teaching Hospital in Prague, Prauge, Czech Republic; 3Institute of Inherited Metabolic Disorders, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Prague, Czech Republic; 4UCL Genetics Institute, London, United Kingdom; 5European Eye Clinic Lexum, Prague, Czech Republic; 6Moorfields Eye Hospital, London, United Kingdom.

Purpose: Congenital hereditary endothelial dystrophy 1 (CHED1) and posterior polymorphous corneal dystrophy 1 (PPCD1) are autosomal dominant inherited corneal endothelial disorders that have been genetically mapped to overlapping loci on chromosome 20p. We recruited extensive pedigrees, comprising over 100 affected individuals, with the aim of identifying the genetic cause(s) of disease.

Methods: Targeted and whole genome sequencing (WGS) was performed on multiple individuals from CHED1 and PPCD1 index families. The variant data were filtered according to allele frequency and potential pathogenicity. Candidate variants were directly sequenced for validation and segregation in the extensive pedigrees, and the promoter region of OVOL2 was directly sequenced as a candidate region in additional families. A dual luciferase reporter assay was used to assess the impact of disease-associated mutations on the activity of the OVOL2 promoter.

Results: Following exclusion of coding, splice-site and copy number variations, two unique variants in a conserved region of the OVOL2 promoter were identified, one segregating with CHED1, and another segregating with PPCD1 in the respective index families. Two further unique variants were identified in additional families by direct sequencing. The four unique mutations lie within a highly conserved 100 bp region of the OVOL2 proximal promoter. In vitro analysis using a dual luciferase reporter assay demonstrated that all four OVOL2 promoter mutations exhibited significantly (P≤0.001) increased transcriptional activity compared to the corresponding wild-type promoter sequence.

Conclusions: Our data establishes CHED1 and PPCD1 as allelic conditions, with CHED1 an extreme of what can be considered a disease spectrum, and implicates transcriptional dysregulation of OVOL2 as a common cause of dominantly inherited corneal endothelial dystrophies. OVOL2 encodes ovo-like 2 zinc finger 2, a C2H2 zinc finger transcription factor that regulates mesenchymal to epithelial transition, and acts as a direct transcriptional repressor of the established PCCD-associated gene, ZEB1. We postulate that the mutations identified introduce cryptic cis-acting regulatory sequence binding sites that drive aberrant OVOL2 expression during development of the corneal endothelium.

Commercial Relationships: Alice E. Davidson, None; Petra Liskova, None; Cerys J. Evans; Lubica Dudakova, None; Lenka Noskova, None; Nikolas Pontikos, None; Vincent Plagnol, None; Martin Filipce, None; Stanislav Knoch, None; Stephen J. Tuft, None; Alison J. Hardcastle, None

Support: Fight for Sight early career investigator award

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Characterization of an Alternative ZEB1 Nuclear Localization Signal in Posterior Polymorphous Corneal Dystrophy 3

Lydia Ann, Ricardo F. Frausto, Evelyn Hanser, Doug Chung, Anthony J. Aldave. UCLA Stein Eye Institute, Irvine, CA.

Purpose: To determine the ability of an alternative nuclear localization signal (aNLS) to mediate nuclear translocation of mutant two-handed zinc-finger E-box binding homeodomain transcription factor 1 (ZEB1) proteins associated with posterior polymorphous corneal dystrophy 3 (PPCD3).

Methods: The full-length sequence of the ZEB1 protein was screened for alternative NLS motifs by in silico analysis. Overexpression DNA constructs containing wild type ZEB1 (ZEB1WT) or mutant ZEB1 (ZEB1PPCD3) (representing mutations associated with PPCD3) cDNA and an N-terminal halo-tag sequence were generated. A construct containing ZEB1 cDNA lacking the classical NLS sequence (ZEB1NLSdel), spanning amino acids 892-998, was used as a control. ZEB1 cDNA containing the PPCD3 mutations with or without deletion of the aNLS sequence were generated using site-directed mutagenesis. These cDNAs were overexpressed in Ishikawa cells, a human cell line. Localization of the exogenous halo-tagged ZEB1 proteins was determined by fluorescence immunocytochemistry using an anti-halo antibody and confocal microscopy.

Results: An aNLS motif (Lys-Lys-Arg) was identified at amino acid positions 274-276 by in silico analysis. ZEB1WT protein localized exclusively within the nucleus, while ZEB1NLSdel demonstrated a marked cytoplasmic localization. Similarly, while ZEB1 mutant proteins p.(Arg325*), p.(Glu495fs), and p.(Val526fs) containing an intact aNLS displayed nuclear localization, deletion of the aNLS sequence for these proteins resulted in the distinct cytoplasmic localization, while the effect was less pronounced with p.(Val526fs). In contrast, deletion of the alternative NLS motif did not impact the distinct cytoplasmic localization observed with p.(Ser638fs) and p.(Gln884fs) mutants.

Conclusions: ZEB1 possesses a functional alternative NLS and is only active in truncated proteins with no more than between 250-360 amino acids downstream of the NLS.

Commercial Relationships: Lydia Ann, None; Ricardo F. Frausto, None; Evelyn Hanser; Doug Chung, None; Anthony J. Aldave, None

Support: R01 EY02082

Program Number: 4822 Poster Board Number: A0038

Examination of sequencing data in three Israeli families with Keratoconus

Wesley A. Goar1, Charles C. Searby2, 3, S Scott Whitmore1, 3, Adam P. DeLuca1, 4, Ahed Imtirat1, None; Edwin M. Stone, None; Ruti Pavari, None; Todd E. Scheetz, None; Val Sheffield, None

Support: National Institutes of Health Predoctoral Training Grant T32GM008629, PI Daniel Eberl

Program Number: 4822 Poster Board Number: A0038

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Wesley A. Goar1, 5, Charles C. Searby2, 3, S Scott Whitmore1, 3, Adam P. DeLuca1, 4, Ahed Imtirat1, None; Edwin M. Stone, None; Ruti Pavari, None; Todd E. Scheetz, None; Val Sheffield, None

Support: National Institutes of Health Predoctoral Training Grant T32GM008629, PI Daniel Eberl

Purpose: Keratoconus (KT) is the most common corneal dystrophy with an occurrence rate of 1 in every 2,000 people. KT causes the cornea to thin with age, becoming conical and leading to myopia, irregular astigmatism and corneal scarring. The age of onset is generally the teenage years with stabilization in the third and fourth decades of life. Incidence of KT does not seem to be more prevalent in a specific ethnic group or gender. Currently, corneal transplantation is the only treatment for KT when visual acuity is no longer correctable by contact lenses. We hypothesize that KT is a genetically heterogeneous disease that is caused by mutations in one of several genes.

Methods: Samples were obtained from 3 Israeli KT families (16 samples) and genotyped using an Affymetrix Genome-Wide SNP 6.0 microarray. The SNP data was analyzed for regions consistent with segregation within each of the families using MERLIN. Three samples with KT (one from each family) were chosen, sequenced using the Illumina HiSeq 2500 platform and analyzed using our exome pipeline. Resulting variants were filtered based upon variant quality, predicted function, and population prevalence (1000 genomes, ExAC). Spurious variants (based upon our local set of 1000+ exomes) and those in regions inconsistent with segregation were removed to create the final variant list, which was annotated with corneal expression (http://genome.uio.edu/otdb) to assist in prioritizing candidates.

Results: No plausible variations were found in these three families in genes previously reported to cause KT. In addition, no single gene with plausible exonic disease-causing variations was shared across all three families. However, a few genes were found that are shared between two of the families and need further follow up. Figure 1 summarizes the family structure and numbers of plausible variations throughout the pipeline.

Conclusions: Our data supports the genetic heterogeneity of KT even within isolated populations. Further work is needed to identify the causative mutations in these families. Due to the isolated population we will continue to pursue these through ascertainment of additional families and family members. This will allow us to narrow the intervals of the genome in which the causative mutations are found.

<table>
<thead>
<tr>
<th>Family</th>
<th>Affected/Healthy Individuals</th>
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Number of total variants found: 144,472, 144,183, 153,708

Number of variants after filtering: 459, 451, 458

Number of final variants: 86, 28, 106

Figure 1. For each family, the individuals available for study, and the variant-level results at multiple stages of filtering are shown.

Commercial Relationships: Wesley A. Goar; Charles C. Searby, None; S Scott Whitmore, None; Adam P. DeLuca, None; Ahed Imtirat, None; Edwin M. Stone, None; Ruti Pavari; None; Todd E. Scheetz, None; Val Sheffield, None

Support: National Institutes of Health Predoctoral Training Grant T32GM008629, PI Daniel Eberl

Program Number: 4824 Poster Board Number: A0040

The mutational spectrum of anterior corneal dystrophies in the UK

Cerys J. Evans1, Alice E. Davidson1, Nicole Cars1, 2, Neyme Velti1, Stephen J. Tuft1, Alison J. Hardcastle, 1 Institute of Ophthalmology, UCL, London, United Kingdom; 2 Moorfields Eye Hospital, London, United Kingdom; 3 Save Sight Institute, The University of Sydney, Sydney, NSW, Australia.

Purpose: Corneal dystrophies are bilateral, inherited disorders that cause corneal opacity and result in visual impairment. The majority of dystrophies that affect the anterior cornea are caused by mutations in TGFBI. We sought to identify the spectrum of mutations causing anterior corneal dystrophies in a large UK cohort of mixed ethnicity.

Methods: Individuals clinically diagnosed with an epithelial, sub-epithelial, or stromal corneal dystrophy were recruited at Moorfields Eye Hospital, London. Each proband was clinically examined

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and provided a blood sample for DNA extraction. Depending on
the phenotype, we screened the corneal dystrophy genes TGFBI,
UBIAD1 or CHST6 by direct sequencing. Mutations upstream of
CHST6, previously identified to be disease causing, were screened
using a PCR based assay. If the proband was mutation negative,
GSN sequencing and/or plasma electrophoresis was performed.
Segregation analysis was undertaken when familial samples were
available.

**Results:** In 68 of 72 probands we identified nine different, previously
reported, TGFBI mutations causing Reis-Bücklers, Thiel-Behnke,
lattice or granular corneal dystrophy. Of the four probands who were
TGFBI-mutation negative; two were subsequently identified to have
mutations in GSN, resulting in a diagnosis of Meretoja Syndrome,
and two had paraproteinemic keratopathy confirmed by plasma
electrophoresis. Interestingly, two probands with epithelial basement
membrane dystrophy (EBMD) were also found to carry a mutation in
TGFBI. There were 20 probands with macular corneal dystrophy;
the majority had different homozygous or compound heterozygous
mutations in CHST6, although p.(Leu200Arg) was found in multiple
probands. Three previously reported mutations were identified in four
probands with Schnyder corneal dystrophy.

**Conclusions:** In this large multi-ethnic cohort a limited spectrum
of only nine mutations was responsible for all TGFBI-associated
disease. Genotype-phenotype correlation was observed for all TGFBI
mutations except p.(Gly623Asp), which was associated with a broad
phenotypic spectrum; including clinical features of lattice corneal
dystrophy, Reis-Bücklers dystrophy or EBMD. We have expanded
the mutational spectrum of CHST6 with the identification of five
novel mutations.

**Commercial Relationships:** Cerys J. Evans, None;
Alice E. Davidson, None; Nicole Carnt, None; Neyme Veli, None;
Stephen J. Tuft, None; Alison J. Hardcastle, None

**Support:** Fight for Sight, Moorfields Eye Charity, National Institute
for Health Research (NIHR) Biomedical Research Centre at
Moorfields Eye Hospital NHS Foundation Trust and UCL Institute
of Ophthalmology.

**Program Number:** 4825 Poster Board Number: A0041
**Presentation Time:** 11:00 AM–12:45 PM

**Mutational Spectrum of Korean Patients with Corneal Dystrophy Man Soo Kim, Eun Chul Kim, Woong-Joo Whang.**

**Purpose:** This study aimed to analyze the spectrum of genetic
aberrations in Korean patients referred and diagnosed with various
types of corneal dystrophies in a single tertiary referral center, and to
characterize the genotype-phenotype correlations in this population.

**Methods:** Patients with corneal dystrophies who presented at Seoul
St Mary’s Hospital, Seoul, Korea, from September 2009 to July 2014
and for whom gene mutation analysis was requested were included
in this study. Genomic DNA was isolated from the peripheral blood
leukocytes, using the QIAmp DNA Mini Kit (Qiagen, Hamburg,
Germany). Polymerase chain reaction (PCR) was carried out using
previously published primer sets for TGFBI, CHST6, UBIAD1,
COL8A2, ZEB1, and SLC4A11. All the coding exons and the flanking
intron/exon boundaries of the above genes were amplified. The
PCR amplicons were bi-directionally sequenced using the Big Dye
terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster
City, CA, USA) on an ABI PRISM 3100 Genetic Analyzer (Applied
Biosystems). The chromatograms were analyzed with the Sequencher
software version 5.0 (Gene Codes, Ann Arbor, MI, USA). Sequence
variants were confirmed by sequencing two or more independent
PCR reactions.

**Results:** A total of 120 probands were included, with a mean age
of 50 years (SD: 18 years) and 70% were female. A total of 26
mutations in 5 genes (14 clearly pathogenic and 12 likely pathogenic)
were identified in 49 probands (41%). Epithelial-stromal TGFBI
dystrophies, macular corneal dystrophy and Schnyder corneal
dystrophy showed 100% mutation detection rates, while endothelial
corneal dystrophies showed lower detection rates of 8.9%. Twenty six
non-duplicate mutations including 8 novel mutations were identified
and mutations associated with Schnyder corneal dystrophy were
identified genetically for the first time in this population.

**Conclusions:** This study provides a comprehensive characterization
of the genetic aberrations in Korean patients and also highlights the
diagnostic value of molecular genetic analysis in corneal dystrophies.

**Commercial Relationships:** Man Soo Kim, None; Eun Chul Kim,
None; Woong-Joo Whang.

**Program Number:** 4826 Poster Board Number: A0042
**Presentation Time:** 11:00 AM–12:45 PM

**Characterization of Fuchs Corneal Dystrophy phenotype in a TCF8 knock-in mouse model John D. Gottschl, Shivakumar Vasanthi, Allen O. Eghrari, Edwin Oh, S Amer Riazuddin, Nicholas Katasans.**

**Purpose:** We previously reported a missense mutation (p.Q840P)
in TCF8 responsible for Fuchs Corneal Dystrophy (FCD) in a large
multigenerational pedigree and a sporadic late-onset case. To further
investigate the physiological characteristics of FCD in a model that
closely mimics human ocular tissue, we developed a knock-in mouse
model harboring the variant homologous to the human mutation in
Tcf8. The following study was undertaken to characterize the FCD
phenotype in the murine model.

**Methods:** Tcf8<sup>Q818P/Q818P</sup> knock-in mice were developed by
constructing a targeting vector using BAC recombineering with
Neo cassette for positive selection of embryonic stem cells. Contact
confocal microscopy was conducted in corneas of wild-type (Wt)
mice and mice heterozygous (Tcf8<sup>Wt/Q818P</sup>) and homozygous (Tcf8<sup>Q818P/</sup>
Q818P) for the pathological variant. Pachymetry was assessed with
peak-to-peak signal intensity from confocal Z-scan with 5 micron
intervals. Endothelial cell density was calculated using the center
method. The extent of guttae on confocal microscopy was assessed
by an independent reviewer on a 1-to-5 scale with reference images.
We utilized paraffin-embedded corneal sections for histopathological
and immunohistochemical analyses.

**Results:** Targeted ES clones and germline transmission were
confirmed by Southern blotting while the mutation was confirmed by
Sanger sequencing following RT-PCR of Tcfc mRNA. We conducted
confocal microscopy of 48 mice ranging from 0.3 to 2.1 years old.
Among 4-month old mice, confocal imaging of 14 eyes from Wt
mice and 14 eyes from mice harboring the mutation (9 homozygous,
5 heterozygous) revealed significantly thicker values compared to Wt
mice in homozygous (95 vs. 118 microns, p<0.01) and marginally
in heterozygous (95 vs. 108 microns, p=0.06) corneas (p<0.001,
WT compared to all mice bearing mutation). Endothelial cell count
was decreased in heterozygous compared to Wt (2289 vs. 2498 cells/
m2, p<0.01) and homozygous (2289 vs. 2544, p=0.03) mice. Compared
to Wt, mean guttate appearance score was significantly
higher among homozygous (P=0.016) and marginally in heterozygous
(p=0.057) mice. Corneal sections of 18-month-old homozygous Tcf8
mice show reduced COL4A3 and COL8A2 expression compared to
WT littermates.

**Conclusions:** We demonstrate a knock-in mouse model with guttate
appearance, endothelial cell loss, and increased corneal thickness,
pathological findings consistent with human FCD.
Commercial Relationships: John D. Gottsch, None; Shivakumar Vasanth, None; Allen O. Eghrari, None; Edwin Oh, None; S Amer Riazuddin, None; Nicholas Katsanis, None
Support: NIH Grant R01 EY016835

Program Number: 4827 Poster Board Number: A0043
Presentation Time: 11:00 AM–12:45 PM
The relationships between COL8A2 mutation and biometric measurements in Fuchs' endothelial corneal dystrophy

Woong-Joo Whang, Man Soo Kim. The catholic university of Korea, Seoul, Korea (the Republic of).

Purpose: To compare clinical measurements according to the presence of collagen type VIII a2 (COL8A2) which is potential susceptibility gene for patients with Fuchs’ corneal dystrophy (FECD).

Methods: A total 56 Korean patients diagnosed with Fuchs’ endothelial cornea dystrophy were enrolled. PCR-SSCP and direct sequencing were used to screen genetic variations in COL8A2. The central corneal thickness (CCT) were measured by ultrasound pachymeter and the endothelial cell counts were measured using Konan corneal specular microscopy. The differences of CCT and endothelial cell counts between both eyes were also analyzed.

Results: The COL8A2 gene was identified in 6 patients. (F:M = 3:3) In another 50 patients, genetic mutation was not found. (F:M = 39:11) No significant differences was found in CCT (602.5 ± 85.7 versus 620.8 ± 110.4, p=0.66), however the differences between both eyes showed statistically significant differences. (70.1 ± 39.0 versus 155.0 ± 40.9, p<0.05) We also identified endothelial cell counts in both groups. 8 eyes of COL8A2 gene group showed significantly lesser endothelial cell count compared with no mutation group. (890.5 ± 157.0 versus 1673.8 ± 201.4, p<0.05)

Conclusions: Our data demonstrated the relatively higher bilaterality in the COL8A2 gene group. The COL8A2 gene group showed lesser endothelial cell counts compared with no mutation group. Genetic mutation would be a useful indicator for predicting the pattern of progression and bilaterality of disease in FECD patients.

Commercial Relationships: Woong-Joo Whang, None; Man Soo Kim

Program Number: 4828 Poster Board Number: A0044
Presentation Time: 11:00 AM–12:45 PM
Vortex pattern of corneal deposits in granular corneal dystrophy associated with the p.(ArgR555WTrp) mutation in TGFBI

Arturo J. Ramirez-Miranda, Juan Carlos Serna-Ojeda, Eung Kweon Kim, Juan Carlos Serna-Ojeda, Eung Kweon Kim, Juan Carlos Serna-Ojeda, Eung Kweon Kim, Antonio J. Aldave, Antonio J. Aldave.

1 Stein Eye Institute, Los Angeles, CA; 2 Cornea and Refractive Surgery, Instituto de Oftalmologia “Conde de Valenciana”, Mexico City, Mexico; 3 Ophthalmology, Yonsei University, Seoul, Korea (the Republic of).

Purpose: To describe two unrelated families with multiple family members demonstrating an atypical vortex pattern of corneal deposits confirmed to be granular corneal dystrophy (GCD1) following identification of the p.(Arg555Trp) mutation in the transforming growth factor β-induced gene (TGFBI).

Methods: A slit lamp examination was performed on individuals from two North-American families, one of Mexican descent and a second of Italian descent. Following DNA extraction from peripheral blood leukocytes or buccal swabs from affected individuals and their unaffected relatives (20 in the Mexican family and 55 in the Italian family), TGFBI screening was performed.

Results: Eight of 20 individuals in the Mexican family and 22/78 in the Italian family demonstrated corneal stromal opacities. Seven of the eight affected individuals in the Mexican family and three of the 22 affected individuals in the Italian family demonstrated an atypical phenotype characterized by a “sea fan” or vortex pattern of superficial stromal corneal deposits originating from the inferior aspect of the cornea. Screening of TGFBI in both families revealed a heterozygous missense mutation (p.Arg555Trp) in exon 12, confirming the diagnosis of GCD1.

Conclusions: GCD1 may be associated with a vortex pattern of anterior stroma deposits. Although this pattern of dystrophic deposits is not recognized by clinicians as a typical phenotype of GCD1, it is consistent with the production of the majority of the TGFBI protein by the corneal epithelial cells.

Commercial Relationships: Jaffer Kattan, None; Juan Carlos Serna-Ojeda, None; Eung Kweon Kim; Arturo J. Ramirez-Miranda, None; Juan C. Zenteno, None; Anthony J. Aldave, None
Support: NEI Grant 1R01 EY022082 (A.J.A.), David Geffen School of Medicine Dean’s Leadership in Health and Science Scholarship, NEI Grant P30 EY000331 (core grant), the Walton Li Chair, the Stott fund and an unrestricted grant from Research to Prevent Blindness

Program Number: 4829 Poster Board Number: A0045
Presentation Time: 11:00 AM–12:45 PM
TGF-β regulate TGFBIp expression through coordination between miR-21 and miR-181a, and Smad signaling in corneal fibroblasts

Eung Kweon Kim, Seung-il Choi, Kye Seo Kim, Tae-im Kim, Hun Lee, Si Yoon Park. 1 Yonsei University, Seoul, Korea (the Republic of); 2 Emory University, Atlanta, GA; 3 International St. Mary’s hospital, Incheon, Korea (the Republic of).

Purpose: Transforming growth factor-β (TGF-β)-induced gene (TGFBIp) protein (TGFBIp) is associated with a granular corneal dystrophy type 2 (GCD2), and plays a role in tumorigenesis. Dosage of TGFBI can affect disease phenotypes of this disease, but the underlying molecular mechanisms have not been fully elucidated. We investigate here the contributions of microRNA (miRNA) and TGF-β to TGFBI expression in corneal fibroblasts.

Methods: Isolation, immortalization, and culture of primary corneal fibroblast were performed. RNA Isolation and Quantitative real-time PCR were done. Levels of miRNAs were normalized to RNU6B. Relative quantification was analyzed by the system software analysis based on 2−ΔΔCt method. The precursor miR-9, miR-21, miR-181a, miR-181a-3p, miR-181a-2-3p and negative miR precursor were transfected at a final concentration of 100 nM with G-Fectin and Lipofectamine™ 2000 reagent (Invitrogen Life Technologies). Western blotting was done with got anti-TGFBIp (R&D Systems, Minneapolis, MN). Statistical comparisons were performed using one-way analysis of variance (ANOVA) followed by Newman–Keuls multiple comparison tests.

Results: Ectopic expression of miR-9, miR-21, and 181α reduce significantly TGFBIp levels. Further, the expressions of miR-21 and miR-181α were also induced by TGF-β1. The expression of miR-21 was 10-fold higher than miR-9 and miR-181α in corneal fibroblasts. Additionally, TGF-β1 expression was significantly higher than TGF-β2 and TGF-β3 in corneal fibroblasts, but levels of all three TGF-β forms are not significantly different between WT and GCD2 corneal fibroblasts. Taken together, these data indicated that TGFBIp expression are regulated by TGF-β positively and TGF-β-induced miR-21 and miR-181α negatively in corneal fibroblasts.

Conclusions: In conclusion, TGFBIp level in corneal fibroblasts is controlled through coordination between miR-21 and miR-181a, and Smad signaling. Pharmacologic modulation of these miRNAs and
A genome-wide association study of central corneal thickness in Latinos

Drew Nannini1, Kristen Goulee1, Mina Torres2, Yii-Der Chen3, Kent Taylor4, James Gauderman5, Jerome I. Rotter6, Rohit Varma7, Xiaoyi (Raymond) Gao1. 1Ophthalmology and Visual Sciences, University of Illinois at Chicago, Chicago, IL; 2Ophthalmology, University of Southern California, Los Angeles, CA; 3Institute for Translational Genomics and Population Sciences, Los Angeles Biomedical Research Institute and Department of Pediatrics and Medicine at Harbor-UCLA, Torrance, CA; 4Preventive Medicine, University of Southern California, Los Angeles, CA.

Purpose: Central corneal thickness (CCT) has been identified as an important risk factor for keratoconus, brittle cornea syndrome, and primary open angle glaucoma. Previous genome-wide association studies (GWAS) of CCT were conducted mostly in European and Asian populations. We hypothesize that GWAS in minority populations may provide novel information for CCT genetics.

Methods: We conducted a population-based GWAS for CCT using 3741 Latinos recruited in the Los Angeles Latino Eye Study (LALES). Study participants were genotyped using the Illumina OmniExpress BeadChip (~730K markers). All study participants were 40 years of age or older. We assessed the association between individual single nucleotide polymorphisms and CCT using linear regression in PLINK, adjusted for age, gender, and principal components of genetic ancestry. Additionally, we performed functional enrichment analyses using IPA.

Results: Compared to our previous report on CCT in Latinos using a smaller dataset (IOVS 2013 54:2435-43), we observed more significant results for rs3118515 (P = 5.9×10^{-8}) within the top SNP associated with CCT in the South Indian study was also significantly associated with CCT in the PheWAS (P = 1.9×10^{-7}). The top WNT7B SNP (rs9330813) was selected for PheWAS using all 45 traits. The association tests were performed using likelihood-ratio test in MERLIN after adjusting for age and sex.

Conclusions: Results suggest that WNT7B variants are significantly associated only with CCT in the South Indian population. This study also demonstrates the value of PheWAS for evaluating correlations between genes and phenotypes. In future studies this collection of clinical trait associations will provide the detailed phenotype data required for a glaucoma-oriented PheWAS that will be useful for identifying clinical features correlated with genes known to be associated with glaucoma.

Commercial Relationships: Drew Nannini, None; Kristen Goulee, None; Mina Torres, None; Yii-Der Chen, None; Kent Taylor, None; James Gauderman, None; Jerome I. Rotter, None; Rohit Varma, None; Xiaoyi (Raymond) Gao, None

Support: NIH Grant R01EY022651, U10EY011753, Departmental Core Grant P30EY001792

Program Number: 4830, Poster Board Number: A0046
Presentation Time: 11:00 AM–12:45 PM

Phenome-wide association study (PheWAS) using glaucoma-related ocular quantitative traits supports WNT7B association with central corneal thickness

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Purpose: Connections between the phenome and genome can be identified through phenome wide association studies (PheWAS) where the association between a collection of gene(s) and a wide range of phenotypes are evaluated. For complex traits, such as glaucoma, PheWAS can correlate a disease gene with subphenotypes that may have specific therapies. Previously using 16 consanguineous pedigrees from South India we measured 45 ocular quantitative traits and completed genome-wide association studies and linkage analyses for each trait. Interestingly, in this dataset the top SNP associated with central corneal thickness (CCT) is located within WNT7B, a gene that has been associated with axial length and corneal curvature in a Japanese study. The purpose of this study is to perform a PheWAS for WNT7B SNPs in the South Indian dataset to identify the full range of ocular phenotypes associated with this gene.

Methods: 240 members of 16 consanguineous pedigrees from South India were measured for 45 ocular quantitative traits. Genotyping was performed using the Illumina HumanOmniExpress platform. The top WNT7B SNP (rs10453441) was previously associated with axial length and corneal curvature and the top SNP associated with CCT (rs9330813) was selected for PheWAS using all 45 traits. The association tests were performed using likelihood-ratio test in MERLIN after adjusting for age and sex.

Results: After Bonferroni correction (P < 0.001), the top SNP from the Japanese study (rs10453441) was significantly associated with CCT (P = 5.9×10^{-8}), but not with corneal curvature, axial length or any other quantitative trait. rs9330813, the top SNP associated with CCT in the South Indian study was also significantly associated with CCT in the PheWAS (P = 1.9×10^{-7}), and was not significantly associated with any other quantitative trait.

Conclusions: The PheWAS results suggest that WNT7B variants are significantly associated only with CCT in the South Indian population. This study also demonstrates the value of PheWAS analyses for evaluating correlations between genes and phenotypes. In future studies this collection of clinical trait associations will provide the detailed phenotype data required for a glaucoma-oriented PheWAS that will be useful for identifying clinical features correlated with genes known to be associated with glaucoma.

Commercial Relationships: Baojian Fan, None; P Ferdina Marie Sharmila, None; N Soumithra, None; S Sripriya, None; David S. Friedman, None; I Vijaya, None; Jonathan L. Haines, None; R George, None; Janey L. Wiggs, None

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Pediatric cataract is the most common form of treatable childhood visual disability. Non-syndromic hereditary forms may be associated with other ocular anomalies in approximately 15% of cases, usually involving the anterior segment. Inherited cataracts are less frequent than the sporadic form, and are predominantly associated with systemic disease. They may also be associated with other ocular anomalies. Cataracts are much more commonly associated with iris colobomas than isolated posterior segment colobomas. The objective of this study is to report the uncommon phenotype of congenital cataract and posterior segment coloboma in a South African family and to investigate its genetic etiology.

**Methods:** Observational case series. DNA was extracted and whole genome sequencing was performed on six affected family members.

**Results:** Twenty-six family members were included, with 20 individuals affected in an inheritance pattern consistent with autosomal dominant. Posterior segment coloboma (predominantly optic disc coloboma) was present in 17 individuals and iris coloboma in 1. Fourteen had evidence of cataract, with anterior polar cataracts present in 17 individuals and iridocorneal in 3 individuals. Variant ranking identified a R208W mutation (dbSNP rs757259413; HGMD CM930572) that was present in all 6 sequenced individuals.

**Conclusions:** This study reports on the uncommon association of congenital cataract with posterior segment coloboma in a South African family. The R208W PA26 mutation known to be associated with aniridia is implicated in the variably expressed cataract-coloboma phenotype seen in this family. This association has not previously been described and should contribute to our understanding of the role PAX6 plays in ocular development and cataractogenesis.

**Commercial Relationships:** Saadiah Goolam, None; Nadia Carstens, None; Mark Ross, Illumina Inc.; David Bentley, Illumina Inc.; Margarida Lopes, Illumina Inc.; John Pedren, Illumina Inc.; Zoya Kingsbury, Illumina Inc.; Trevor R. Carmichael, None; Michele Ramsay, None; Susan E. Williams, None.

Cortical cataracts involved in this study. The coding exons and adjacent intronic regions of CRYBA1 gene were analyzed by cycle sequencing.

**Results:** DNA sequencing of the gene revealed a c.272_274delGAG mutation in exon 4, which resulted in a glycine residue deletion at position 91 (p.G91del). This mutation was identified in all of the affected individuals in these two families but was not found in the 192 control chromosomes. Lens examinations in ten affected members showed bilateral nuclear cataracts in Family 1 while another two affected sibling of Family 2 displayed bilateral lamellar cataract.

**Conclusions:** Our results identify that the c.272_274delGAG mutation in CRYBA1 is responsible for the autosomal dominant congenital nuclear or lamellar cataract disease in these two Chinese families.

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**Program Number:** 4833 **Poster Board Number:** A0049

**Presentation Time:** 11:00 AM–12:45 PM

**Mutation analysis of CRYBA1 gene in 185 Chinese families with congenital cataracts**

**Purpose:** Pediatric cataract is the most common form of treatable childhood blindness and is both clinically and genetically heterogeneous. This study is aimed at identification of the mutations in CRYBA1 gene in congenital cataracts patients in a Chinese population.

**Methods:** One hundred and eighty-five families with congenital cataracts involved in this study. The coding exons and adjacent intronic regions of CRYBA1 gene were analyzed by cycle sequencing.

**Results:** DNA sequencing of the gene revealed a c.272_274delGAG mutation in exon 4, which resulted in a glycine residue deletion at position 91 (p.G91del). This mutation was identified in all of the affected individuals in these two families but was not found in the 192 control chromosomes. Lens examinations in ten affected members showed bilateral nuclear cataracts in Family 1 while another two affected sibling of Family 2 displayed bilateral lamellar cataract.

**Conclusions:** Our results identify that the c.272_274delGAG mutation in CRYBA1 is responsible for the autosomal dominant congenital nuclear or lamellar cataract disease in these two Chinese families.

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**Presentation Time:** 11:00 AM–12:45 PM

**Genome-wide trans-ancestry meta-analysis identifies new susceptibility loci for age-related cortical cataract**

**Purpose:** Age-related cataract is a leading cause of visual impairment worldwide, especially in developing countries with limited access to cataract surgery. Cortical cataract is the second most prevalent type of age-related cataract, yet knowledge about its genetic etiology is lacking. We aimed to identify genetic variants influencing age-related cortical cataract.

**Methods:** We conducted a meta-analysis of genome-wide association studies (GWAS) for age-related cortical cataract, involving 9,055 individuals from 5 cohorts of European ancestry and 7,987 from 6 Asian cohorts. Study individuals were genotyped using either Illumina or Affymetrix platforms. After stringent quality controls, genotype data in each cohort were imputed to the 1000 Genomes reference panel. Severity of cortical lens opacity was measured based on lens photos according established protocols. The phenotype data were rank transformed and normalized before analysis. The genotype and phenotype association was assessed using linear regression analysis under an additive effect model, adjusting for age, gender and principal components. Meta-analysis was conducted under an
inverse-variance-weighted fixed-effect model and two random-effects models (DerSimonian-Laird and Han-Eskin).

Results: The combined analysis of both Asian and Caucasian cohorts revealed one genome-wide significant locus and two suggestive susceptibility loci for cortical cataract with low cross-cohort heterogeneity. The most significant locus influencing cortical cataract was observed at chromosome 20q12 ($p = 8.4 \times 10^{-8}$), followed by two loci at chromosome 8q11 ($p = 7.3 \times 10^{-4}$) and 3q26 ($p = 1.3 \times 10^{-4}$). Earlier studies showed that genes at these loci are expressed in ocular tissue and/or involved in development of the eye.

Conclusions: Our study is the first GWAS on age-related cortical cataract. The results yield insights at the gene level for the pathogenesis of age-related cortical cataract.

Commercial Relationships: Wanting Zhao; Rob P. Igo, None; Ekaterina Yonova-Doing, None; Gyungah Jun, None; Caroline C. Klaver, None; Barbara E. Klein, None; Christopher J. Hammond, None; Ching-Yu Cheng, None; Jie Jin Wang, None; Sudha K.伊yengar, None

Program Number: 4835 Poster Board Number: A0051
Presentation Time: 11:00 AM–12:45 PM

Congenital cataracts: new diagnoses using next-generation sequencing in sporadic and familial cases

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Purpose: There is marked genetic heterogeneity in congenital cataracts. Cases are familial or sporadic and provision of genetic information is especially difficult in sporadic cases. Apparently non-syndromic cases may have a subtle syndromic diagnosis. To improve genetic diagnosis, we applied a next-generation sequencing (NGS) strategy in a large Australian cohort of familial and sporadic congenital cataract cases.

Methods: We studied 22 familial and 24 sporadic apparently non-syndromic congenital cataract probands. These patients were seen in the genetic eye clinic of a major referral centre over a period of 12 years. The TruSeq Custom Amplicon and TruSight One (Illumina Inc., CA, USA) NGS platforms were used to examine 32 cataract-associated genes. Sanger sequencing was performed for variant confirmation and segregation studies.

Results: We identified pathogenic variants in 73% of familial and 68% of sporadic congenital cataract cases. In almost two-thirds (20/33) of these cases, this resulted in new information about the diagnosis and/or inheritance pattern. This included one family where a mutation in a different cataract gene (MAF) was found in the affected father, with confirmed paternity, compared with the causative disease gene (NHS) identified in his two sons. The majority of familial cases were due to autosomal dominant mutations in crystallin or gap junction genes, with two autosomal recessive cases due to mutations in these genes, and two families were found to have X-linked NHS mutations. Sporadic cases were mostly due to de novo autosomal dominant mutations in a several genes including crystallins, gap junctions, MAF, MIP and VIM and there were two de novo X-linked BCOR cases. Two novel nonsense mutations on the same allele were found in CRYBB2 in a sporadic case, and in another family we identified the first “run-on” mutation affecting CRYBB3. Severe microphthalmia and sclerocornea, with cataracts, was associated with a novel GJA8 mutation. PAX6 mutations were identified in two patients with complex and variable cataract phenotypes.

Conclusions: Our results demonstrate that targeted NGS in presumed non-syndromic congenital cataract patients provided new diagnostic and/or inheritance information in almost two-thirds of cases. This led to improvements in management and genetic information, and illustrates the important emerging role of NGS in patient care in congenital cataracts.

Commercial Relationships: Alan Ma, None; John R. Grigg, None; Bruce Bennets, None; Robyn V. Jamieson, None

Support: Ophthalmic Research Institute of Australia

Program Number: 4836 Poster Board Number: A0052
Presentation Time: 11:00 AM–12:45 PM

Refractive error and susceptibility to myopia in mice are inherited as quantitative trait

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Purpose: Refractive eye development is controlled by both environmental and genetic factors. However, contribution of genetic factors accounts for at least 70% of variance in refraction in humans. The purpose of this study was to determine whether genetic background influences refractive eye development and susceptibility to experimental myopia in mice.

Methods: Using a high-resolution automated eccentric infrared photorefractor, normal refractive eye development and susceptibility to form-deprivation myopia was compared in 11 genetically distant mouse strains, i.e., A/J, C57BL/6J, CAST/EiJ, CE/J, CZECHII/EiJ, NOD/ShiLtJ, NZO/HILtJ, PWK/PhJ, WSB/EiJ, and 129S1/svlmj. The myopic shift in refraction in the deprived eyes was compared with the control eyes.

Results: There was marked genetic heterogeneity in congenital cataracts. Cases are familial or sporadic and provision of genetic information is especially difficult in sporadic cases. Apparently non-syndromic cases may have a subtle syndromic diagnosis. To improve genetic diagnosis, we applied a next-generation sequencing (NGS) strategy in a large Australian cohort of familial and sporadic congenital cataract cases.
that refractive state of the eye and susceptibility to myopia in mice represent quantitative traits.

Commercial Relationships: Andrei V. Tkatchenko, None; David T. Burke, None; Tatiana V. Tkatchenko, None 
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Program Number: 4837 Poster Board Number: A0053
Presentation Time: 11:00 AM–12:45 PM
Large-scale microRNA expression profiling identifies retinal miRNA-mRNA signaling pathways underlying form-deprivation myopia in mice

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Purpose: Postnatal refractive eye development is regulated by a vision-driven signaling cascade originating in the retina. Failure of this process often leads to the development of myopia associated with changes in ocular tissue gene expression. Although differential expression of coding genes underlying development of myopia has been a subject of intense investigation, the role of non-coding genes such as microRNAs in refractive eye development and in the development of myopia is largely unknown. In this study we explored miRNA-associated miRNA expression profiles in the retina and sclera of mice with experimental myopia using microarrays.

Methods: Twelve P24 C57BL/6J mice were subjected to 10 days of monocular visual form deprivation. Total RNA was isolated from the retina and sclera of myopic and control eyes using mirVana miRNA isolation kit (Life Technologies, Grand Island, NY) according to the manufacturer’s instructions. MiRNA expression profiling was carried out using Agilent Mouse microRNA Microarray (release 12.0) (Agilent Technologies, Santa Clara, CA). Raw gene expression data were normalized using quantile normalization procedure, and log2-transformed. This was followed by the removal of absent features and outliers. The normalized data were then analyzed using ANOVA to identify differences in miRNA expression levels between myopic and control eyes. Differentially expressed miRNAs were identified using an FDR-adjusted P-value threshold of 0.05 and a cutoff of 2-fold change in expression.

Results: We found a total of 53 differentially expressed miRNAs in the retina and no differences in miRNA expression in the sclera of C57BL/6J mice after 10 days of visual form deprivation, which induced -6.93 ± 2.44 D (P < 0.000001, n = 12) of myopia. We also identified their putative mRNA targets among miRNAs found to be differentially expressed in myopic retina and potential signaling pathways involved in the development of form-deprivation myopia using miRNA-mRNA interaction network analysis. Analysis of miRNA-associated signaling pathways revealed that myopic response to visual form deprivation in the retina is regulated by a small number of highly integrated signaling pathways.

Conclusions: Our findings highlight extensive involvement of miRNAs in the regulation of refractive eye development, and in the development of myopia through the retinal gene regulation.

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Program Number: 4838 Poster Board Number: A0054
Presentation Time: 11:00 AM–12:45 PM
A Systematic Genome-wide Meta-analyses for Spherical Error and Axial Length in Asian Population

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Purpose: Myopia has two important endophenotypes, axial length (AL) and spherical equivalent (SE), all previous myopia related genome-wide association studies (GWASs) were analyzing AL or SE alone. We conducted a multivariate GWASs by jointly analyzing AL and SE for myopia in Asian cohorts from a subset of CREAM consortium.

Methods: The univariate regression modeling for the Orthogonal Decomposition Analysis (RegODA) method was used in our multivariate GWASs. We performed a multivariate meta-analysis of genome-wide association studies on AL and SE in Chinese children from the Guangzhou Twin Eye Study (GTES n=1055) and Singapore Cohort Study of the Risk Factors for Myopia (SCORM, n=915) and 6795 Asian adults from Singapore Epidemiology of Eye Disease (SEED), and Singaporean Chinese in the Strabismus, Amblyopia, and Refractive Error Study (STARS).

Results: We identified three novel genes associated with myopia: CMPK1 derived from SNP rs60078183 (P=3.77×10-8), PARPBP derived from SNP rs11204213 (P=1.52×10-8), and ZNF488 derived from SNP rs11111226 (P=2.67×10-8). Interestingly, we found that the signals from these three novel genes were mainly origin from AL, but not significant in SE.

Conclusions: In this multivariate genome-wide meta-analyses, we found three novel genes for myopia in Asia. More studies were needed for validation of our study findings.

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Program Number: 4839 Poster Board Number: A0055
Presentation Time: 11:00 AM–12:45 PM
Linkage Analysis of Common Myopia using Whole Exome Genotyping in Highly Aggregated Ashkenazi Families Replicates Loci on Chromosomes 1 and 7

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Purpose: To perform linkage analyses on highly aggregated Ashkenazi Jewish families with common myopia.

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**Methods:** Our family-based study design involves recruitment of 2,000 members of the Jirel population, a small endogamous ethnic group from the Jiri region of Nepal. Participants undergo a comprehensive eye examination to document ocular biometrics such as central corneal thickness (CCT) and intraocular pressure (IOP), known risk factors for glaucoma. A variance components method is employed to determine the underlying genetic architecture (heritability, pleiotropy) of all measured ocular-related traits (e.g., CCT, IOP) and disease end-points (e.g., glaucoma). The Jirel participants belong to a single extended pedigree containing >62,000 pair-wise relationships that are informative for genetic analysis. The Jirel pedigree has 80% power to detect an additive genetic heritability as low as 6.5% and a genetic correlation between these traits as low as 4.4%.

**Results:** Our first data collection session yielded the recruitment of 229 members of the Jirel population to the study. Their mean (range) age at exam was 45.7 (13-85) years. Mean (median) CCT values: OD 530.6 (534.0) µm, OS 528.8 (536.5) µm. Mean (median) IOP values: OD 14.2 (14.0) mmHg, OS 14.2 (14.0) mmHg. We have recently completed our second data collection session and have recruited an additional 308 members of the Jirel population to the study. By our third data collection session (April 2016) we anticipate a total of approximately 850 Jirels to be recruited to the study, allowing us to then determine initial heritability and pleiotropy estimates to explain the basic underlying genetic architecture of these clinically important ocular measures.

**Conclusions:** Evaluating the genetic architecture of ocular biometry, especially in under-studied populations from the developing world, may yield new information on the biological pathways underlying VI, and thus may be an important step to help address global burden associated with VI.

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