155 Cornea Development
Sunday, May 07, 2017 3:15 PM–5:00 PM
Exhibit/Poster Hall Poster Session
Program #/Board # Range: 959–968/A0307–A0316
Organizing Section: Cornea

Program Number: 959 **Poster Board Number:** A0307
Presentation Time: 3:15 PM–5:00 PM
Effect of Wnt/β-Catenin Signaling Modifiers on Self-Renewal of Human Limbal Epithelial Progenitor Cells Grown from Limbal Biopsy Explants
Hyun Jung Lee[1], J Mario Wolosin[2], So-Hyang Chung[1].
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Purpose: Wnt signaling plays an important role in the regulation of self-renewal in stem cells. Here we investigated the effect of modulation of β-catenin, the primary transducer of the Wnt signaling canonical pathway, on the growth and differentiation of the corneal epithelial progenitors that outgrow from limbal biopsy explants.
Methods: Explants were made in control medium or medium complemented with IWP2, a Wnt signal blocker, or CHIR99021, an indirect β-catenin activator. We determined outgrowth size from limbal biopsy explants, cell size, protein and mRNA expression of markers for stem/precursor cells for corneal epithelial differentiation, and colony formation efficiency (CFE). Comparative experiments with the two modifying agents were performed in cultures of freshly isolated limbal epithelial cells grown in low calcium medium.
Results: CHIR99021 inhibited limbal explant outgrowth rate and expression of the proliferation marker Ki67. Concurrently, IWP2 increased but CHIR99021 decreased (a) the cell density; b) the expression of the stem/precursor marker p63α; c) the expression of the limbal stem cell marker ABCG2; and d) the CFE from outgrowth cells. CHIR99021 also enhanced the expression of the differentiation marker Krt12. In the isolate limbal epithelial cell cultures the effect of the two Wnt-related agents on ABCG2 expression, function, and CFE were in opposite directions to the effects seen on the limbal explant cultured cells. In spite of the divergence of the effects, measurements of the effect of IWP2 or CHIR99021 on total β-catenin indicated that in both systems IWP2 decreased and CHIR99021 increased total β-catenin, respectively.
Conclusions: This result suggests that activation of the canonical Wnt pathway has a negative impact on stem/precursor cells in outgrowths from limbal explant cultures.
Commercial Relationships: Hyun Jung Lee, None; **J Mario Wolosin**, None; **So-Hyang Chung**, None

Program Number: 960 **Poster Board Number:** A0308
Presentation Time: 3:15 PM–5:00 PM
Spatiotemporally regulated ablation of *Klf5* results in dysregulated epithelial homeostasis in mature mouse corneas
Chelsea L. Loughner[1], Doreswamy Kenchegowda[1, 4], Sudha Swamynathan[1], Shivalingappa K. Swamynathan[3, 2].
[1]Ophthalmology, University of Pittsburgh School of Medicine, Pittsburgh, PA; [2]Fox Center for Vision Restoration, University of Pittsburgh School of Medicine, Pittsburgh, PA; [3]Ophthalmology and Cell Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA; [4]Medicine, University of Maryland School of Medicine, Baltimore, MD.
Purpose: Previously, we reported that Le-Cre-mediated conditional ablation of Krüppel-like transcription factor-5 (*Klf5*) in the developing murine ocular surface results in abnormal cornea, conjunctiva, and the eyelids. Here, we employ Tet-On system for spatiotemporally regulated ablation of *Klf5* to examine its functions in mature mouse corneal epithelial (CE) homeostasis.
Methods: Cre expression was induced in 8-week-old ternary transgenic *Klf5*$^{LoxP/LoxP}$/ *Krt12*$^{rtTA/rtTA}$/ *Tet-O-Cre* (*Klf5*$^{\Delta/\Delta CE}$) mouse corneas by doxycycline administered through chow, to ablate *Klf5* in the CE. The wild type (WT) and *Klf5*$^{\Delta/\Delta CE}$ corneal *(i)* histology, *(ii)* cell proliferation, and *(iii)* Klf5-target gene expression were examined using *(i)* hematoxylin and eosin (H&E), and periodic acid-Schiff's reagent (PAS)-stained sections, *(ii)* Ki67 expression, and *(iii)* qPCR and immunostaining, respectively. The effect of Klf5, Klf4 and Oct1 on gastrokine-1 (*Gkn1*) promoter activity was determined by transient transfection in human corneal limbal epithelial (HCLE) cells.
Results: *Klf5* was efficiently ablated within 15 days of doxycycline administration in *Klf5*$^{\Delta/\Delta CE}$ corneas (23% of WT), resulting in disrupted epithelial basement membrane, decreased cell proliferation (51% of WT), and irregular stratification. *Klf5*$^{\Delta/\Delta CE}$ corneas displayed decreased expression of basement membrane laminin (55% of WT), corneal crystallin transketolase (*Tkt*; 36% of WT), *Gkn1* (17% of WT), uroplakin-1b (*Upk1b*; 14% of WT), and *Upk3b* (11% of WT) that promote permeability barrier, and desmosomal protein desmoglein-1a (*Dsg1a*; 13% of WT). *Gkn1* promoter activity was stimulated 7-fold by Klf4, 19-fold by Klf5, 2.75-fold by Oct1, and 41-fold in a synergistic manner by Klf5 and Oct1.
Conclusions: Spatiotemporally regulated ablation of *Klf5* in mature mouse CE results in disrupted epithelial basement membrane, dysregulated cell proliferation and stratification, accompanied with decreases in *Gkn1*, *Dsg1a*, *Upk1b*, *Upk3b*, and *Tkt* expression, consistent with a prominent role for Klf5 in mature CE homeostasis.
Commercial Relationships: Chelsea L. Loughner, None; **Doreswamy Kenchegowda**, None; **Sudha Swamynathan**, University of Pittsburgh School of Medicine (P); **Shivalingappa K. Swamynathan**, University of Pittsburgh School of Medicine (P)
Support: NEI, NIH Grant R01EY026533; NEI, NIH Grant P30 EY08098; Unrestricted grants from 'Research to Prevent Blindness' and the 'Eye and Ear Foundation of Pittsburgh'

Program Number: 961 **Poster Board Number:** A0309
Presentation Time: 3:15 PM–5:00 PM
Exogenous TGFβ3 promotes mouse corneal stroma development
Lingling Zhang[1], Lung-Kun Yeh[2, 3], Yujin Zhang[1], Yuka Okada[4, 1], Yen-Chiao Wang[1], Chia-Yang Liu[1]. [1]Indiana University, BLOOMINGTON, IN; [2]Department of Ophthalmology, Chang-Gung Memorial Hospital, Taoyuan, Taiwan; [3]Chang-Gung University College of Medicine, Taoyuan, Taiwan; [4]Wakayama Medical University, Wakayama, Japan.
Purpose: We previously reported that loss of β-catenin in corneal stromal keratocytes triggered corneal precocious maturation, coincident with up-regulation of various growth factors including BMP4 and TGFβ3. This study attempts to examine the potential roles of TGFβ3 during cornea development.
Methods: Primary corneal stroma fibroblasts were treated with BMP4, TGFβ3 or PBS (control) and related gene expressions were determined by qRT-PCR and western blots. In addition, either BMP4 or TGFβ3 was injected subcutaneously into C57BL6 neonates from postnatal day 0 (P0) to P7. Mice were sacrificed at P8 and were subjected to histological examinations by HE staining and TEM. Gene expressions were determined by western blots and IHC immunostaining.

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Results: BMP4 injection causes mouse epithelial layer increase to more than 10 layers instead of 1-2 cell layers in littermate control mice, while it has no influence on stromal maturation. On the other hand, TGFβ3 promotes corneal stromal maturation but has little effect on epithelial cell stratification and differentiation. Primary cell culture experiments showed that the expressions of *Col1a1, Kera and Lum* were ≥2-fold increased after TGFβ3 treatment for 24hrs in the corneal stromal fibroblasts, while no increases were detected after BMP4 treatment. TEM revealed that TGFβ3 promotes mouse corneal stroma maturation by increasing stromal cell density and cell activates (more ER and Golgi), extracellular matrix formation/ organization.
Conclusions: These data suggested that canonical Wnt signaling modulates Bmp4 and TGFβ3 expression which in turn facilitate epithelial stratification and stromal maturation during corneal development.
Commercial Relationships: Lingling Zhang, None; **Lung-Kun Yeh**, None; **Yujin Zhang**, None; **Yuka Okada**, None; **Yen-Chiao Wang**, None; **Chia-Yang Liu**, None
Support: NIH RO1 EY21501, EY 23086, CMRPG3E1522, CMRPG3E1521 and 104-2314-B-182A-097-MY3

Program Number: 962 **Poster Board Number:** A0310
Presentation Time: 3:15 PM–5:00 PM
Developmental expression of SV2 in embryonic chicken corneal epithelium
James K. Kubilus, Christopher Talbot, Thomas Linsenmayer. Integrative Physiology and Pathobiology, Tufts University School of Medicine, Boston, MA.
Purpose: Within the cornea, intraepithelial corneal nerves (ICNs) help to protect the cornea by acting as the afferent limb of the blink reflex, as well as by modulating the production of tears. Without ICNs, the cornea is more susceptible to damage. In addition to these functions, ICNs are also thought to play a role in the health and homeostasis of the cornea through the release of trophic factors. These trophic factors have been shown previously to support the cells of the corneal epithelium. Though the ICNs innervating the cornea are thought to terminate predominately as "simple" free nerve endings, the mechanisms involved in their release of trophic factors is unclear. Here, we sought to investigate this mechanism by determining the expression pattern of SV2, a marker of synaptic vesicles within the cornea during development.
Methods: Embryonic chicken anterior eyes from embryonic day 4 (E4) through E17 were dissected and either fixed or frozen fresh for immunohistochemistry with antibodies against SV2. Transmission electron microscopy (TEM) was used to observe morphological changes. Western blot was used to further examine the expression of SV2.
Results: The embryonic cornea of the chicken is innervated in a series of precise stages, with nerves first appearing in the corneal epithelium between E10 and E11. Immunohistochemistry showed that SV2 labeling did not appear in the epithelium until after this time point, suggesting that SV2 is specific for vesicles within the nerves. This punctate SV2 labeling was present in all levels of the corneal epithelium, but localized predominately at the basolateral borders between cells in both the subbasal nerve plexus as well as within the apical squamous layer. Western blot of corneal epithelial lysates showed that corneal SV2 migrated at a different molecular weight than SV2 from brain. Also, unlike brain derived SV2, this migration was not affected by the deglycosylating enzyme PNGASE.
Conclusions: Our data show that the appearance of SV2 correlates with the appearance of nerves in the cornea. However, corneal SV2 is different in its size and glycosylation. This suggests that the mechanisms though which ICNs release trophic factors are likely not as "simple" as previously thought and may be unique to the cornea.
Commercial Relationships: James K. Kubilus, None; **Christopher Talbot**, None; **Thomas Linsenmayer**, None
Support: NIH Grant R01EY023569

Program Number: 963 **Poster Board Number:** A0311
Presentation Time: 3:15 PM–5:00 PM
Overexpression of TGF-a is detrimental to the corneal epithelial development
Yen-Chiao Wang[1], Yujin Zhang[1], Lingling Zhang[1], Yuka Okada[1, 2], Chia-Yang Liu[1]. [1]School of Optometry, Indiana University, BLOOMINGTON, IN; [2]Wakayama Medical University, Wakayama, Japan.
Purpose: Our previous study showed that conditional overexpression of TGFα in mouse keratocytes resulted in corneal epithelial hyperproliferation followed by subsequent degeneration. In this study, we examined the potential mechanism of the corneal degeneration caused by TGF-a.
Methods: *KeraR/tet-O-TGFα* bitransgenic (TG) mice were generated by naturally crossing *KeraR* driver mice which express rtta specifically in mouse stromal keratocytes with the effector mice *tet-O-TGFα*. TG mice were administered with doxycycline (Dox) for TGFα overexpression from embryonic day 12.5 (E12.5) to different developing stages including E14.5, E16.5, E18.5, postnatal day 0 (P0) and P7. Single transgenic littermates were used as controls (CTR). Mouse samples were subjected to Western blot, HE staining and transmission electron microscope (TEM) and immunofluorescence staining (IFS) to evaluate ocular surface conditions.
Results: TGFα overexpression by the corneal stroma of TG mice after Dox induction was confirmed by Western blot. Although, the expressions of K12 keratin and Pax6 in the central corneal region remained intact, while gradually lost in corneal peripheral region. On the other hand, Keratin 13 expression was extended in corneal peripheral region. Furthermore, the hyperproliferative epithelial cells were not connected with desmosomes in the TG as compared with those in CTR at P7.
Conclusions: These data suggest that loss of desmosomes in cell-cell junction could be a critical reason to the corneal degeneration. Overexpression of TGF-alpha not only triggered an aberrative proliferation of corneal epithelial cells but lacking the formation of desmosomes to tight them together via intermediate filament. In addition, overexpression of TGF-a may deplete limbal epithelial stem cell phenotype and lead to conjunctivalization. Together, our data indicated that excess of TGF-a is detrimental to corneal epithelium development.
Commercial Relationships: Yen-Chiao Wang, None; **Yujin Zhang**, None; **Lingling Zhang**, None; **Yuka Okada**, None; **Chia-Yang Liu**, None

Program Number: 964 **Poster Board Number:** A0312
Presentation Time: 3:15 PM–5:00 PM
Pigment epithelial-derived factor promotes niche reconstruction in rabbit limbal regeneration
Nai-Wen Fan[1], Tsung-Chuan Ho[2], Shu-I Yeh[3], Yeou-Ping Tsao[3, 2]. [1]Ophthalmology, Taipei Veterans General Hospital, Taipei, Taiwan; [2]Department of Medical Research, Mackay Memorial Hospital, Taipei, Taiwan, Taipei, Taiwan; [3]Department of Ophthalmology, Mackay Memorial Hospital, Taipei, Taiwan.
Purpose: The microenvironment or "niche" plays an important role in regulating the fate of limbal stem cells. Our previous animal study showed that pigment epithelial derived factor (PEDF) accelerates the regeneration of limbal epithelium in limbal lesion created by partial

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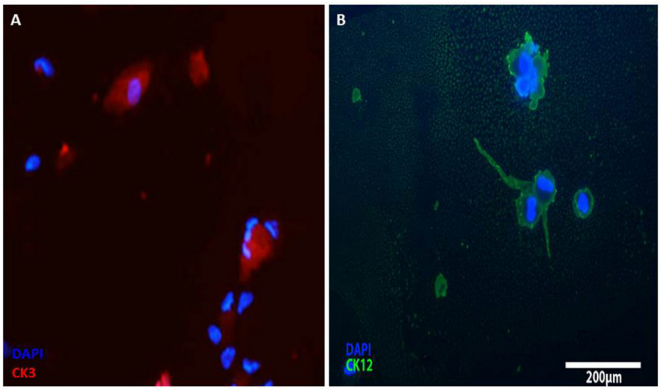
limbal excision. The reconstruction of limbal niche in the process of limbal epithelial repair remains elusive. This study was to investigate the effect of PEDF-derived short peptide 29-mer on the renewal of limbal niche.
Methods: New Zealand albino rabbits were used in this study. Limbal excision (180 degree) was performed surgically to remove complete limbal epithelium and partial limbal stroma. Topical 29-mer containing ointment once a day for 2 weeks was applied immediately after surgery. The repair of limbal niche at 2 weeks was assessed by immunofluorescence staining with antibodies for N-cadherin, CD90, CD105, c-kit, ABCG2. Corneal stromal stem cells (CSSC) were harvested from normal rabbit limbus and phenotypic evaluation was assessed by the expression of CD90. Inhibitors for morphogen pathways were used to investigate the cross-talk between morphogen pathways and PEDF signaling in regulating CSSC proliferation and markers.
Results: Immunofluorescence analysis revealed that eyes treated with the 29-mer significantly increased the number of CD90, CD105, and N-cadherin positive cells in the anterior stroma of repaired limbus compared to vehicle treatment ($P < 0.05$). Interestingly, presence of c-kit, ABCG2 positive stromal cells were noted in 29-mer treated eyes but not in control eyes. This suggests that 29-mer may also mediate recruitment of BM-derived mesenchymal cells to the limbal wound. In cultured CSSC, western blotting showed upregulation of CD90 and cyclin D1 proliferation marker, and the effect was decreased after treated with the Inhibitors for Wnt and Hedgehog.
Conclusions: PEDF facilitate limbal niche reconstruction with evidence of presenting CD90, CD105, c-kit ABCG2 and N-cadherin positive stromal cells. The beneficial effect of PEDF 29-mer on limbal niche cell may involve in morphogen pathways.
Commercial Relationships: Nai-Wen Fan, None; **Tsung-Chuan Ho**, None; **Shu-I Yeh**, None; **Yeou-Ping Tsao**, None
Support: This work is supported by grants from Taipei veterans general hospital and Mackay Memorial Hospital, Taipei, Taiwan

Program Number: 965 **Poster Board Number:** A0313
Presentation Time: 3:15 PM–5:00 PM
Can corneal limbal proteins drive the trans-differentiation of dental pulp stem cells into corneal epithelium like cells?
M C. Hillarby[1], Josh Smith[1], Susan Shawcross[2], Julian Yates[3], Arun Brahma[4], Fiona Carley[4], Abid Ali[1]. [1]Division of Pharmacy and Optometry, University of Manchester, Manchester, United Kingdom; [2]Division of Cell Matrix Biology & Regenerative Medicine, University of Manchester, Manchester, United Kingdom; [3]Division of Dentistry, University of Manchester, Manchester, United Kingdom; [4]Manchester Royal Eye Hospital, Manchester, United Kingdom.
Purpose: Limbal stem cell deficiency results in the loss of normal corneal epithelial cell homeostasis and results in loss of vision. Stem cell transplantation may restore the limbal stem cells and replenish the corneal epithelium. The aim of this study was to assess whether proteins secreted by corneal limbal explants, keratinocyte growth factor (KGF) and type IV collagen (Col4), could drive Dental Pulp Stem Cells (DPSC) trans-differentiation into corneal epithelium-like cells (CEC) or precursor cells.
Methods: CD90 positive DPSC were extracted from adult teeth and cultured with 1) a non-contact co-culture with limbal explants; 2) KGF supplemented media and 3) Col4 supplemented α-MEM growth media. After eight days, inverted light microscopes was used to review histological changes that occurred in each group and immunocytochemistry and RT-PCR were conducted to detect changes in DPSC expression of mesenchymal stem cell marker CD90, CEC markers cytokeratin 12 (KRT 12) and cytokeratin 3 (KRT 3) and conjunctival markers cytokeratin 13 (CK13) and cytokeratin 19 (CK19).
Results: DPSCs co-cultured with limbal explants or in media supplemented with either Col4 or KGF all demonstrated corneal like morphology and expression of CEC markers cytokeratin 3 and 12, consistent with the trans-differentiation into corneal epithelial like cells.
Conclusions: These findings may contribute to establishing a standardised culture protocol for DPSC-CEC-like trans-differentiation without relying on non-contact co-culture with limbal explants and facilitate research in to stem cell therapy for LSCD.



Immunohistochemistry staining showing cytokeratin 3 (A) and 12 (B) expression in DPSC cultured with Col4
Commercial Relationships: M C. Hillarby, None; **Josh Smith**, None; **Susan Shawcross**, None; **Julian Yates**, None; **Arun Brahma**, None; **Fiona Carley**, None; **Abid Ali**, None

Program Number: 966 **Poster Board Number:** A0314
Presentation Time: 3:15 PM–5:00 PM
Altered fate of corneal epithelial cells in *Dstn*^{corn1} mice
Yuyun Zhu[1], Sharolyn Kawakami-Schulz[1], Wei-Hua Lee[1], Akihiro Ikeda[1], Winston W. Kao[2], Sakae Ikeda[1]. [1]Department of Medical Genetics, University of Wisconsin-Madison, Madison, WI; [2]Department of Ophthalmology, University of Cincinnati, Cincinnati, OH.
Purpose: *Dstn*^{corn1} mice, deficient mutants of the actin depolymerizing factor destrin, display epithelial hyperproliferation, inflammation and neovascularization in the cornea. In the *Dstn*^{corn1} corneal epithelium, we observed keratin 12 (Krt12)-positive cells that are also positive for keratin 15 (Krt15), a marker of corneal epithelial progenitor cells that normally reside in the limbus. The purpose of this study is to test the hypothesis that abnormal actin dynamics lead to transformation of Krt12-positive corneal epithelial cells into the less differentiated state in *Dstn*^{corn1} mice.
Methods: The *mTmG* double fluorescent cre reporter system was used to genetically label specific cell populations for cell lineage tracing. Specifically, we generated mice with the genotypes of *Dstn*$^{corn1/corn1}$; *Krt12*$^{rtTA/rtTA}$; tetO-cre; *mTmG/+*, and *Dstn*$^{corn1/+}$; *Krt12*$^{rtTA/rtTA}$; tetO-cre; *mTmG/+*. Doxycycline (dox) was administered through diet for 10 days to label the differentiated Krt12-positive cells with green fluorescence (mG), during which mT reporter gene cassette was deleted by cre expressed in Krt12-positive cells. The mG was chased upon the withdraw of dox from the diet for 0, 2 and 4 days, and excised corneas were subjected to immunohistochemistry using the Krt15 antibody. The corneal area positive for mG and the total corneal area were quantified using ImageJ. Analysis of variance was used with Tukey's honest significant difference test for statistical analysis.

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Results: The mG-positive area normalized to the total corneal area was the largest immediately after the completion of dox administration (day 0), and decreased in time both in control and *Dstn*^{corn1} mice. The mG-positive corneal area was less in *Dstn*^{corn1} mice compared to control mice at each time point. In the *Dstn*^{corn1}cornea, we observed mG labeled cells that were also stained by Krt15 antibody.
Conclusions: Our findings indicate that the Krt12-positive terminally differentiated corneal epithelial cells transform to the less differentiated state in the corneal epithelium of *Dstn*^{corn1} mice. The mG-positive area was larger in control mice compared to *Dstn*^{corn1} mice at each time point, suggesting that cells in the *Dstn*^{corn1} corneal epithelium may have a higher renewal rate compared to control mice.
Commercial Relationships: Yuyun Zhu; **Sharolyn Kawakami-Schulz**, None; **Wei-Hua Lee**, None; **Akihiro Ikeda**, None; **Winston W. Kao**, None; **Sakae Ikeda**, None
Support: NIH R01 EY016108

Program Number: 967 **Poster Board Number:** A0315
Presentation Time: 3:15 PM–5:00 PM
Evaluation of the effects of RB-35 on the viability and growth of a corneal epithelium cell line
blanca elizabeth martinez baez, Rosario Gulías-Cañizo, Valeria Sanchez-Huerta, Federico Castro Muñoz-Ledo, Lorena Zendejas Reyes. Cornea, Asociación para Evitar la Ceguera en México "Hospital Dr. Luis Sánchez Bulnes" IAP, Ciudad de Mexico, Mexico.
Purpose: RB-35 has shown to possess antioxidant properties due to its free radical scavenging activities and its protective effects against lipid peroxidation. Since oxidative damage plays a major role in many ocular surface diseases, we aimed to evaluate the effects of RB-35 in a corneal epithelium cell line as preliminary evidence of its therapeutic potential.
Methods: A rabbit corneal epithelium cell line was used, RCE-1(5T5), which reproduces in vitro the differentiation and proliferation processes normally present in primary cultures of corneal epithelium. The experimental strategy included: Colony forming assay to evaluate cell growth, flow cytometry to evaluate cell viability and immunofluorescence to evaluate cell phenotype. All experiments were performed in triplicate.
In the colony forming assay, the cells are seeded with the standard density for a 35 mm plate (3 x 10^4) on day 0; on day 1 control cells receive corneal medium + EGF and treated cells receive corneal medium + EGF + RB-35 at different concentrations (10 μM, 20 μM, 30 μM, 50 μM, 100 μM). On day 3, cells are harvested and reseeded with a seeding density of 500 cells per 60 mm plate, and the growth is evaluated on a daily basis until the culture forms colonies. At this point, colonies are stained with rhodamine.
To evaluate cell viability, we used 7-aminoactinomycin D, a fluorescent intercalator that undergoes a spectral shift upon association with DNA, which stains non-viable cells. These experiments were analyzed by flowcytometry.
We also performed the immunofluorescence staining protocol for keratin 3, a marker of terminal differentiation specific of corneal epithelial cells, to evaluate cell characteristics.
Results: We demonstrate a colony forming efficiency similar to the control, which rules out an anti-proliferative effect on cell growth. The percentages of colony forming efficiency between control cells and treated cells did not show statistical significance. Moreover, in the viability assay, we observed a statistical significant difference favoring RB-35.
Conclusions: RB-35 does not induce cytotoxic effects in a concentration range of 10 to 100 μM, and it demonstrates a protective effect evidenced by reduction of apoptosis without inducing cell phenotypic changes. These results give the first positive evidence to continue evaluating RB-35 as a potential treatment for ocular conditions.

Condition	Number of colonies	Colony forming efficiency
Negative control (without EGF)	173	34%
Positive control (with EGF)	212	42%
RB-35 10 μM	176	35%
RB-35 20 μM	190	38%
RB-35 30 μM	186	37%
RB-35 50 μM	201	40%
RB-35100 μM	199	41%

Condition	Viability (%)
Control	81.34*
RB-35 10 μM	91.57*
RB-35 20 μM	91.45*
RB-35 30 μM	94.79*
RB-35 50 μM	94.66*
RB-35 100 μM	89.97*

*p< 0.005

Commercial Relationships: blanca elizabeth martinez baez, None; **Rosario Gulías-Cañizo**, None; **Valeria Sanchez-Huerta**, None; **Federico Castro Muñoz-Ledo**, None; **Lorena Zendejas Reyes**, None

Program Number: 968 **Poster Board Number:** A0316
Presentation Time: 3:15 PM–5:00 PM
A novel *CYP1B1* mutation with the Von Hippel's description of an internal corneal ulcer phenotype
Tania Albavera, Valeria Oliva, Alejandro Navas, Oscar Chacon, Juan C. Zenteno, Enrique O. Graue-Hernandez, Mirena Astiazarán, Antonio Bermudez. Instituto de Oftalmologia Conde de Valenciana, Distrito Federal, Mexico.
Purpose: To report a mutation of *CYP1B1* in a new born with a rare phenotype consisting of bilateral corneal opacification and elevated intraocular pressure without the classic features of a dysgenesis of anterior segment or congenital glaucoma
Methods: The new born presented with diffuse corneal edema and bilaterally elevated IOP. Ophthalmological examination, ultrasound and UBM were performed, congenital infections were ruled out. Genomic DNA of the patient and both of his parents was extracted from peripheral blood leukocytes according to standard procedures. The 2 coding exons and the flanking intronic regions of the *CYP1B1* gene were amplified by PCR. Direct automated Sanger sequencing was performed. All samples were analyzed in a3130 Genetic Analyzer. Wild type and mutated *CYP1B1* sequences were compared manually. The patient underwent penetrating keratoplasty and goniotomy in a single surgical time. The button was subjected to histopathological examination. Findings and outcomes were recorded
Results: The patient is the first child of young, healthy, consanguineous parents. In the ophthalmological examination the

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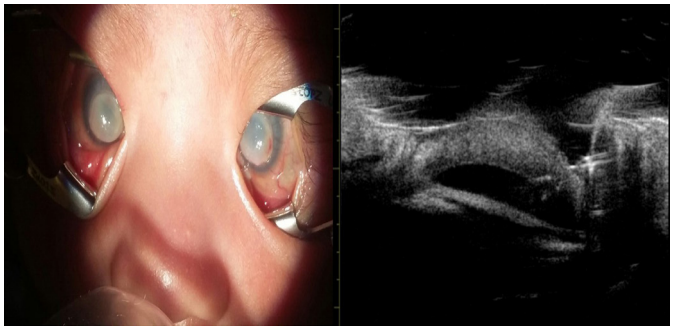
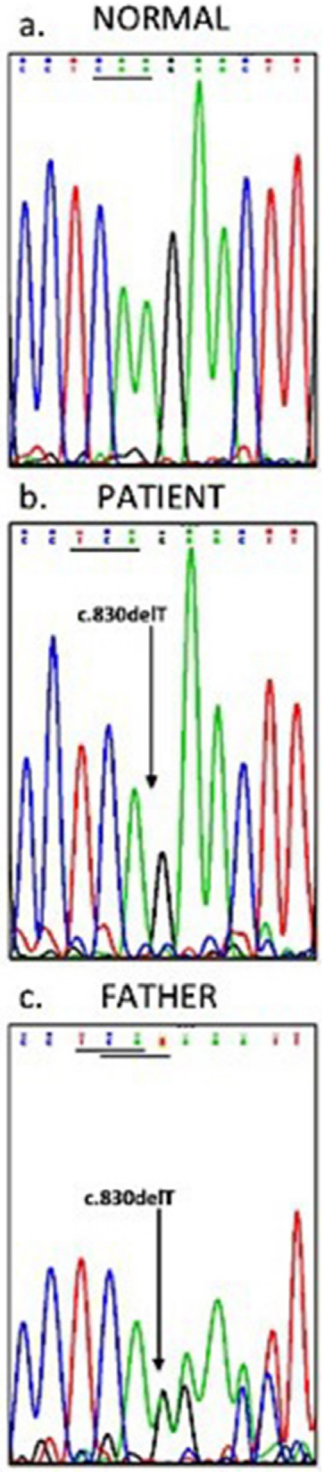
VA was light Perception for both eyes. IOP was 55 and 45 mmHg respectively. Corneal diameter was 10mm for both eyes. Ultrasound reported an optic cup suggestive of glaucoma. In the UBM corneal thickness was 3.23 and 3.42mm respectively and keratoiridolenticular dysgenesis were not found. *CYP1B1* gene analysis demonstrated homozygosity for a 1bp deletion in exon 2 (c.830delT). This pathogenic variant is predicted to result in a frameshift and introduction of a premature stop codon immediately after the deletion site (p.L277*). Parental DNA analysis demonstrated that both parents were heterozygous carriers for the same variant. After PKP and goniotomy IOP was normalized and corneal button was clear. Histophatological examination of the corneal button revealed loss of Bowman's membrane in the central cornea, fibrosis of the stroma, absence of endothelial cells and loss of Descemet's membrane centrally

Conclusions: This study supports the role of CYP1B1 as a causative gene in anomalies of the anterior segment. Furthermore, this emphasizes the broad range of phenotypic expression for CYP1B1 mutations and its role in eye development. The mutation reported in this study is not present in public variant databases. There is scant evidence of a CYP1B1 mutation with this phenotipic presentation





Commercial Relationships: Tania Albavera, None; **Valeria Oliva**, None; **Alejandro Navas**, None; **Oscar Chacon**, None; **Juan C. Zenteno**, None; **Enrique O. Graue-Hernandez**, None; **Mirena Astiazarán**, None; **Antonio Bermudez**, None

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