Anterior Corneal Aberrations in Relation to Severity of Fuchs Endothelial Dystrophy

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**Purpose:** In corneas requiring endothelial keratoplasty for Fuchs endothelial corneal dystrophy (FECD), high-order aberrations from the anterior corneal surface are higher than normal and remain higher after keratoplasty. In this study, we examined the changes in anterior corneal aberrations over a range of severity of FECD.

**Methods:** In a cross-sectional study, 70 corneas of 44 patients with FECD (mean age, 67 years; range, 42-83 years) and 67 normal corneas of 35 control participants (mean age, 58 years; range, 40-80 years) were examined by slit-lamp and Scheimpflug imaging. Clinical grade of FECD was based on the presence and extent of guttae and the presence or absence of clinically evident edema (modified Krachmer grades 1-6). FECD was categorized as mild (grades 1-2), moderate (grades 3-4), or advanced (grades 5-6). Corneas of control subjects were devoid of any central guttae (grade 0). Aberrations from the anterior corneal surface were measured from Scheimpflug images (Pentacam, Oculus), and wavefront errors over a 6 mm-diameter optical zone were expressed as Zernike polynomials through the 6th order. High-order aberrations, expressed as the root-mean-square of wavefront errors, were compared between severities of FECD and normal by using generalized estimating equation (GEE) models to account for any correlation between fellow eyes of the same subject, and with an adjustment for age.

**Results:** Over a 6 mm-diameter optical zone, total anterior high-order aberrations in FECD (0.63 ± 0.31 μm) were higher than normal (0.45 ± 0.17 μm, p=0.002). Aberrations were higher in moderate FECD (0.64 ± 0.32 μm, n=27) and advanced FECD (0.68 ± 0.33 μm, n=21) compared to normal (p=0.03 for both comparisons), whereas there was no difference between mild FECD (0.58 ± 0.27 μm, n=22) and normal (p=0.10, minimum detectable difference, 0.14 μm [α=0.05, β=0.20]).

**Conclusions:** The increase in anterior corneal high-order aberrations, which affect visual acuity after endothelial keratoplasty, begins earlier in the course of FECD than the onset of clinically detectable corneal edema. Anterior corneal changes over the course of FECD suggest the possibility of suboptimal vision even after endothelial keratoplasty for moderate FECD.

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that Rho kinase (ROCK) inhibitor suppresses the apoptosis of cultured corneal endothelial cells (CECs). At the onset of apoptosis, membrane blebbing is caused by phosphorylation of myosin light chain (MLC) following activation of ROCK. The purpose of this present study was to investigate whether or not ROCK inhibitor suppresses CEC apoptosis by inhibiting blebbing.

**Methods:** Monkey corneal endothelial cells (MCECs) were treated by ultraviolet (UV) radiation to induce apoptosis, and then treated with 10μM blebbistatin (inhibitor of myosin II) or 10μM Y-27632 ROCK inhibitor. To elucidate the involvement of MLC in apoptosis, apoptotic cells in the MCECs treated by UV radiation were evaluated by Annexin V staining. Cleavage of caspase 3 and Poly (ADP-ribose) polymerase (PARP) were also evaluated by western blotting. Phosphorylation of MLC after UV radiation was evaluated by western blotting and immunostaining. The numbers of blebbing cells was evaluated by phase contrast microscopy.

**Results:** In the UV-radiated MCECs, blebbistatin and Y-27632 significantly decreased UV-induced Annexin V-positive apoptotic CECs (14.8±2.6% and 7.8±1.4%, respectively) compared to the controls (36.2±2.3%) (p<0.01). In addition, cleavages of caspase 3 and PARP induced by UV radiation were decreased both by blebbistatin and Y-27632. Western blotting and immunostaining showed that MLC phosphorylation was induced by UV radiation and suppressed by blebbistatin and Y-27632. The percentages of blebbing cells among floating cells post UV radiation were 57.5±3.1% in the control, while those in the MCECs treated by blebbistatin and Y-27632 were significantly decreased (26.1±3.6%, 15.2±1.3%, respectively) (p<0.01).

**Conclusions:** The findings of the present study indicate that ROCK inhibitor suppresses CEC apoptosis by inhibiting blebbing caused by MLC phosphorylation, yet the blebbing independent pathway requires further investigation. Modulation of CEC apoptosis by ROCK inhibitor might be a useful therapeutic tool for corneal endothelial disease.

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**Presentation Time:** 4:30 PM–4:45 PM

**The involvement of transforming growth factor beta in excessive extracellular matrix production of corneal endothelial cells in Fuchs’ endothelial corneal dystrophy**

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**Purpose:** Fuchs’ endothelial corneal dystrophy (FEDC) is characterized by progressive loss of corneal endothelial cells (CECs), thickening of Descemet’s membrane and deposition of extracellular matrix (ECM) in the form of guttae. However, the molecular mechanism by which ECM is produced in FEDC has yet to be elucidated. Here we demonstrate the involvement of epithelial-to-mesenchymal transition (EMT)-related genes in the excessive ECM production by CECs in FECD.

**Methods:** Human CECs (HCECs) derived from 3 FECD patients and HCECs derived from 3 normal donor corneas were cultured and then immortalized by SV40 and hTERT to produce iFEDC and iHCEC cell lines. To elucidate ECM production, iFEDC and iHCEC cells were cultured in Transwell® Inserts, and ECM deposition was analyzed by hematoxylin-eosin staining. Type-I and type-IV collagen and fibronectin were evaluated by real-time polymerase chain reaction (PCR) and immunostaining. EMT-related gene (Snail1, Snail2, and ZEB1) levels were analyzed by real-time PCR. To elucidate the involvement of Snail1 and ZEB1 in the excessive ECM production, siRNA was used to knockdown those two genes. To evaluate the involvement of the transforming growth factor beta (TGF-β) signaling pathway, cells were treated with TGF-β or with SB431542 (an inhibitor of TGF-β type-1 ALK receptors).

**Results:** ECM thickness was significantly increased in iFEDC compared to iHCEC (6.65±0.82μm and 3.14±0.64μm, respectively) (p<0.01). PCR and immunostaining revealed increased production of type-I collagen, type-IV collagen, and fibronectin in iFEDC compared to iHCEC. In iFEDC, expression of Snail1 and ZEB1 was significantly increased (p<0.01), while Snail2 was not increased. TGF-β markedly increased the expression of Snail1 and ZEB1 associated with the increased production of ECM both in FECD and iHCEC. On the other hand, siRNA of Snail1 and ZEB1 suppressed ECM association of iFEDC (p<0.01). Moreover, SB431524 significantly suppressed the expression of Snail1 and ZEB1, as well as the production of ECM (p<0.01).

**Conclusions:** Our findings demonstrate that increased levels of EMT-related genes may induce excessive ECM production in FEDC and that inhibition of the TGF-β signaling pathway may be a potential therapeutic target for the treatment of FEDC.

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**Presentation Time:** 4:45 PM–5:00 PM

**Menadione Induces Endothelial Mesenchymal Transition in Human Corneal Endothelial Cells**

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**Purpose:** Menadione induces endothelial mesenchymal transition (EMT)-related genes in human corneal endothelial cells (HCECs), which is characterized by the loss of functional endothelial cells due to thickening of Descemet’s membrane and deposition of extracellular matrix (ECM) in the form of guttae. The hypothesis of this study was to quantify endothelial cell stress response and EMT changes in telomerase-immortalized human endothelial cells (HCEntC-21T) in response to oxidative stress.

**Methods:** HCEntC-21T were grown to confluence and then treated with 100 μM of menadione bisulfite (MN) for 1-5 hours in low glucose Dulbecco’s Modified Eagle Medium (DMEM). Non-treated cells served as controls. Morphology was assessed by phase-contrast microscopy.
microscopy every hour during treatment. Rosette formation was quantified and adjusted to the cell number. Subcellular localization of EnMT markers (vimentin, E-cadherin, N-cadherin, and alpha-smooth muscle actin) was determined using immunofluorescence confocal microscopy.

**Results:** MN treatment resulted in endothelial cell rosette formation, where cells clustered around circular areas of distinct cell loss. There was a time-dependent increase in loss of cell hexagonality and development of fibroblast-like morphology. There was a linear rise in the number of rosettes formed in response to MN treatment, with significant increase at 5 hours (37±5.72) compared with 1 hour (6.0±0.82; p=0.01). MN treatment resulted in positive discrete cytoplasmic staining of vimentin and alpha-smooth muscle actin as compared to non-treated controls, which were negative for both antibodies. Staining of E-cadherin and N-cadherin was more intense in MN-treated samples as compared to controls.

**Conclusions:** MN treatment results in endothelial morphologic changes seen in FED. There is an upregulation of EnMT markers during corneal endothelial cell rosette formation in response to MN treatment. These findings suggest that EnMT may play an important role in FED pathogenesis.

Representative confocal images of non-treated (A) and treated (B) telomerase-immortalized human endothelial cells (HCEnC-21T). Discrete cytoplasmic vimentin staining (B) was noted in cells treated with 100μM of Menadione for 5 hours. Final magnification: 400X with 4 zoom.

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

**Comparing the Transcriptome of Ex Vivo Endothelium with Cultured Human Corneal Endothelial Cells**

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**Purpose:** To compare the gene expression profile of ex vivo human corneal endothelium with that of primary and established cultures of human corneal endothelial cells (HCEnC) and to determine the utility of immortalized corneal endothelial cells as a cell culture model of in vivo corneal endothelium.

**Methods:** Descemet membrane with the attached corneal endothelium was separated from 5 eye bank donor corneas. Total RNA was isolated from ex vivo HCEnC and triplicate cultures of primary and immortalized corneal endothelial cells (HCEC-12, HCEnC21T, HCEC-B4G12) and processed for downstream gene expression analysis using RNA-sequencing technology. Hierarchical clustering and principle component analysis (PCA) were performed to determine relationships between the samples. The expression of genes that are considered functional markers of HCEnC were compared and differences in expression were analyzed statistically.

**Results:** Primary and immortalized corneal endothelial cells demonstrated polygonal and cobblestone morphology characteristic of ex vivo corneal endothelium. Hierarchical clustering and PCA demonstrated underlying variance between the datasets from each RNA source. Primary cells showed the strongest relationship with ex vivo endothelium, while the cell lines were more distantly related to ex vivo samples. Ex vivo and primary cells showed the highest number and percentage of commonly expressed genes, while ex vivo and HCEC-B4G12 cells demonstrated the lowest number and percentage of commonly expressed genes. The mRNA levels of AQP1 and ATP1A1 were reduced in the cell lines, while ZO1 was increased compared to levels in the ex vivo samples. SLC4A11, ZEB1, TCF4 and COLAA2 demonstrated significant differential expression in the cultured cells that was most pronounced in the cell lines, while expression of LOXHD1 and AGBL1 was undetectable in ex vivo, primary and immortalized HCEnCs.

**Conclusions:** Our findings caution against reaching the conclusion that the expression of a few functional markers defines the parameters for validating the characteristics of immortalized corneal endothelial cells. The cell lines remain a valuable tool in the absence of robust primary cell culturing techniques, but should be used with an understanding of their limitations. Where possible, follow up validation experiments in animal models or humans should be considered.

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**Presentation Time:** 5:15 PM–5:30 PM

**Fuchs Corneal Dystrophy in African Americans**

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**Purpose:** Since its description in Austria one century ago, Fuchs Corneal Dystrophy (FCD) has often been thought of a disease primarily found among individuals of European origin. Studies of FCD in African-Americans are lacking; we therefore sought to characterize the clinical features and severity of FCD in African-Americans.

**Methods:** All individuals with Fuchs Corneal Dystrophy undergoing cataract surgery by two surgeons at a single institution from 2010-2013 were studied retrospectively and records reviewed for biometric data. Exclusion criteria included history of intraocular surgery, trauma, or inflammation.

**Results:** A total of 76 patients with FCD underwent cataract surgery by one surgeon from 2010-2013, of which 62 were Caucasian and 5 African-American. When compared with 76 unaffected control patients who underwent cataract surgery by the same surgeon in the same time period, no significant association was found between race and affection between Caucasian and African-American individuals (67 Caucasian, 5 African-American, p=0.58 by Fisher exact test). To confirm these findings, we examined a cohort of 88 FCD patients from a second surgeon and obtained similar results with no significant association (p=0.07). We then examined age, severity, and central corneal thickness in 186 Caucasian and 18 African-American eyes with FCD in these cohorts, the combination of the two surgeons. Despite similar age (71.2 African-American, 70.6 Caucasian, p=0.78) and severity by Krachmer grading (2.61 African-American, 2.80 Caucasian, p=0.25), there was a significant difference noted in central
corneal thickness between both groups (581 microns in African-Americans, 613 microns in Caucasians, p<0.01).

**Conclusions:** African-Americans with FCD demonstrate decreased corneal thickness relative to affected Caucasians. Further study is necessary to explore if corneal edema develops at different rates in the two population groups, or if this is solely secondary to baseline differences in corneal thickness attributed to race.

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