Loss of Corneal Nerves and Corneal Haze in Fuchs’ Endothelial Corneal Dystrophy

Patients with the TCF4 Trinucleotide Repeat Expansion

Matthew Gillings¹*, Andrew Mastro¹*, Xunzhi Zhang², Kelly Kiser¹, Jane Gu¹, Chao Xing²,³,⁴, Danielle Robertson¹, W. Matthew Petroll¹, V. Vinod Mootha¹,²

¹Department of Ophthalmology, ²Eugene McDermott Center for Human Growth and Development, ³Department of Bioinformatics, ⁴Department of Population and Data Sciences, University of Texas Southwestern Medical Center, Dallas, Texas

*These authors contributed equally to this work.

Corresponding Authors:
W. Matthew Petroll
V. Vinod Mootha
The University of Texas Southwestern Medical Center
Department of Ophthalmology
5323 Harry Hines Blvd, Dallas, TX 75390 – 9057
Matthew.petroll@utsouthwestern.edu
Vinod.mootha@utsouthwestern.edu

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Abstract

Objective: Seventy percent of Fuchs’ endothelial corneal dystrophy (FECD) cases are caused by an intronic trinucleotide repeat expansion in the transcription factor 4 gene (TCF4). The objective of this study was to characterize the corneal sub-basal nerve plexus and corneal haze in FECD patients with (RE+) and without the trinucleotide repeat expansion (RE-) and to assess the correlation of these parameters with disease severity.

Design: Cross-sectional, single-center study

Participants: Fifty-two eyes of 29 subjects with a modified Krachmer grade of FECD severity from 1 to 6 were included in the study. Fifteen of the 29 subjects carried an expanded TCF4 allele length of ≥40 CTG repeats (RE+).

Main Outcomes and Measures: In-vivo confocal microscopy assessments of corneal nerve fiber length (CNFL), corneal nerve branch density (CNBD), corneal nerve fiber density (CNFD), and anterior corneal stromal backscatter (haze); Scheimpflug tomography densitometry measurements of haze in anterior, central, and posterior cornea layers.

Results: Using confocal microscopy, we detected a negative correlation between FECD severity and both CNFL and CNFD in the eyes of RE+ subjects (Spearman ρ=-0.45, P-value=0.029 and ρ=-0.62, P-value=0.0015 respectively) but not in the eyes of RE- subjects. Additionally, CNFD negatively correlated to the repeat length of the expanded allele in the RE+ subjects (Spearman ρ=-0.42, P-value=0.038). We found a positive correlation between anterior stromal backscatter and severity in both the RE+ and RE- groups (ρ=0.60, P-value= 0.0023 and ρ=0.44, P-value=0.024 respectively). The anterior, central, and posterior Scheimpflug densitometry measurements also positively correlated with severity in both the RE+ and RE- groups.
(P=5.5 × 10⁻⁵, 2.5 × 10⁻⁴, and 2.9 × 10⁻⁴, respectively, after adjusting for the expansion status in a pooled analysis. However, for patients with severe FECD (Krachmer grades 5 & 6), the posterior densitometry measurements were higher in RE+ group than in RE- group (P<0.05).

**Conclusions:** Loss of corneal nerves in FECD supports the classification of the *TCF4* trinucleotide repeat expansion disorder as a neurodegenerative disease. Haze in the anterior, central, and posterior cornea correlate with severity irrespective of genotype. Quantitative assessments of corneal nerves and corneal haze may be useful to gauge and monitor FECD disease severity in RE+ patients.

**Introduction**

Fuchs’ endothelial corneal dystrophy (FECD) is a common age-related degenerative disorder affecting four percent of whites in the United States and the leading indication for keratoplasty in the developed world.¹² FECD is characterized by the progressive loss of the normal morphology and cell density of the corneal endothelium accompanied by diffuse thickening of its basement membrane (Descemet’s membrane) with focal excrescences called guttae.³ Patients experience symptoms of glare, diurnal fluctuation in vision, and loss of vision as a result of the guttae, endothelial cell loss, corneal edema, and scarring.⁴

Recently, the potential for treating FECD has been transformed by the discovery that an intronic trinucleotide repeat expansion in the transcription factor 4 gene (*TCF4*) accounts for 70% of FECD cases in the United States.⁵-⁸ Expansions of greater than 40 CTG repeats at this gene locus confer significant risk for the development of FECD.⁶ Expanded CUG repeat RNA
molecules accumulate in the nuclei of corneal endothelial cells of subjects with the triplet repeat expansion. These mutant repeat RNA species bind and functionally sequester the muscleblind-like (MBNL) family of splicing factors resulting in mis-splicing of their target exons. We recently observed that mis-splicing of MBNL-sensitive genes and the aberrant expression of key extracellular matrix genes occur early in the disease course in the corneal endothelium of presymptomatic subjects with the repeat expansion and foreshadow the upregulation of molecular pathways related to fibrosis, mitochondrial dysfunction, and immune cell activation, pathogenic changes observed late in the FECD disease course.

These observations open the possibility of preventing FECD disease onset or progression with molecular therapies early in the disease course before the onset of irreversible loss of endothelial cells, fibrosis, and corneal edema. We and others have proposed therapeutic strategies that target the TCF4 trinucleotide repeat expansion including use of gene editing, antisense oligonucleotides, duplex RNAs, trinucleotide repeat-targeting dCas9, and small molecules that bind with repeat RNA.

The slow rate of FECD disease progression and endothelial cell loss, however, poses challenges to clinical trial design and selection of outcome measures to assess efficacy of potential molecular therapies over a reasonable duration of time. The validation of corneal imaging biomarkers that correlate to FECD severity in patients with the trinucleotide repeat expansion may facilitate clinical trials of precision medicines.

Although FECD is primarily a disease affecting the corneal endothelium, structural and morphologic changes occur in all the pre-Descemet’s corneal layers that are detectible by both
in-vivo confocal microscopy and Scheimpflug tomography. Studies on heterogenous FECD cohorts have revealed that loss of the sub-basal nerve plexus and stromal haze correlate to disease severity,\textsuperscript{19-23} but it is unknown whether these changes are applicable to patients with the $TCF4$ repeat expansion. In this study, we aimed to quantify the changes of the sub-basal nerve plexus and stroma utilizing in-vivo confocal microscopy and Scheimpflug tomography and to assess whether these changes correlate with disease severity in FECD patients with the $TCF4$ trinucleotide repeat expansion (RE+) as well as in patients without the expanded repeat (RE-).

**Methods**

**Study Participants**

This was a cross-sectional, single-center study. The study was conducted in compliance with the tenets of the Declaration of Helsinki and with the approval of the institutional review board of the University of Texas Southwestern Medical Center (UTSW). All study subjects were recruited from the cornea referral practice at UT Southwestern. After informed consent, subjects underwent a complete eye examination including slit lamp bio-microscopy by a cornea fellowship-trained ophthalmologist (VVM) to assess the corneal endothelium using the modified Krachmer FECD grading scale: grade 0: no central guttae; grade 1: up to 12 scattered central guttae; grade 2: ≥12 scattered central guttae; grade 3: 1- to 2-mm confluent central guttae; grade 4: 2–5 mm of confluent central guttae; grade 5: >5-mm confluent central guttae without stromal edema; grade 6: >5-mm confluent central guttae with stromal edema.\textsuperscript{24} Genomic DNA from peripheral blood of subjects was used to genotype the $TCF4$ CTG18.1 triplet repeat polymorphism using a combination of short tandem repeat (STR) and triplet repeat primed
polymerase chain reaction (TP-PCR) assays as we have previously described.\textsuperscript{5-7} An allele length of $\geq 40$ CTG repeats was considered an expanded allele as in previous studies.\textsuperscript{7}

Fifty-two eyes with FECD Krachmer grades 1-6 from 15 individuals with the repeat expansion and 14 individuals without the repeat expansion were selected from our UTSW FECD cohort for this imaging sub-study conducted from July 2018 through December 2020. On the study visit for corneal imaging, the subjects were re-examined by the same investigator (VVM) using slit-lamp bio-microscopy to document the current Krachmer grade disease severity of their eyes. Eyes of subjects that had previously undergone keratoplasty or had a history of prior herpes simplex or zoster keratitis were excluded. Eyes of subjects with diabetes mellitus, contact lens use without corneal fluorescein staining, or mild dry eye ($\leq$ grade 1 DEWS dry eye severity grading scheme\textsuperscript{25}) at the time of imaging were not excluded. Available medical records of subjects with diabetes were reviewed for details of treatment and glycemic control.

**Corneal Imaging**

Fifty-two eyes of 29 patients underwent Scheimpflug Pentacam imaging (OCULUS, Wetzlar, Germany) of which forty-nine eyes of 27 patients also underwent in vivo confocal microscopy through-focusing (CMTF) with the Heidelberg Retina Tomograph with Rostock Cornea Module (HRT-RCM, Heidelberg Engineering, GmBH Dosenheim, Germany) that was modified for remote-controlled scanning and real-time image streaming.\textsuperscript{26-28} The investigators performing the imaging data acquisition and analyses (M.G., A.M., K.K., J.G., M.P., D.R.) were masked to the Krachmer grade severity of disease and genotype of the study subjects. One group of
investigators (A.M., J.G.) analyzed the Scheimpflug imaging data independently and were
masked to the CMTF data analysis performed by the second group of investigators (M.G., K.K.).

Scheimpflug tomography on all the subjects was performed in standardized low ambient light
conditions in a room without windows located in the ophthalmic imaging suite of the Aston
Ambulatory Care Center eye clinic at UT Southwestern Medical Center. For Scheimpflug
imaging, the “Densitometry” display of each eye was derived by the instrument’s software
(Pentacam version 1.22r05) and exported as a high-resolution image. The “Densitometry”
images provided by the Pentacam software were evaluated for quantitative measure of corneal
backscatter (haze). Densitometry values are expressed in grayscale units (GSU) and range from 0
(completely clear) to 100 (completely opaque). The densitometry data are broken down by the
software into an anterior 120µm layer (epithelium, sub-basal nerve plexus, and anterior stroma),
central layer (mid-stroma), and posterior 60µm layer (posterior stroma, Descemet’s membrane,
and endothelium). Densitometry data is further broken down into concentric rings composed of a
central 0-2mm, 2-6mm, 6-10mm and 10-12mm optical zones. In this study, we analyzed only the
central 0-6mm optical zone because this is the most applicable to visual function. To calculate
densitometry values for the central 0-6mm optical zone, the 0-2mm and 2-6mm zones were
combined using methods previously described by Hirabayashi KE, et al.29

Confocal microscopy through-focusing (CMTF) imaging was performed as previously
described.28 Briefly, prior to CMTF, eyes were anesthetized with one drop proparacaine. An
ophthalmic lubricant gel (Systane gel, Alcon, Fort Worth, Texas) was used to optically couple
the Tomocap to the objective and to applanate the cornea. Several continuous CMTF scans were
obtained from endothelium to epithelium to maintain contact with the cornea during the scan.

Images were collected from the central region of the cornea using the HRT streaming software function with the acquisition rate set to 30 frames per second, a lens speed of 60 μm/sec and a step size between images of approximately 2 μm. The field of view for each 384x384 pixel image was 400x400 μm. CMTF scans were obtained with the gain manually set to 20. The same HRT-RCM confocal microscope was used to obtain images of the sub-basal nerve plexus. For these nerve scans, the autogain feature was enabled and image depth was controlled manually to focus on the sub-basal nerve plexus, collecting sequences of images of multiple distinct regions of the cornea.

CMTF analysis was performed using a custom software program as previously reported.28,30 The area under the image intensity versus depth curve for the first 50 μm of anterior stroma was calculated, using a baseline set at a pixel intensity of 15. The beginning of the anterior stroma was identified as the interface between the sub-basal nerve plexus and the underlying stroma. Anterior stromal backscatter (haze) was expressed in arbitrary confocal backscatter units (CBUs), which are defined as μm x pixel intensity. CMTF analysis was completed by a single observer (M.G.) who was masked to the subject’s Krachmer grade and genotype. CMTF curves with evidence of excessive movement or those that lacked clear sub-basal nerve plexus and anterior stromal peaks were excluded.

The nerve image scans were analyzed using MetaMorph. Images with nerves in focus across the entire image were selected for analysis, and if necessary multiple images were aligned to keep the nerve plexus in focus across the entire image.31 As previously reported, eight unique images
that had less than approximately 20% overlap were used for analysis to approximate the value of
the entire sub-basal nerve plexus.32 The nerves were traced using the MetaMorph multi-line
tracing tool, and the total nerve length was calculated and reported in arbitrary units per frame as
the corneal nerve fiber length (CNFL). The number of individual nerves excluding branches per
image was recorded as the corneal nerve fiber density (CNFD). Branches were defined as nerve
fibers less than approximately 50% of the longer continuous nerve segment. The total number of
branch points per image was recorded as the corneal nerve branching density (CNBD). The sub-
basal nerve plexus imaging analysis was performed by two observers (K.K., M.G.) who were
masked to the Krachmer grade and genotype.

Statistical analysis

Demographic and clinical information was summarized as counts and percentage except age was
summarized by median. Scheimpflug imaging measurements were summarized as median and
interquartile range (IQR). Comparisons of the demographic and clinical phenotypic features
between the RE+ and RE- groups were performed using a 2-sample t test for age and Fisher’s
exact test for binary traits. Comparisons of confocal and Scheimpflug imaging parameters
between eyes of the same severity in the RE+ and RE- groups were made using the Wilcoxon-
Mann-Whitney test. Spearman’s rank correlations between the imaging measurements and the
Krachmer grade were calculated and tested in the two groups, separately. In the RE+ group,
Spearman’s rank correlations between the imaging measurements and repeat lengths of the
expanded allele were also calculated and tested. We also used multiple linear regression models
adjusting for the expansion status to examine the correlations. Software R (version 4.0.0) was
used for statistical analysis. P < 0.05 was considered statistically significant.
Results

Demographics of Study Subjects
Study participant demographics and pertinent past medical history are summarized in Table 1. There is an approximately 2:1 female to male ratio in both the RE+ and RE- groups which is consistent with the natural female predisposition for FECD. Details of the management and glycemic control in the study subjects with diabetes mellitus are summarized in Supplemental Table 1. The Krachmer grades and trinucleotide repeat expansion status of the eyes in the entire cohort are shown in Table 2. A list of the repeat lengths for the RE+ group is shown in Supplemental Table 2 available at www.ophthalmologyscience.org.

In-Vivo Confocal Microscopy
Analysis of the in-vivo sub-basal nerve plexus images revealed statistically significant negative correlations of CNFL and CNFD with the Krachmer grade in the RE+ eyes ($\rho=-0.45$, $P=0.029$, and $\rho=-0.62$, $P=0.0015$, respectively) but not in the RE- eyes (Figures 1A, 1B & 1C; Table 3). There was no statistically significant correlation between CNBD and the Krachmer grade in either RE+ or RE- group, but a multiple linear regression model adjusting for the expansion status (RE+ or RE-) did show a significant negative correlation in the entire cohort ($P=0.026$; Figure 1D; Table 3). The CNFL and CNFD values were significantly lower in the RE+ eyes with severe FECD (Krachmer grade 5 or 6) compared to the RE- eyes with severe FECD ($P<0.05$; Table 3).
In the CMTF scans, the anterior stromal backscatter (haze) was visualized and quantified in the form of a peak immediately following the peak normally associated with the sub-basal nerve plexus (Figures 2A & 2B). For quantification of anterior stromal haze, a consistent 50 microns slice of the anterior corneal stroma underlying the sub-basal nerve plexus that includes this anterior stromal haze peak was imaged (Figure 2B). There was a positive correlation between anterior stromal haze and the Krachmer grade in both the RE+ and RE- groups ($\rho=0.60$, $P=0.0023$, and $\rho=0.44$, $P=0.024$, respectively; Figure 2C; Table 3).

Scheimpflug Tomography

The anterior (120 $\mu$m layer of the cornea), central, and posterior (60 $\mu$m layer) densitometry measurements were all positively correlated with the Krachmer grade in both the RE+ and RE- eyes ($P=5.5 \times 10^{-5}$, $2.5 \times 10^{-4}$, and $2.9 \times 10^{-4}$, respectively, adjusting for the expansion status; Figures 3 A, 3B, 3C, & 3D; Table 3). For patients with severe FECD (Krachmer grades 5 & 6), the posterior densitometry measurements were higher in the RE+ group than in the RE- group ($P<0.05$) ($P<0.05$; Table 3).

Correlation of Imaging Parameters to Repeat Length

Correlations of confocal imaging parameters (CNFL, CNBD, CNFD, anterior corneal stromal backscatter) and Scheimpflug tomography densitometry measurements to repeat length of the expanded allele in the RE+ group are shown in Supplemental Figures 1-7 available at www.ophthalmologyscience.org. CNFD negatively correlated to the repeat length of the expanded allele in the RE+ subjects (Spearman $\rho=-0.42$, P-value=$0.038$) (Supplemental Figure
available at www.ophthalmologyscience.org). We found no statistically significant correlations between the other 6 imaging parameters and repeat length.

Discussion

Nucleotide repeat expansions are associated with more than fifty human diseases and they primarily exhibit a neurodegenerative phenotype. Like peripheral neurons, corneal endothelial cells are derived from neural crest tissue, express neuronal markers, and are post-mitotic after birth. Our observation that loss of the sub-basal nerve plexus correlates with disease severity in FECD patients further supports the classification of the TCF4 trinucleotide repeat disorder as a neurodegenerative disease. Although we also observed loss of corneal nerve fiber length and density loss in FECD patients without the expansion, their correlations to disease severity did not reach statistical significance. In other neurodegenerative disorders mediated by DNA repeat expansions such as myotonic dystrophy, alleles with longer repeat length correlate to increased disease severity and earlier onset. Here, we found that the corneal nerve fiber density negatively correlated to the repeat length of the expanded allele in FECD patients with the expansion.

Loss of corneal nerves occurs early in the disease course well before the onset of corneal edema in FECD subjects with the expanded repeat. The mechanism by which the attenuation of the sub-basal corneal nerves occurs in FECD is not known but may result from loss of corneal endothelium triggered by the mutant repeat RNA. Corneal endothelial cells are known to produce neuropeptides including vasoactive intestinal peptides that maintain endothelium in their
differentiated state and prevent cellular apoptosis; these neuropeptides may also be relevant to
corneal nerve homeostasis.\textsuperscript{19,39,40} Additionally, corneal endothelial cells express vascular
endothelial growth factor and nerve growth factor vital to maintenance of axons and neuronal
growth.\textsuperscript{41} However, attenuation of sub-basal nerve plexus observed in diabetes and herpes
simplex keratitis may result in loss of the normal morphology and density of endothelial cells.\textsuperscript{19}
These findings support an alternate hypothesis that the primary loss of corneal nerves and
decreased levels of neuropeptides contributes to endothelial disease pathogenesis in FECD.\textsuperscript{19}
A limitation of our study was the inclusion of patients with diabetes mellitus or mild dry eye
which may have also contributed to loss of corneal nerves.\textsuperscript{42,43} However, the prevalence of these
common, age-related co-morbid conditions were comparable in the RE+ and RE- groups.
Unlike the loss of the sub-basal nerve plexus which is particularly striking in patients with the
\textit{TCF4} triplet repeat expansion, corneal backscatter or haze in the various layers of the cornea
does not appear to be specific to FECD patients with the expansion. By confocal microscopy,
we were able to measure the backscatter in the 50 microns of stroma underlying the sub-basal
nerves in CMTF scans of the central cornea and establish that the previously observed
correlation between anterior stromal backscatter and disease severity\textsuperscript{22} applies to both RE+ and
RE- FECD patients. It is currently unknown why anterior corneal haze in the form of keratocyte
activation develops early in the FECD course before the onset of clinically detectable edema.
Anterior corneal haze increases further in late FECD which has been attributed to corneal
edema.\textsuperscript{44} Interestingly, the anterior stromal haze improves only partially after successful
endothelial keratoplasty and resolution of corneal edema.\textsuperscript{28,45-47} These observations suggest that anterior stromal haze represent irreversible damage.

We found positive correlations between Scheimpflug densitometry measurements of the anterior 120 \( \mu \)m, central, and posterior 60 \( \mu \)m corneal layers and FECD severity in our entire cohort as has been previously reported in a heterogenous group of patients.\textsuperscript{23} We found these correlations in both RE+ and RE- patients. Interestingly, Scheimpflug densitometry of the anterior 120 \( \mu \)m corneal layer has been shown to strongly correlate to the endothelial pump function in FECD subjects assessed by measurements of the rate of recovery of cornea edema from contact-lens induced hypoxia.\textsuperscript{48}

Increased densitometry of the posterior 60 \( \mu \)m layer of the cornea in FECD may be explained by the higher posterior keratocyte density in the pre-Descemet’s stromal layers,\textsuperscript{19} fibrosis, edema, as well as the thickening of Descemet’s membrane and guttae observed in FECD. A recent study reported that FECD subjects with higher posterior corneal densitometry measurements had more visual disability.\textsuperscript{49} Our finding in this study of higher posterior corneal densitometry in eyes with severe FECD in RE+ patients compared to eyes with severe FECD in RE- patients may help account for our previous observations that RE+ FECD patients are at a higher risk for disease progression and need for keratoplasty than their RE- FECD counterparts.\textsuperscript{50,51}

The slow rate of endothelial cell loss may limit the utility of endothelial cell density as an endpoint to assess efficacy of a potential molecular therapy in a clinical trial setting. Additionally, measuring corneal endothelial cell morphology and density may be challenging
and inaccurate in FECD using specular microscopy. The guttae obscure the visualization of
overlying endothelial cells.\textsuperscript{52} Additionally, the variation in the regional distribution and
confluence of guttae can contribute to markedly different endothelial cell densities of the same
patient’s cornea.\textsuperscript{52} Confocal microscopy may be an alternative to specular microscopy to
evaluate regional differences of endothelial cell density in guttae and non-guttae areas of corneas
of patients with severe FECD.\textsuperscript{53} Scheimpflug tomography can generate useful pachymetry and
posterior elevation maps providing functional assessments of the health of the corneal
endothelium in FECD.\textsuperscript{54} However, since these Scheimpflug imaging parameters related to the
thickness of the cornea are disturbed only in late FECD after significant loss of the endothelium
and the onset of edema, they may not be ideal as endpoints to test the efficacy of molecular
therapies intended to slow progression in patients with mild or moderate disease.

The morphological and structural changes in the pre-Descemet’s layers of the cornea that occur
early in the FECD disease course and correlate with severity are pertinent to understanding the
natural history of the disorder and therapeutic development. Quantitative assessments of corneal
nerves and corneal haze may be useful outcome measures for therapeutic trials intended to slow
or halt disease pathogenesis before the onset of significant and irreversible corneal haze and
endothelial cell loss. However, longitudinal studies are certainly warranted to understand the
natural history of disease progression of these imaging parameters in FECD patients with the

\textbf{Figure Legends}
Figure 1. Sub-basal Nerve Plexus in FECD. A. Representative confocal microscopy through focusing (CMTF) image of subbasal nerve plexus from eye with mild FECD (Krachmer grade = 2) of RE+ patient (Left Panel) compared to eye with severe FECD (Krachmer grade = 5) of RE+ patient (Right Panel). B. Corneal nerve fiber length (CNFL) vs. FECD severity. C. Corneal nerve fiber density (CNFD) vs. FECD severity. D. Corneal nerve branching density (CNBD) vs. FECD severity. Spearman correlation between CMTF subbasal nerve plexus imaging parameters of CNFL, CNFD, and CNBD and modified Krachmer grade of FECD disease severity from 1-6.

Figure 2. Corneal Backscatter (Haze) of Anterior Stroma in FECD. A. Representative CMTF image of keratocytes and reflectivity in anterior stroma of eye with mild FECD (KG = 2) of RE+ patient compared to eye with severe FECD (KG = 5) of RE+ patient. B. CMTF scan showing peaks corresponding to subbasal nerve plexus and anterior stroma haze in FECD subject. Anterior stromal haze was defined as the area under the CMTF curve of the first 50 microns of stroma underlying the subbasal nerve plexus and is reported in arbitrary confocal backscatter units (CBU). C. Anterior stromal haze (CBU) vs. FECD disease severity from 1-6. Spearman correlation between CBU and modified Krachmer grade of FECD disease severity from 1-6.

Figure 3. Corneal Densitometry (Backscatter or Haze) of Anterior, Middle, and Posterior Corneal Layers. A. Representative Scheimpflug densitometry measurements of eye with mild FECD (Krachmer grade 1) of RE+ patient (Left Panel) compared to eye with severe FECD (Krachmer grade 6) of RE+ patient. B. Densitometry of anterior 120μm corneal layer vs. FECD disease severity from 1-6. C. Densitometry of central corneal layer vs. FECD disease severity from 1-6. D. Densitometry of posterior 60μm corneal layer vs. FECD disease severity from 1-6.
Spearman correlation between densitometry of anterior, central, and posterior corneal layers and modified Krachmer grade of FECD disease severity from 1-6.

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References


Table 1. Baseline Characteristics.

<table>
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<tr>
<th>No. (%) of Participants</th>
<th>With Repeat Expansion (RE+) (n = 15)</th>
<th>Without Repeat Expansion (RE-) (n = 14)</th>
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<td>4 (27)</td>
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<tr>
<td>Female</td>
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<td>10 (71)</td>
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<td>Race/ethnicity</td>
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<td>Asian/Indian</td>
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<tr>
<td>Diabetes Mellitus*</td>
<td>3 (20)</td>
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<tr>
<td>Contact Lens Use</td>
<td>1 (7)</td>
<td>1 (7)</td>
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<tr>
<td>Dry Eye Disease</td>
<td>3 (20)</td>
<td>6 (42)</td>
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</table>

Table 1. Baseline Participant and Ocular Characteristics. With the exception of race/ethnicity (p=0.02), no differences were found to be significant between the two populations. Fisher exact tests were used for all characteristics except for age where a two-tailed t-test was used. *Details of management and control of diabetes mellitus are summarized in Supplemental Table 1.
Table 2. Summary of Eyes by Krachmer Grade and Expansion Status.

<table>
<thead>
<tr>
<th>Krachmer Grade</th>
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<th>RE- (n = 26)</th>
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Table 2. Krachmer grade of Fuchs’ endothelial corneal dystrophy for the 52 eyes of 29 patients.
Table 3. Confocal and Scheimpflug Imaging Parameters stratified by FECD severity measured by the Krachmer grade.

<table>
<thead>
<tr>
<th></th>
<th>FECD eyes without the Repeat Expansion (RE-)</th>
<th>FECD Eyes with Repeat Expansion (RE+)</th>
<th>P-value $^d$</th>
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<tbody>
<tr>
<td></td>
<td>Mild (9 eyes)</td>
<td>Moderate (9 eyes)</td>
<td>Severe (8 eyes) $^b$</td>
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<td>Confocal Nerve Fiber Length $^a$</td>
<td>2252.37 (731.33)</td>
<td>1643.56 (1659.38)</td>
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<td>Confocal Nerve Fiber Density</td>
<td>4.38 (1.00)</td>
<td>4.40 (2.45)</td>
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<td>Confocal Nerve Branching Density</td>
<td>6.88 (4.45)</td>
<td>5.50 (14.45)</td>
<td>5.56 (1.42)</td>
</tr>
<tr>
<td>Confocal Anterior Stroma 50 µm Backscatter (CBU)</td>
<td>3152.50 (573.80)</td>
<td>3524.90 (744.60)</td>
<td>3697.25 (1450.50)</td>
</tr>
<tr>
<td>Scheimpflug Anterior 120 µm Backscatter (GSU)</td>
<td>19.87 (7.35)</td>
<td>23.17 (2.85)</td>
<td>23.39 (8.21)</td>
</tr>
<tr>
<td>Scheimpflug Mid Cornea (GSU)</td>
<td>16.61 (1.89)</td>
<td>17.01 (1.90)</td>
<td>17.20 (3.31)</td>
</tr>
<tr>
<td>Scheimpflug Posterior 60 µm (GSU)</td>
<td>11.29 (3.01)</td>
<td>12.05 (2.42)</td>
<td>12.74 (0.62)</td>
</tr>
</tbody>
</table>

Median and interquartile range are presented. Mild: Krachmer grade (KG) = 1 or 2; moderate: KG= 3 or 4; severe KG= 5 or 6. CBU: confocal backscatter units; GSU: Grayscale Units

$^a$ CNFL units are arbitrary units given by the tracing software in Metamorph.

$^b$ Sub-basal nerve plexus parameters were unable to be assessed for one of these eyes.

$^c$ Three of these eyes did not undergo confocal imaging.

$^d$ Association between the parameter and Krachmer grade by fitting a linear regression model adjusting for the expansion status.

*Significant difference between eyes of the same severity in RE+ and RE- groups (p < 0.05) by the Wilcoxon-Mann-Whitney test.
Subbasal Nerve Plexus in Mild FECD

Subbasal Nerve Plexus in Severe FECD
Anterior Stromal Haze in Mild FECD

Anterior Stromal Haze in Severe FECD
Expansion
- No $\rho=0.44$, $P=0.024$
- Yes $\rho=0.60$, $P=0.0023$

CBU (arbitrary confocal backscatter units)

Krachmer Grade
Densitometry in Mild FECD

Densitometry in Severe FECD

<table>
<thead>
<tr>
<th></th>
<th>0 - 2mm</th>
<th>2 - 6mm</th>
<th>6 - 10mm</th>
<th>10 - 12mm</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior (120μm)</td>
<td>26.7</td>
<td>24.9</td>
<td>34.7</td>
<td>32.9</td>
<td>29.7</td>
</tr>
<tr>
<td>Center layer</td>
<td>16.6</td>
<td>17.3</td>
<td>28.2</td>
<td>32.3</td>
<td>23.3</td>
</tr>
<tr>
<td>Posterior (60μm)</td>
<td>11.7</td>
<td>14.0</td>
<td>22.0</td>
<td>30.6</td>
<td>13.1</td>
</tr>
<tr>
<td>Total</td>
<td>18.0</td>
<td>16.7</td>
<td>28.3</td>
<td>31.9</td>
<td>24.0</td>
</tr>
</tbody>
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<th>6 - 10mm</th>
<th>10 - 12mm</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior (120μm)</td>
<td>31.8</td>
<td>29.4</td>
<td>41.6</td>
<td>41.8</td>
<td>35.9</td>
</tr>
<tr>
<td>Center layer</td>
<td>19.1</td>
<td>18.7</td>
<td>30.6</td>
<td>26.0</td>
<td>24.1</td>
</tr>
<tr>
<td>Posterior (60μm)</td>
<td>16.2</td>
<td>16.5</td>
<td>25.6</td>
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<tr>
<td>Total</td>
<td>22.4</td>
<td>21.5</td>
<td>32.6</td>
<td>31.6</td>
<td>27.0</td>
</tr>
</tbody>
</table>
Precis

Loss of corneal sub-basal nerve plexus and corneal haze correlate to disease severity of Fuchs’ endothelial corneal dystrophy in patients with the trinucleotide repeat expansion in the transcription factor 4 (TCF4) gene.