

# Association of Smoking and Other Risk Factors With Fuchs' Endothelial Corneal Dystrophy Severity and Corneal Thickness

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**PURPOSE.** We investigated effects of smoking and other risk factors on the development of advanced Fuchs' endothelial corneal dystrophy (FECD) and on central corneal thickness (CCT).

**METHODS.** Eyes from Caucasian probands, affected and unaffected family members, and unrelated controls matched for age from the FECD Genetics Multi-Center Study ( $n = 2044$  subjects) were examined. Univariate and multivariate models, adjusted for family correlations, were used to determine the effect of smoking, sex, diabetes, and age on FECD case/control status and CCT.

**RESULTS.** In a multivariate model, sex and smoking were associated significantly with advanced FECD (grades 4-6) development ( $P = 0.016$  and  $P = 0.047$ , respectively). Female sex increased odds by 34%. Smoking increased odds by 30%. In a multivariate model, diabetes was associated with an increase of 9.1  $\mu\text{m}$  in average CCT ( $P = 0.021$ ). Female sex was associated significantly with a decrease in average CCT by 6.9  $\mu\text{m}$  ( $P = 0.015$ ). Smoking had no significant effect on CCT in any model. As shown previously, advanced FECD was associated with large increases in CCT (31.4-94.2  $\mu\text{m}$ ).

**CONCLUSIONS.** Smoking was associated with an increased risk of advanced FECD and self-reported diabetes was associated with increased CCT. Further study of the impact of smoking and diabetes on FECD development and changes in corneal thickness is warranted.

Keywords: FECD, corneal endothelial cells, smoking

Fuchs' endothelial corneal dystrophy (FECD) is characterized by progressive loss of corneal endothelial cells associated with guttae formation.<sup>1-3</sup> The corneal endothelium normally maintains corneal transparency by its endothelial barrier and pump functions. Tight junctions between endothelial cells prevent water from entering, and Na<sup>+</sup>/K<sup>+</sup> ATPase-dependent pumps actively transport fluid out of the cornea and into the aqueous humor to maintain corneal clarity.<sup>2</sup> When the number of endothelial cells becomes critically low, the cornea swells, resulting in hazy vision and, eventually, vision loss. FECD has a prevalence of approximately 4% in the United States, and is one of the most common indications for penetrating and endothelial keratoplasty.<sup>4</sup> While FECD is strongly heritable, the exact pathogenesis is unknown; however, several hormonal, genetic, and environmental factors are postulated to have a role in disease severity.<sup>2,4-7</sup>

Smoking and environmental tobacco smoke have been associated with increased risk of developing and worsening of several ocular diseases, including cataracts, age-related macular

degeneration, and Graves ophthalmopathy.<sup>8-12</sup> Prior studies have demonstrated that smoking and diabetes were associated with increased central corneal thickness (CCT).<sup>13-16</sup> Prolonged smoking has been associated with increased guttae seen on specular microscopy.<sup>17</sup> Several in vitro and ex vivo cell models show increased oxidative stress-induced apoptosis in FECD endothelial cells compared to normal corneal endothelial cells.<sup>18,19</sup> Other studies have found increased rates of cell loss, increased permeability, and decreased pump function in FECD endothelium versus normal corneal endothelium.<sup>3,20,21</sup> We hypothesize that since smoking increases oxidative stress, it leads to accelerated endothelial cell apoptosis and, thus, could be an additional risk factor for FECD development and severity. To our knowledge, no epidemiologic studies to date have evaluated the effects of smoking on FECD. Our goal was to determine whether smoking increases the risk of developing advanced FECD (grades 4-6).<sup>4</sup> In addition, Kopplin et al. showed that increasing FECD grade was associated with increasing CCT, even in early/mild FECD, and, therefore,

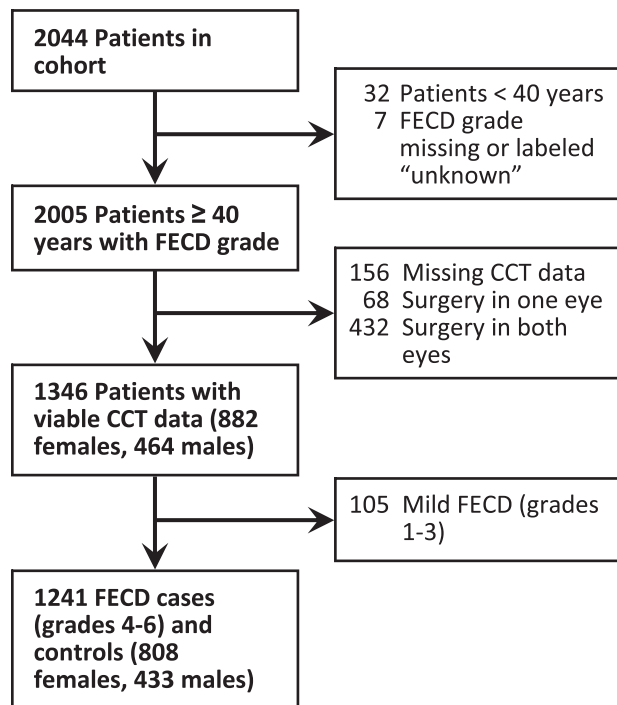


FIGURE. Subject disposition.

suggested that CCT be used as a clinical marker for monitoring FECD progression.<sup>5</sup> Since CCT is an important indicator of FECD severity, we also examined whether smoking and/or other covariates affected this outcome.

## METHODS

Caucasian subjects ( $n = 2044$ ) were selected from the FECD Genetics Multi-Center Study cohort.<sup>4,5</sup> A subset analyzed previously<sup>5</sup> for the relationship of CCT to FECD grade was included in the current analysis. Our cohort was selected so that confounders for CCT, such as prior penetrating and endothelial keratoplasty, and intraocular surgery within a year of the procedure, were eliminated. Appropriate ethical approvals were obtained from Institutional Review Boards at all participating sites. Written informed consent was obtained from all participants after explaining the nature of the study, which was conducted in accordance with the principles of the Declaration of Helsinki. Detailed inclusion and exclusion criteria have been described previously.<sup>4,5</sup> Briefly, cases consisted of probands and their affected family members with FECD. Controls consisted of unaffected family members and unrelated subjects without FECD. Unrelated controls were matched to be five years older than probands. As FECD is an age-related progressive disease, unrelated controls were matched to be older than cases to reduce misclassification.

Demographic information, and ocular and systemic medical histories were obtained through a standardized questionnaire. Subject age, sex, smoking history (never versus ever smoked), and self-reported history of diabetes also were recorded. A slit-lamp biomicroscopic examination by a cornea fellowship-trained ophthalmologist was performed to determine the extent of corneal guttae as well as presence of stromal and epithelial edema according to an established FECD 7-point severity grading system adopted from the study of Krachmer et al.<sup>1,4</sup> Specular microscopy was not used to assess FECD severity, since this technique is useful only for early disease

staging and not suitable when guttae confluence develops in more advanced disease. CCT was measured three times and averaged, using ultrasonic pachymeters from multiple manufacturers that had been calibrated internally.<sup>5</sup>

## Statistical Analysis

Analysis was done with subject-level data, and not eye-level data because left and right eyes had very similar FECD grades (Pearson's correlation coefficient = 0.962). The eyes from subjects were divided into four categories for analysis: probands, affected family members, unaffected family members (FECD grade 0), and unrelated controls with normal corneas. Enrollment under the genetic study design emphasized advanced FECD probands and affected siblings with FECD grades 4 or greater in at least one eye, resulting in small sample sizes in groups with FECD grades 1 to 3. Using the generalized estimating equations (GEE) approach, as implemented in the GEE package in the statistical programming language R, two models (univariate and multivariate) were fitted to estimate the effect of smoking and diabetes on FECD development and CCT. GEE accounts for the within-family correlation of FECD values among related individuals and adjusts the uncertainty of parameter estimates accordingly. We excluded age from the univariate analysis, because unrelated controls were slightly older than unrelated cases by design. However, age still is a confounder for the effects of smoking and diabetes on FECD, and so we included age in the multivariate models to control for misleading associations between these predictors and FECD due to age; models without age also were tested, but were not materially different. Analyses of FECD status as the outcome used a binomial model with logistic link function, whereas analyses of CCT used a linear model with Gaussian error structure. Both models included an exchangeable correlation among family members. To test for differences in odds ratio for smoking between males and females, we added an interaction term between sex and smoking in the analysis on the entire sample.  $P < 0.05$  was considered significant, without correction for multiple analyses. We conducted a sensitivity analysis to examine the possible influence of age differences between cases and controls on our effect estimates for smoking on FECD. Random subsets of 457 of the 784 advanced FECD cases were paired with the 457 controls in regression analyses, and the effect estimate for smoking on FECD was compared to the mean age of the cases relative to that of the controls over 1000 independent replicates.

## RESULTS

In total, 2044 Caucasian subjects were considered for this study. Only data from the Caucasian cohort were used to reduce heterogeneity in the sample. Of these subjects, 32 subjects had early onset FECD (age <40) and were excluded along with 7 other subjects with FECD grade marked as "unknown" or missing. Of the remaining 2005 subjects, 656 had missing CCT data. The majority of them ( $n = 432$ ) had penetrating keratoplasty in both eyes, so CCT was not performed (see Figure for subject disposition). Three subjects were outliers with CCT <100  $\mu\text{m}$  and also were excluded. Of the remaining 1346 subjects with usable CCT data, 784 had advanced, 457 had no, and 105 had mild FECD. CCT measurements were highly consistent within eyes: the average coefficients of variation (CV; SD divided by the mean) for right and left eyes were 0.84% and 0.76%, respectively, and no eye had a CV of more than 10%. Table 1 shows that the distribution of morning (between 7 AM and 12 PM) and afternoon

TABLE 1. Timing of CCT Measurement With Respect to FECD Grade

Timing of CCT Measurement	FECD Grade		
	Controls (Grade 0)	Mild (Grades 1–3)	Advanced (Grades 4–6)
Morning, 7 AM–12 PM	45.7%	40.0%	40.3%
Afternoon, 12:01 PM–5 PM	53.5%	58.1%	57.3%
Evening, 5:01 PM–7 PM	0.8%	1.9%	2.4%

(between 12:01 PM and 5 PM) CCT measurements was similar across unaffected controls, mild cases of FECD (grades 1–3), and advanced FECD (grades 4–6).

First, we studied sex, smoking, and diabetes, and their effects on advanced FECD (i.e., cases, severity grades 4–6), and subsequently also included age in a multivariate analysis. Analysis was done on 1241 Caucasian subjects, including 784 subjects with advanced FECD and 457 subjects with no FECD (grade 0). Table 2 shows the baseline characteristic of these two subgroups. There was a significantly greater percentage of females in cases versus controls ( $P = 0.013$ , Table 2). Despite controls being matched to be on average 5 years older than cases, the mean difference in age was only approximately two years because there were more cases than controls in this analysis. Per original study design to recruit advanced FECD cases, the average FECD grade in the cases was 5.09 (Table 2). CCT was significantly greater in cases than in controls by a mean of 56  $\mu\text{m}$  ( $P < 0.001$ , Table 2). There were significantly more smokers in cases than in controls ( $P = 0.022$ ). Cases and controls did not differ in presence of diabetes history.

In multivariate models, female sex and smoking were associated significantly with development of FECD, with 34% ( $P = 0.016$ ) and 30% ( $P = 0.047$ ) excess odds, respectively (Table 3). The effect of smoking on FECD was robust to differences in mean age between cases and controls, as determined by sensitivity analysis (results not shown). Since female sex was noted to have an effect on advanced FECD development, a subgroup analysis of FECD based on sex was performed (Table 4). In the female cohort ( $n = 808$ ), the effect of smoking and diabetes was more pronounced, with an odds ratio (OR) of 1.37 and 1.55, respectively, in the multivariate analysis. However, these results only trended toward significance ( $P = 0.065$  and  $P = 0.071$ , respectively). The data did not show that smoking and diabetes have significant effects on FECD development in males (OR = 1.08,  $P = 0.71$  and OR = 1.26,  $P = 0.33$ , respectively). Although the ORs for smoking were different in the stratified male and female samples, a combined analysis, including an interaction term between sex and smoking, did not show a significant difference (interaction

TABLE 2. Presence of Smoking and Diabetes in Advanced FECD Cases (Grades 4–6) and Controls

	Cases, $n = 784$	Controls, $n = 457$	$P$ Value
Age, y	68.4 $\pm$ 11.3	70.2 $\pm$ 10.0	0.003
Female, $n$ (%)	531 (67.7)	277 (60.6)	0.01
FECD grade	5.09 $\pm$ 0.76	0.00 $\pm$ 0.00	-
CCT, $\mu\text{m}$ in worse eye	613 $\pm$ 59	557 $\pm$ 34	<0.001
Smoker, $n$ (%)	592 (75.5)	317 (69.4)	0.02
Diabetes, $n$ (%)	104 (13.3)	49 (10.7)	0.22

$P$  value for significance of differences between cases and controls by  $t$ -test (age, CCT) or by Pearson's  $\chi^2$  test (all other variables). No  $P$  value given for FECD grade because case/control status was determined by FECD grade.

TABLE 3. Univariate and Multivariate Associations Between Selected Covariates and Advanced FECD (Grades 4–6,  $n = 1241$ )

Covariate	Univariate Associations		Multivariate Associations	
	OR (95% CI)	$P$ Value	OR (95% CI)	$P$ Value
Sex	1.38 (1.09, 1.74)	0.007	1.34 (1.16, 1.71)	0.02
Smoking	1.36 (1.06, 1.75)	0.02	1.30 (1.00, 1.68)	0.047
Diabetes	1.28 (0.93, 1.77)	0.13	1.37 (0.99, 1.89)	0.06

OR = 1.02, 95% confidence interval [CI] = [0.62, 1.66],  $P = 0.94$ ).

A breakdown by sex, smoking, and diabetes status, and their relationship to CCT is shown in Table 5. There were 1346 subjects, including all FECD grades and controls (882 female subjects and 464 male subjects), who had available CCT data and were included in this analysis. In a multivariate model, presence of diabetes significantly increased CCT on average by 9.1  $\mu\text{m}$  ( $P = 0.021$ ). Being female was associated with a significant average decrease of 6.9  $\mu\text{m}$  in CCT ( $P = 0.015$ ). Smoking was not associated with an increase or decrease in corneal thickness in univariate and multivariate models.

Increasing FECD grade was associated with an increase in CCT. For advanced FECD, the magnitude and significance of CCT change increased as disease severity increased (Table 5). The most pronounced and significant increase in CCT was from grades 4 to 6 (Table 5).

A similar sex-stratified analysis also was performed for CCT (Table 6). The historical reporting of diabetes had a larger effect in females ( $n = 882$ ) compared to the total cohort ( $n = 1346$ ). CCT in diabetic females increased by 19.4  $\mu\text{m}$  ( $P = 0.001$ ) and 13.1  $\mu\text{m}$  ( $P = 0.0077$ ) in the univariate and multivariate analyses, respectively. Again, when looking at the male cohort ( $n = 464$ ), we did not find a significant effect of diabetes on CCT. However, advanced FECD increased CCT significantly regardless of sex ( $P < 0.001$ ).

## DISCUSSION

While many studies have demonstrated the positive correlation between smoking and several ocular disorders, few have looked at the effects of smoking and other modifiable risk factors on corneal endothelial function, or the presence and degree of FECD. Our study found that smoking increased the odds of advanced FECD development by 30% when modeled with other risk factors. The Reykjavik Eye Study, a large, population-based, cross-sectional study, showed that smoking one pack per day for 20 years increased guttae by 2-fold in an

TABLE 4. Univariate and Multivariate Associations Between Selected Covariates and Advanced FECD (Grades 4–6) Separated by Female and Male Sex

Covariate	Univariate Associations		Multivariate Associations	
	OR (95% CI)	$P$ Value	OR (95% CI)	$P$ Value
Females only, $n = 808$				
Smoking	1.34 (0.96, 1.87)	0.09	1.37 (0.98, 1.91)	0.07
Diabetes	1.52 (0.94, 2.45)	0.09	1.55 (0.96, 2.50)	0.07
Males only, $n = 433$				
Smoking	1.14 (0.77, 1.69)	0.51	1.08 (0.72, 1.63)	0.71
Diabetes	1.15 (0.73, 1.81)	0.54	1.26 (0.79, 2.00)	0.33

TABLE 5. Univariate and Multivariate Associations Between Selected Covariates and CCT Among All FECD Grades and Controls ( $n = 1346$ )

Covariate	Univariate Associations		Multivariate Associations	
	Effect (95% CI)	P Value	Effect (95% CI)	P Value
Sex	-2.1 (-8.8, 4.7)	0.55	-6.9 (-12.4, -1.3)	0.02
Smoking	3.2 (-3.8, 10.1)	0.38	0.5 (-5.1, 6.1)	0.86
Diabetes	12.6 (3.3, 21.8)	0.008	9.1 (1.4, 16.8)	0.02
FECD grade 1	13.1 (0.6, 25.6)	0.04	12.9 (0.2, 25.6)	0.05
FECD grade 2	5.8 (-5.7, 17.3)	0.32	5.7 (-5.8, 17.1)	0.33
FECD grade 3	6.3 (-5.8, 18.4)	0.31	6.3 (-6.4, 18.9)	0.33
FECD grade 4	36.3 (28.8, 43.8)	<0.001	36.1 (28.4, 43.8)	<0.001
FECD grade 5	45.0 (38.1, 52.0)	<0.001	31.4 (22.2, 40.6)	<0.001
FECD grade 6	91.6 (84.2, 98.9)	<0.001	94.2 (86.6, 101.9)	<0.001

Effects are in units of  $\mu\text{m}$  CCT. FECD grade effects are relative to baseline of FECD grade 0.

Icelandic population.<sup>17</sup> The seven-year cumulative data from that study showed that the incidence of guttae was highest in patients aged 55 to 64.<sup>22</sup>

Cigarette smoke also has been shown to decrease rabbit corneal epithelial cell growth in vivo and in vitro, in a time and dose-dependent manner. Longer exposures progressively flattened the growth curve.<sup>14</sup> Buddi et al. detected increased concentrations of nitrotyrosine and malondialdehyde, byproducts of oxidative damage, in corneas from FECD patients compared to normal corneas.<sup>23</sup> In addition, Jurkunas et al. demonstrated increased hydrogen peroxide release from FECD endothelium after treatment with a pro-oxidant.<sup>19</sup> Azizi et al. also suggested that FECD corneal endothelial cells were more susceptible to oxidative stress and p53-induced apoptosis due to impaired, down-regulated, or suboptimal antioxidant defense mechanisms.<sup>18</sup> Cigarette smoke contains many reactive oxygen species, which further increase the oxidative burden.<sup>24</sup> These additive effects theoretically could accelerate endothelial cell apoptosis, leading to increased endothelium dysfunction, loss, and guttae formation.

In our study, smoking was not associated with an effect on CCT in subjects with any FECD grade or in controls. The Funagata Eye Study, a Japanese population-based, cross-

sectional study, reported that active smoking was associated with an average increase in CCT of 13.7  $\mu\text{m}$  in a univariate model, and an average increase in CCT of 11.8  $\mu\text{m}$  in a multivariate model.<sup>13</sup> The difference between our study and the Funagata study may be explained by several factors: (1) Our study looked at a specific population subset, targeting those with FECD traits, (2) we did not measure pack-years or distinguish active smokers from past smokers, and (3) the susceptibility of CCT to smoking could differ by race.

Diabetes, like smoking, is linked to increased production of free radicals and impaired antioxidant defense capabilities.<sup>25-27</sup> However, unlike smoking, self-reported diabetes was not associated with increased risk of advanced FECD development in this study. We found that diabetes increased CCT among all FECD cases and controls. The Funagata Eye Study, which looked at hyperglycemia and diabetes in relation to CCT, found that with every 1% increase in hemoglobin A1c, there was a mean CCT increase of 8.26  $\mu\text{m}$  when adjusted for age and sex.<sup>13</sup> Patients with impaired glucose tolerance or diabetes had a 7.81  $\mu\text{m}$  increase in mean CCT when adjusted for covariables.<sup>13</sup> In the Singapore Malay Eye Study, diabetes was associated with a 6.5  $\mu\text{m}$  increase in CCT independent of other factors.<sup>15</sup> Our data showing that diabetes is an independent

TABLE 6. Univariate and Multivariate Associations Between Selected Covariates and CCT Among All FECD Grades and Controls, Separated by Female and Male Sex

Covariate	Univariate Associations		Multivariate Associations	
	Effect (95% CI)	P Value	Effect (95% CI)	P Value
Females only, $n = 882$				
Smoking	5.5 (-2.7, 13.7)	0.19	1.2 (-5.8, 8.3)	0.73
Diabetes	19.4 (7.7, 31.1)	0.001	13.1 (3.5, 22.8)	0.008
FECD grade 1	9.3 (-5.7, 24.4)	0.22	11.1 (-4.2, 26.4)	0.16
FECD grade 2	1.2 (-14.7, 17.1)	0.88	1.3 (-14.9, 17.4)	0.88
FECD grade 3	10.8 (-3.0, 24.5)	0.12	12.0 (-2.3, 26.3)	0.10
FECD grade 4	33.2 (23.9, 42.5)	<0.001	33.6 (24.0, 43.1)	<0.001
FECD grade 5	42.3 (34.3, 50.4)	<0.001	41.7 (33.7, 49.6)	<0.001
FECD grade 6	82.9 (74.6, 91.3)	<0.001	82.2 (73.9, 90.6)	<0.001
Males only, $n = 464$				
Smoking	-0.2 (-12.3, 11.9)	0.97	0.0 (-13.0, 13.0)	0.99
Diabetes	2.6 (-11.9, 17.0)	0.73	5.4 (-10.5, 21.2)	0.51
FECD grade 1	8.6 (-16.5, 33.7)	0.50	7.6 (-20.0, 35.2)	0.59
FECD grade 2	18.8 (-9.2, 46.7)	0.19	19.8 (-12.3, 51.9)	0.23
FECD grade 3	-22.1 (-77.5, 33.3)	0.43	-24.8 (-83.0, 33.3)	0.40
FECD grade 4	40.9 (24.6, 57.1)	<0.001	39.7 (21.0, 58.4)	<0.001
FECD grade 5	47.9 (31.3, 64.5)	<0.001	46.3 (27.2, 65.5)	<0.001
FECD grade 6	107 (91, 123)	<0.001	106 (88, 124)	<0.001

Effects are in units of  $\mu\text{m}$  CCT. FECD grade effects are relative to baseline of FECD grade 0.

factor for increasing CCT, on average, by 9.1  $\mu\text{m}$ , is consistent with both studies.

The pathophysiology of how diabetes causes thicker corneas is not known, but is hypothesized to be due to abnormal functioning and decreased corneal endothelial cells. Several studies show that hyperglycemia contributes to increased production of mitochondrial superoxide, a reactive oxygen species.<sup>25-27</sup> Superoxide overproduction is accompanied by increased nitric oxide and peroxynitrite, which damage DNA and deplete cellular stores of the antioxidant NADH.<sup>25-27</sup> Hyperglycemia also has been shown to cause endothelial cell dysfunction exhibited by increased abnormal endothelial cell morphology<sup>28,29</sup> and increased corneal endothelial cell permeability with decreased pump rates.<sup>30</sup> Analogous to smoking, increased oxidative stress from diabetes may lead to accelerated endothelial cell death, especially in FECD cases, where endothelial defense mechanisms already are impaired.<sup>18,19</sup> However, we found that diabetes increased CCT only in females, and was not associated with advanced FECD development. If diabetes and hyperglycemia cause endothelial cell death and dysfunction, we would expect that comorbid diabetes also would increase risk of advanced FECD. Further studies to investigate this hypothesis are needed.

While others have reported that FECD is three times more common in females,<sup>2</sup> our data showed only a 34% increased odds for advanced FECD developing in females ( $P = 0.016$ ). Interestingly, we also found a significant decrease in CCT associated with being female. Whether sex affects CCT still is in debate. Some research has found increased CCT in males compared to females in adult Caucasians,<sup>31</sup> and Turkish<sup>32,33</sup> and Singaporean<sup>34</sup> children. However, other studies reported no significant differences in CCT between male and female subjects.<sup>35-40</sup> To our knowledge, studies that do show a difference in CCT among sexes report that females have lower CCT values than males, which is consistent with our study results.

A subgroup analysis was performed to see if there was a difference in effect of smoking and diabetes between females and males. There were increased effects of smoking on advanced FECD development and diabetes on CCT in the female cohort based on multivariate analyses (OR 1.37,  $P = 0.065$  and 13.1  $\mu\text{m}$  increase,  $P = 0.0077$ , respectively). In males, these associations become nonsignificant (OR 1.08,  $P = 0.71$  and 5.4  $\mu\text{m}$  increase,  $P = 0.51$ , respectively), although the direction of the effect is similar between males and females. The sex difference may represent a true dichotomy of these effects between men and women, or the lack of significant associations in men simply may be due to smaller sample size. We tested for the interaction between sex and smoking, and found it to be nonsignificant, consistent with our earlier observation that the direction of effect is similar between males and females.

Our study had several limitations, including its cross-sectional nature, and the lack of a more detailed smoking and diabetes history, including a defined measure of diabetes severity. Subjects were not followed longitudinally; thus, the long-term effects that smoking and diabetes have on FECD progression could not be discerned. Self-reported smoking did not include number of pack-years smoked, current versus past smokers, or years since quitting. This may have contributed to the high percentage of smokers in both groups (cases and controls). Self-reported diabetes did not include severity, duration, and type of diabetes. Many studies report that smoking is an independent risk factor for type 2 diabetes, and that smoking cessation is associated with weight gain and subsequent increased risk of diabetes in the short term (first five years after quitting).<sup>41,42</sup> Future studies should evaluate interventions, such as smoking cessation and strict blood

glucose control, on FECD disease progression. It also should be acknowledged that the measurement of CCT by ultrasound does not necessarily reflect the impact of FECD, smoking, and diabetes on each of the layers of the cornea, and assumes that the increase in thickness is derived primarily from endothelial cell dysfunction. Also, CCT measured by ultrasound may not be as accurate as CCT measure by tomography.

In summary, smoking appears to increase the risk of advanced FECD development, while diabetes was correlated with increased corneal thickness, but not FECD severity. We speculate that smoking and diabetes cause increased oxidative stress, which accelerates endothelial cell apoptosis in FECD patients. Thus, smoking and diabetes may have additive detrimental effects on corneal endothelial function, especially in FECD cases. These modifiable risk factors appear only to have effects in women and should be studied in a larger cohort. The results from our study should prompt counseling FECD patients on smoking cessation and tight glycemic control.

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## APPENDIX

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