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Associations between measures of oestrogen exposure and severity of Fuchs endothelial corneal dystrophy

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ABSTRACT

Aims To determine the associations between measures of oestrogen exposure and Fuchs endothelial corneal dystrophy (FECD) severity.

Methods Clinic-based cross-sectional study of 32 postmenopausal women and 11 men with mild or severe FECD, age>55 years. Participants completed questionnaires for data on demographics, anthropometric factors, medical history and potential risk factors for FECD. Women completed an additional reproductive history questionnaire used to calculate the months of lifetime oestrogen exposure. Slit-lamp biomicroscopy, specular microscopy, corneal Scheimpflug tomography and laboratory testing (*TCF4* repeat expansion quantification, total estradiol, free estradiol, sex hormone binding globulin (SHBG), calcaneal bone density) were performed. Logistic regression models were developed to predict FECD severity based on three-way interactions of each oestrogen exposure measure, sex and *TCF4* genotype.

Results 43 patients were enrolled in the study (mild FECD: 17 women, 3 men; severe FECD: 15 women, 8 men). Serum-free estradiol was higher in the severe compared with mild FECD group (0.21±0.2 vs 0.09±0.1 pg/mL; p=0.046). When stratified by sex, men showed no significant associations between oestrogen measures and FECD severity. However, in women, the odds of severe FECD were increased with more months of lifetime oestrogen exposure (all women; log OR (95% credible interval): 1.3 (0.14 to 4.3)), higher concentrations of free estradiol (all women; 2.1 (0.0049 to 10)), greater % free estradiol (only women without *TCF4* repeat expansion; 1.3 (0.16 to 3.8)) and higher concentrations of SHBG (only women with *TCF4* repeat expansion; 2.2 (0.45 to 9.1)).

Conclusions While the application of these data is constrained by the limited number of participants, a clinic-based sample, small number of men compared with women and single-point measures of serum hormone measures, these data suggest that FECD severity is associated with higher oestrogen exposures in women but not men and may be dependent on *TCF4* repeat expansion status.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ In all populations studied to date, Fuchs endothelial corneal dystrophy (FECD) is more common in women than men.

WHAT THIS STUDY ADDS

⇒ This study identifies a relationship between oestrogen exposure and FECD severity. Higher measures of oestrogen exposure are associated with severe FECD in women, but not in men.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ These clinical observations support laboratory observations that find a toxic role for oestrogen in FECD pathophysiology and may account for the greater prevalence of FECD in women than men. Larger clinical studies are needed to determine if varying oestrogen exposure affects FECD outcomes.

INTRODUCTION

Fuchs endothelial corneal dystrophy (FECD) is a disease of the corneal endothelium and a leading cause of corneal blindness. In FECD, progressive degenerative protrusions (guttae) of the corneal endothelial basement membrane accompany loss of corneal endothelial cells. Subsequent functional loss of the corneal endothelium leads to corneal opacification with vision loss. The pathogenesis of FECD is multifactorial, with both genetic and environmental factors implicated in the development and progression of disease.[1–3]

Late-onset FECD typically presents around the fourth to fifth decades of life and has a higher prevalence in women than in men. Several studies have documented the prevalence of guttae in adult populations. In the Reykjavik Eye Study, 11% of women and 7% of men over age 55 had corneal guttae.[2 4] In a Japanese study of participants over age 40, guttae were detected in 5.8% of women and 2.4% of men.[5] In a Singaporean and Japanese study of participants over age 50, guttae were detected in 8.5% of Singaporean women and 4.4% of Singaporean men, and in 5.5% of Japanese women and 1.5% of Japanese men.[6]

Regardless of geographic distributions, women have a higher prevalence of FECD than men.

The disparity in FECD prevalence between men and women increases in the sixth decade of life,[4 7] shortly after most women enter menopause. Many other age-related diseases such as cardiovascular disease and osteoporosis are also associated with shifts in prevalence around menopause age in women.[8] Diseases with sex disparities in prevalence, such as breast cancer and osteoporosis, have strong associations with oestrogen exposure.[9] Oestrogen levels decrease in women following menopause (premenopausal reference range: 15–350 pg/mL; postmenopausal reference range: <10 pg/mL) but remain relatively stable throughout life in men (10–40 pg/mL).[10–12] Oestrogen has also been shown to have beneficial effects against cellular changes in age-related diseases.[13 14] We therefore hypothesised that oestrogen exposure would be protective in FECD. In this study, we investigated the association between FECD severity and measures of oestrogen exposure.

METHODS

Study design

Clinic-based cross-sectional study of measures of oestrogen exposure in individuals with mild and severe FECD. Strengthening the Reporting of Observational Studies in Epidemiology cross-sectional reporting guidelines were used.[15]

Participant recruitment

Participants were recruited from the cornea subspecialty clinic of the principal investigator (PI) of this study (SPP) at the Ross Eye Institute, University at Buffalo, between July 2019 and November 2021. All participants were >55 years old, and women were postmenopausal. Individuals with a history of or signs of bilateral complex cataract surgery or other intraocular surgery, other than uncomplicated cataract surgery with posterior chamber intraocular lens (completed >1 year prior to enrolment), were excluded from the study. Only one eye of a patient was needed to meet the eligibility criteria for that individual to participate in the study. The study protocol included matching participants with mild and severe FECD for age (±5 years) and sex. However, these criteria could only be loosely maintained after March 2020 due to the COVID-19 pandemic. To reduce the need for repeat visits to the clinic, interested individuals were recruited at the time of routine clinic visits, thus matching by age and sex was not as rigorous after the first 10 participants. Because we were looking to identify measures of oestrogen exposure relating to disease severity, not presence of disease, we excluded those with absence of FECD.

Data collection

Each enrolled participant was administered a questionnaire (online supplemental appendix 1) assessing demographic and anthropometric indices, as well as smoking history, medical history, family history, ultraviolet light exposure and reproductive history (women only). Ultraviolet light exposure score was calculated based on participant responses to survey questions "how often do you wear a sun-blocking hat when outside" and "how often do you wear contact lenses, glasses, or sunglasses when outside". Participant responses 'always', 'sometimes' or 'never' corresponded to scores of 2, 1 and 0, respectively, and responses to the two questions were summed (possible scores 0–4). For women, reproductive lifespan was calculated as the interval between age at menarche and menopause. Estimated lifetime oestrogen exposure was calculated in months as reproductive lifespan – duration (in months) breastfeeding – (1.5)(#pregnancies without breastfeeding) + months of postmenopausal hormone replacement therapy. If women did not breastfeed following a pregnancy, 1.5 months were subtracted to approximate the typical delay before ovulatory cycles resume.

Each participant had an eye examination by slit-lamp biomicroscopy by the PI of the study (SPP). FECD was graded using a modified Krachmer grading scale,[16] and participants with mild (grade 1–2; non-confluent guttae) or severe (grade 5–6; confluent guttae>5 mm diameter with or without corneal oedema) FECD were included in the study. While primary grading of mild FECD was by slit-lamp biomicroscopy examination, presence of guttae was confirmed by at least one classic gutta observed by specular microscopy (Konan Specular Microscope X, Konan Medical) in any one of the five imaging fields of the microscope. In addition, the central field was required to have less than 50% of area occupied by guttae to be considered mild FECD. There were no imaging criteria for grading of severe FECD because it was easily observed and graded by slit-lamp biomicroscopy. For all participants, corneal thickness was measured (DGH pachymeter, DGH Technology, Inc), and specular microscopy (Konan Specular Microscope X) and corneal Scheimpflug tomography (Pentacam HR, Oculus) imaging were obtained. Tomographic features of advanced FECD in the central 4 mm of the cornea were evaluated on the '4 Map Refractive' output of the Pentacam HR according to established criteria.[17] Patients had blood drawn for laboratory testing (Kaleida Health, Department of Pathology and Laboratory Medicine, Buffalo, New York, USA) to determine serum total estradiol, serum-free estradiol (liquid chromatography tandem mass spectrometry; equilibrium dialysis) and sex hormone binding globulin (SHBG; electrochemiluminescence immunoassay). Patients also underwent bone densitometry testing using quantitative calcaneal ultrasound (Achilles Express, GE Healthcare) with measures of broadband ultrasound attenuation (BUA) and speed of sound (SOS), measures which were averaged from readings of both right and left calcaneal bones.

Blood was drawn for genotyping to determine trinucleotide repeat expansion length in the *TCF4* gene that is commonly associated with FECD. *TCF4* repeat length was considered expanded if >50 repeats were present.

Genomic DNA was extracted from buffy coat preparations using the Puregene Blood Core Kit (QIAGEN, Germany, Cat. #158023). DNA concentration was quantified using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, USA). DNA was subjected to PCR (Platinum PCR Supermix, Invitrogen, Carlsbad, California, USA) using primers flanking the trinucleotide repeat of the *TCF4* gene (F: 5′-aatccaaaccgccttccaagt-3′; R: 5′-caaaacttccgaaagccatttct-3′). PCR products were separated by electrophoresis on agarose gels (Lonza, Basel, Switzerland) to make initial determination of allele sizes. Amplified PCR products were also sent to Azenta (Burlington, Massachusetts, USA) for fragment length analysis, with resulting data analysed in Peak Scanner software (Applied Biosystems, Foster City, California, USA).

For five samples with inconclusive data on allele size by PCR, Southern blot was performed according to published protocols.[18] Briefly, DNA was digested with EcoRI (New England Biolabs, Ipswitch, Massachusetts, USA) and resulting fragments were separated overnight by agarose gel electrophoresis. The gel was incubated in denaturation solution (0.5 N NaOH, 1.5 M NaCl) and washed in neutralisation buffer (0.5 M Tris–HCl, 1.5 M NaCl). The DNA was transferred to a nylon membrane (Roche, Basel, Switzerland) and UV crosslinked. A 392 bp ^{32}P-labelled DNA probe designed against the *TCF4* sequence adjacent to the CTG repeat was incubated with the membrane to detect the *TCF4* sequence and associated CTG trinucleotide repeat expansion. The membrane was washed and imaged using a GE Typhoon phosphorimager (Boston, Massachusetts, USA).

Statistical analyses

Prior to the initiation of this study, which was prior to the COVID-19 pandemic, effect size calculations had been performed for each measure of oestrogen exposure for an anticipated enrolment of 65 pairs of matched participants. The effect size calculations are presented in online supplemental appendix 2.

Descriptive summaries and exploratory statistical comparisons of potential risk factors associated with FECD severity, by severity category (mild vs severe; tables 1 and 2) and by sex (table 3), were conducted using χ^2 tests or Fisher's exact tests for categorical risk factors, depending on whether all categories had $n > 5$ or not, respectively, and t-tests for quantitative risk factors. Due to the small sample sizes and the exploratory nature of these comparisons, raw p-values, calculated as descriptive measures, are reported in tables 1–3 without adjustment for multiplicity or inflation in frequentist type I error probabilities. Our main inferential analyses focused on Bayesian posterior probability-based uncertainty quantification to infer the associations of various risk factors with FECD severity, after controlling for sex differences, *TCF4* genotypes and multiple confounding covariates. Specifically, we used a set of Bayesian logistic regression models to predict FECD severity (mild=0, severe=1) based on linear three-way interactions of each oestrogen exposure measure ($n = 10$ measures with a separate model for each measure), sex (for measures applicable to both sexes) and *TCF4* genotype as covariates. Additionally, the models included effects of confounding covariates (race, smoking, current body mass index (BMI), BMI age 18, current height, height age 18, current weight and weight age 18). To address the high dimensionality of the explanatory variables relative to the moderate sample sizes, we employed weakly informative priors (online supplemental appendix 2) and fitted the models using Markov chain Monte Carlo computational methods.[19] The weakly informative priors for model parameters enable shrinkage of the estimates (posteriors) of small effects towards zero. This approach provides implicit control over the frequentist type I error across data replicates.[20 21] However, we emphasise the Bayesian nature of our inferences from the main statistical analyses, evaluated through posterior probabilities conditioned on the observed dataset (as opposed to sampling variability over data replicates).

From the fitted models, we inferred ORs of having severe FECD for a 1 SD increase in the associated oestrogen exposure measure, separately for each sex and *TCF4* genotype-level combinations to understand sex and genotype-specific effects of the exposure on FECD severity. Statistical significance was determined at 95% (Bayesian posterior) probability levels.

There were a few missing data points in our analysis. One participant declined to provide a blood sample (missing total estradiol, free estradiol, SHBG and *TCF4* repeat status). Another participant declined to disclose weight and BMI current and at age 18. *TCF4* analysis was inconclusive for a third participant. Furthermore, serum total estradiol concentration in some patients was low and left-censored. The missing observations for the covariates and the censored observations for serum total estradiol concentration were multiply imputed (n=100 imputed data sets)[22 23]; and inferences from the imputed data sets were obtained by combining the posterior draws from all imputed data sets prior to computing the (mixture) posterior summaries (posterior median and 95% equi-tailed credible intervals). Detailed description of the statistical methods can be found in online supplemental appendix 2. The computations involving multiple data imputations and Bayesian model fitting were performed using statistical software R and probabilistic programming language Stan.

RESULTS

Characteristics of the study population by FECD severity

There were 43 participants in this study, of whom 23 had severe FECD. Mean age was similar between the mild and severe FECD groups (mean±SD: 66.8±9.2and 71.4±8.8 years, respectively). The racial mix of the participants (88.4% white

Table 1 Assessment of potential factors associated with FECD severity

	Mild	Severe	P value*
Number of participants	20	23	
Sex			0.138
Women	17	15	
Men	3	8	
Age (years)	66.8±9.2	71.4±8.8	0.097
Race/ethnicity			0.110
White	16	22	
Black	4	1	
Other	0	0	
Objective measures of FECD severity			
Corneal thickness (µm)	551.5±44.1	625.3±66.2	<0.001
Endothelial cell density (cells/mm^2)	2008±537	–†	
Scheimpflug imaging criteria			
Loss of regular isopachs (%)	20.0	84.2	<0.001
Displacement of the thinnest point (%)	10.0	73.6	<0.001
Focal posterior depression (%)	40.0	84.2	0.004
TCF4 repeat expansions	4/18	17/23	<0.001
Anthropometric factors			
Current height (in)	64.3±2.9	66.1±5	0.144
Height at age 18 (in.)	65.2±2.3	66.6±4.3	0.160
Current weight (lbs)	152.2±42.6	171.0±41.7	0.155
Weight at age 18 (lbs)	133.8±27.7	135.5±23.8	0.828
Current BMI (kg/m^2)	25.9±6.9	27.8±7.2	0.388
BMI at age 18 (kg/m^2)	22.1±4.0	21.5±3.1	0.585
Smoking history			
Ever smoker (%)	40.0	39.1	0.953
Total pack-years of ever smokers	17.2±16.1	14.1±13.8	0.680
Ultraviolet light exposure score	2.3±0.9	2.6±1.1	0.390
Medical and family history			
Diabetes (%)	25.0	17.4	0.541
Hypertension (%)	45.0	78.2	0.024
Heart disease (%)	20.0	4.3	0.110
Family history FECD (%)	15.0	30.4	0.232
Family history AMD (%)	20.0	30.4	0.434
Percentage pseudophakic	29.4	57.1	0.020
Measures of oestrogen exposure			
Serum total estradiol (pg/mL)	5.6±8.2	11±12.3	0.102
Serum-free estradiol (%)	1.7±0.5	1.9±0.6	0.178
Serum-free estradiol (pg/ml)	0.09±0.1	0.21±0.2	0.046
SHBG (nmol/L)	63.6±32	72.2±57.9	0.548
Bone density SOS (m/s)	1555.4±1.2	1552.9±7.1	0.113
Bone density BUA (dB/MHz)	102.5±14.6	102.6±19.5	0.994

Continued

Table 1 Continued

	Mild	Severe	P value*

*Statistical analyses were performed using χ^2 tests for categorical variables (when n>5 for all categories), Fisher's exact test for categorical variables (when n<5 for at least one category), and t-tests for quantitative variables. The raw p values are reported as descriptive, unadjusted summaries for exploratory comparisons.
†Not measurable due to confluent central guttae.
AMD, age-related macular degeneration; BMI, body mass index; BUA, broadband ultrasound attenuation; FECD, Fuchs endothelial corneal dystrophy; OCP, oral contraceptive pills; SHBG, sex hormone binding globulin; SOS, speed of sound; TCF4, transcription factor 4.

participants and 11.6% black participants) was representative of our local population in Erie County, New York, USA. Characteristics of the study population are shown in table 1.

Additional testing was performed to objectively measure FECD severity and reinforce FECD severity categorisation. These analyses included one eye of each participant: the eligible eye where only one eye met study criteria and the right eyes only where both eyes were eligible. (Analyses with left eyes showed similar results.) Pachymetry and Scheimpflug imaging corneal tomographic signs of advanced FECD were more frequently seen in the severe compared with mild groups (table 1).[17] *TCF4* repeat expansion was also more frequently seen in participants with severe FECD.

Multiple additional factors were compared between the study groups (table 1). Significantly more participants with severe disease had hypertension and pseudophakia. While pseudophakia may impact corneal oedema, it is not known to influence the extent of guttae as used for differentiation of the mild and severe FECD groups in this study. Serum-free estradiol was significantly higher in the severe FECD group compared with the mild FECD group. Reproductive history (women only) showed fewer total number of pregnancies in those with severe FECD (table 2). Oestrogen measures by FECD severity in women showed a statistically significant difference in bone density by SOS only. Because of a potential role of ultraviolet light exposure and FECD,[24–26] we also compared ultraviolet light exposure scores by FECD severity in men (mild: 3.7±1.1; severe: 2.6±1.1; p=0.149) and women (mild: 2.1±0.7; severe: 2.5±1.1; p=0.166) and found no significant differences.

Characteristics of the study population by sex

We further characterised our study population by sex (table 3). There were 11 men and 32 women. Men

Table 2 Assessment of potential factors associated with FECD severity in women only

	Mild	Severe	P value*
Number of women	17	15	
Measures of oestrogen exposure			
Serum total estradiol (pg/mL)	2.6±2.6	6.6±12.8	0.255
Serum-free estradiol (%)	1.7±0.05	1.8±0.17	0.429
Serum-free estradiol (pg/mL)	0.04±0.05	0.11±0.17	0.143
SHBG (nmol/L)	66.9±33.1	85.54±65.2	0.295
Bone density SOS (m/s)	1555.8±0.7	1553.7±1.1	<0.001
Bone density BUA (dB/MHz)	101.5±15.3	97.7±20.3	0.556
Reproductive history			
Age at menarche (years)	12.4±0.9	12.8±1.6	0.442
Age at menopause (years)	49.2±7.0	49.9±4.0	0.706
Reproductive lifespan (years)	36.8±7.1	37.1±4.9	0.864
Lifetime oestrogen exposure (months)	452.3±82.8	469.0±65.8	0.532
Ever pregnant (%)	64.7	93.3	0.051
Total number of pregnancies	3.5±1.4	2.4±0.8	0.028
Age at first pregnancy (years)	24.1±5.9	25.6±4.4	0.476
Total duration breastfeeding (months)	26.7±28.8	16.2±11.2	0.600
Duration OCP use (years)	8.4±7.2	10.6±9.8	0.605

*Statistical analysis performed using t-tests for quantitative variables. The raw p values are reported as descriptive, unadjusted summaries for exploratory comparisons.
BUA, broadband ultrasound attenuation; FECD, Fuchs endothelial corneal dystrophy; OCP, oral contraceptive pills; SHBG, sex hormone binding globulin; SOS, speed of sound.

Table 3 Assessment of potential factors associated with sex

	Men	Women	P value*
Number of participants	11	32	
FECD severity			0.140
Mild	3	17	
Severe	8	15	
Age	71.3±10.4	68.6±8.8	0.451
Race/ethnicity			0.590
White	9	29	
Black	2	3	
Other	0	0	
Anthropometric factors			
Current height (in.)	70.5±3.7	63.4±2.4	<0.001
Height at age 18 (in.)	70.2±3.0	64.5±2.4	<0.001
Current weight (lbs)	187.2±24.8	153.1±44.4	0.004
Weight at age 18 (lbs)	156.3±27.8	127.0±19.9	0.006
Current BMI (kg/m^2)	26.7±4.7	26.9±7.8	0.923
BMI at age 18 (kg/m^2)	22.4±4.3	21.6±3.3	0.572
TCF4 repeat expansions>50	6/11	15/30	0.796
Smoking history			
Ever smoker (%)	55.0	34.0	0.301
Total pack-years of ever smokers	13.2±18.0	3.7±7.7	0.143
Ultraviolet light exposure score	2.9±1.0	2.3±1.0	0.070
Medical and family history			
Diabetes (%)	18.2	21.9	0.795
Hypertension (%)	72.7	59.4	0.429
Heart disease (%)	18.2	9.4	0.432
Family history FECD (%)	18.2	25.0	0.644
Family history AMD (%)	18.2	28.1	0.514
Percentage pseudophakic	21.4	36.4	0.300
Measures of oestrogen exposure			
Lifetime oestrogen exposure (months)	NA	469.0±65.8	
Serum total estradiol (pg/mL)	20.4±4.4	4.6±9.2	<0.001
Serum-free estradiol (%)	1.9±0.8	1.8±0.5	0.591
Serum-free estradiol (pg/mL)	0.4±0.2	0.1±0.1	<0.001
SHBG (nmol/L)	45.4±25.0	76.4±51.3	0.013
Bone densitometry SOS (m/s)	1551.9±10.3	1554.8±1.4	0.370
Bone densitometry BUA (dB/MHz)	110.7±13.3	99.7±17.7	0.041

*Statistical analyses were performed using χ^2 tests for categorical variables (when n>5 for all categories), Fisher's exact test for categorical variables (when n<5 for at least one category) and t-tests for quantitative variables. The raw p-values are reported as descriptive, unadjusted summaries for exploratory comparisons.
AMD, age-related macular degeneration; BMI, body mass index; BUA, broadband ultrasound attenuation; FECD, Fuchs endothelial corneal dystrophy; SHBG, sex hormone binding globulin; SOS, speed of sound; TCF4, transcription factor 4.

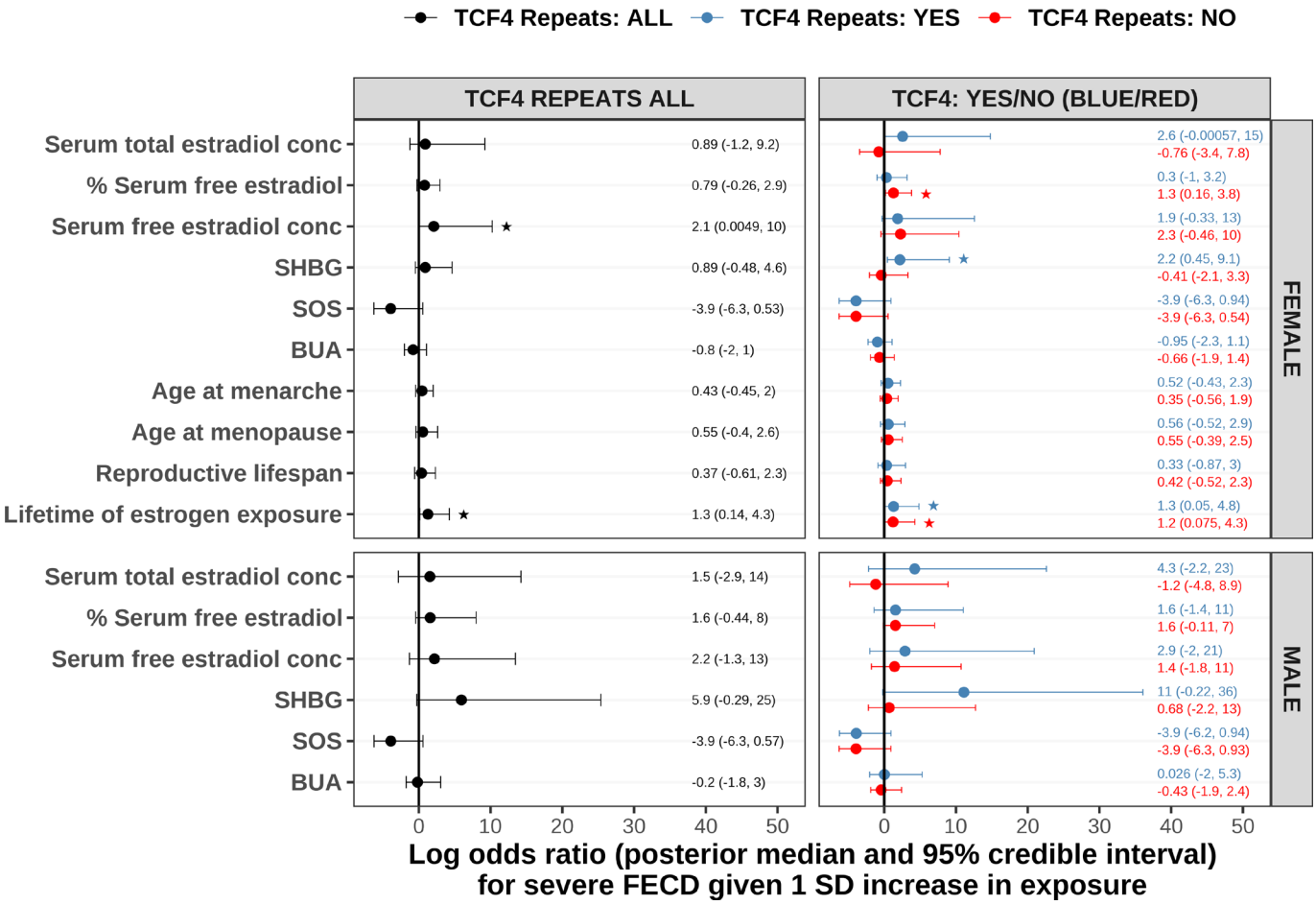

Figure 1 Grouped forest plots illustrating the change in log ORs for severe FECD due to 1 SD change in each oestrogen exposure measure for different sex and *TCF4* trinucleotide repeat expansion status. Asterisks beside the error bars represent statistical significance at the 95% (Bayesian posterior) probability levels. BUA, broadband ultrasound attenuation; FECD, Fuchs endothelial corneal dystrophy; SHBG, sex hormone binding globulin; SOS speed of sound; *TCF4*, transcription factor 4.

were significantly taller and heavier, both currently and at age 18. Men also had significantly higher total and free estradiol, SHBG, and BUA values compared with women.

Factors predictive of FECD severity

We evaluated the interactions between sex, *TCF4* genotype and each oestrogen exposure measure for predicting FECD severity while adjusting for confounding variables (race, smoking, current BMI, BMI age 18, current height, height age 18, current weight and weight age 18). Figure 1 visualises the findings with grouped forest plots (estimates and credible intervals are also provided in online supplemental appendix 2). For each oestrogen exposure measure except SOS, the association with severe FECD had a positive trend, with an increase in the exposure level resulting in a mostly positive (95% credible intervals) change in log odds of severe FECD. A few statistically significant (95% credible intervals excluding zero) associations were identified. Specifically, in women with the *TCF4* repeat expansion present, SHBG demonstrated a significantly positive association with severe FECD. This association was not significant in women without the *TCF4* repeat expansion. In addition, an increase in % free estradiol resulted in a significantly increased odds of severe FECD in women, but only in those without the *TCF4* repeat expansion. A similar measure, free estradiol (in pg/mL) was also significantly positively associated with severe FECD, but only when the groups with and without *TCF4* repeat expansion were combined, and only in women. Larger number of months of lifetime oestrogen exposure (measure for women only) was also associated with increased odds of severe FECD independent of *TCF4* repeat expansion status. There were no significant associations between FECD severity and oestrogen measures for men.

DISCUSSION

In this study, we identified significant positive associations between FECD severity and higher levels of SHBG, free estradiol concentration, % free estradiol and lifetime oestrogen exposure. The associations with SHBG and % free estradiol measures were dependent on *TCF4* repeat

expansion status. None of the findings were significant in men.

The significant findings in women support our results from our recent laboratory data. We found significant decreases in the levels of ATP in corneal endothelial cells from women but not men in response to oxygen stress. In addition, estradiol disrupted mitochondrial morphology in cells from women but not men.[27] Likewise, Liu *et al* observed more prominent disruption of the corneal endothelium in female mice compared with male mice in response to ultraviolet light exposure.[24] Disruption of mitochondria and cellular energetics is fundamental in FECD pathophysiology.[28–30] These clinical and cellular data suggest that the corneal endothelial cells in women are more susceptible to the effects of oestrogens than in men and could potentially relate to FECD severity and the disparity in FECD prevalence between men and women.

In contrast to the laboratory data and findings from this study, in our previous study of women in the Women's Health Initiative Observational Study, a population-based cohort, we observed a statistically significant decreased risk of incident FECD in women who reported active postmenopausal hormone therapy use at study baseline compared with those who had never used hormone therapy.[31] These studies likely had different findings due to different study designs and questions. The Women's Health Initiative study examined the incidence of FECD diagnosis but was unable to detail FECD severity because those data were not present in the Medicare claims data used to identify cases. The current study used detailed grading of participants' corneas to categorise by FECD severity. Oestrogen may have differential influences on disease risk compared with the progression of disease severity.

In addition to the association with oestrogens, we also found a significant direct association between SHBG and FECD severity. SHBG is a protein that binds androgens and oestrogens in the serum and contributes to the relative amounts of circulating free estradiol.[32] Thus, we measured SHBG anticipating that SHBG and free estradiol would have opposite associations with FECD severity. However, we found that both measures had positive associations with FECD, suggesting that SHBG may have effects independent of sex hormone levels, which has been seen with a variety of diseases. For example, low SHBG concentrations are associated with polycystic ovarian syndrome, metabolic syndrome, type 2 diabetes mellitus,[33] non-alcoholic fatty liver disease[34] and ischaemic stroke.[35] High SHBG concentrations are associated with osteoarthritis and rheumatoid arthritis.[36] At this time, it is unclear what mechanism may underlie the association between SHBG concentration and FECD severity. SHBG levels are heritable,[37] like FECD, and perhaps associated genes cosegregate. Alternatively, DNA methylation is altered in the corneal endothelium of patients with FECD and concentrations of SHBG can influence the DNA methylation of multiple genes, although the overlap between involved genes is not known.[38–41]

We looked for differences in general medical history and family history in those with mild versus severe FECD in our cohort. While the presence of hypertension has shown no difference between those with and without FECD in prior population studies,[5 42] diabetes is known to affect the corneal endothelium[43] and multimorbidity is known to increase FECD risk.[44] In our cohort, we found significant differences in the presence of hypertension (greater in those with severe FECD compared with mild FECD), but there were no differences in diabetes or heart disease status by FECD severity status. These findings may indicate a complex interplay between systemic disease and development of FECD (presence or absence) compared with FECD severity (mild or severe).

The development and progression of FECD are affected by both genetic and external factors. For example, in our previous study of 343 patients with FECD, we showed a positive association between BMI and FECD severity in women but not men that was independent of *TCF4* genotype.[45] (The current study was not designed to investigate the association between BMI and FECD.) In the model developed in this study, we included the interaction of *TCF4* genotype along with sex and oestrogen exposure measures for predicting FECD severity. Our data showed that exposure measures did have significantly different associations with FECD severity based on *TCF4* repeat status. Most notably, SHBG had a positive association with FECD severity only in women with the *TCF4* repeat, and % serum-free estradiol was positively associated with FECD severity only in women without the *TCF4* repeat. Lifetime oestrogen exposure was positively associated with FECD severity independent of *TCF4* repeat status. Thus, across the spectrum of oestrogen measures tested, there are both genotype-dependent and genotype-independent factors affecting FECD severity. Identifying non-genetic, modifiable, external factors may assist in the future development of therapeutics for the treatment or prevention of FECD, especially with knowledge regarding whether a person is of a high-risk genotype for FECD.

While our study has strength and novelty in its comparison of oestrogen measures by FECD severity compared with FECD prevalence or incidence, there were a few limitations. Overall, our enrolment was lower than anticipated (disrupted by the COVID-19 pandemic). We anticipated enrolment of 65 pairs of matched subjects (online supplemental appendix 2), but were able to enrol only 43 individuals. Furthermore, matching by age and sex was less rigorous towards the end of the study and far fewer men were enrolled than anticipated. The small sample of men in this study may limit the ability to detect significant associations. Our recruitment from a cornea clinic population may also not fully represent the distribution of risk factors present in a population-based sample, especially for those with mild FECD who would not necessarily seek care because symptoms are typically absent with mild FECD. Additionally, our measures of oestrogen and SHBG were limited to

single timepoint measurements and may not reflect variation across the lifespan.

While studies of other diseases have shown protective benefits of oestrogens, data from our study suggest that increased oestrogen exposures are associated with increased FECD severity in a genotype-dependent fashion in women but not men. These novel findings provide new insights into the role oestrogens may have in FECD severity, particularly in understanding the higher burden of FECD that is found in women.

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