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Genetic screen of African Americans with Fuchs endothelial corneal dystrophy

Mollie A. Minear,[1] Yi-Ju Li,[1,2] Jacqueline Rimmler,[1] Elmer Balajonda,[3] Shera Watson,[1] R. Rand Allingham,[3] Michael A. Hauser,[1] Gordon K. Klintworth,[3,4] Natalie A. Afshari,[5] Simon G. Gregory[1]

(The last two authors contributed equally to this work.)

[1]Duke Molecular Physiology Institute, Duke University Medical Center, Durham, NC; [2]Department of Biostatistics and Bioinformatics, Duke University Medical Center, Durham, NC; [3]Duke Eye Center, Duke University Medical Center, Durham, NC; [4]Department of Pathology, Duke University Medical Center, Durham, NC; [5]Shiley Eye Center, University of California San Diego, La Jolla, CA

Purpose: Fuchs endothelial corneal dystrophy (FECD) is a genetically heterogeneous disorder that has been primarily studied in patients of European or Asian ancestry. Given the sparse literature on African Americans with FECD, we sought to characterize the genetic variation in three known FECD candidate genes in African American patients with FECD.

Methods: Over an 8-year period, we enrolled 47 African American probands with FECD. All participants were clinically examined with slit-lamp biomicroscopy, and when corneal tissue specimens were available, histopathologic confirmation of the clinical diagnosis was obtained. The coding regions of known FECD susceptibility genes collagen, type VIII, alpha 2 (*COL8A2*); solute carrier family 4, sodium borate transporter, member 11 (*SLC4A11*); and zinc finger E-box binding homeobox 1 (*ZEB1* [also known as *TCF8*]) were Sanger sequenced in the 47 probands using DNA isolated from blood samples.

Results: Twenty-two coding variants were detected across the *COL8A2*, *SLC4A11*, and *ZEB1* genes; six were nonsynonymous variants. Three novel coding variants were detected: a synonymous variant each in *COL8A2* and *SLC4A11* and one nonsynonymous variant in *ZEB1* (p.P559S), which is predicted to be benign and tolerated, thus making its physiologic consequence uncertain.

Conclusions: Variation in the *COL8A2*, *SLC4A11*, and *ZEB1* genes is present in only a small fraction of our African American cases and as such does not appear to significantly contribute to the genetic risk of FECD in African Americans. This observation is on par with findings from previous sequencing studies involving European or Asian ancestry patients with FECD.

Fuchs endothelial corneal dystrophy (FECD; OMIM 136800) is a slowly progressive late-onset disorder that is a leading indication for corneal transplantation in the United States [1,2]. Symptoms typically manifest when patients are in their 50s or 60s, and women are disproportionately more frequently affected compared to men [1,3-5]. Although no formal epidemiological studies have been performed to assess the prevalence or incidence of this disease, FECD may affect as much as 4% of the American population over the age of 40 years [3]. However, in one genetically isolated population on Tangier Island in Virginia, a sample of half the inhabitants over the age of 50 suggested a prevalence rate as high as 11% [6]. Corneal transplant data reveal a disparate worldwide distribution of FECD, with Western regions such as the United States, Canada, and Europe reporting more FECD cases than the Middle East, Asia, Oceania, and South America [2]. To date, corneal transplants remain the sole treatment for advanced FECD. The genetic and environmental risk factors that contribute to this debilitating condition are still not fully understood.

In 2001, Biswas and colleagues published the first report of a variant that segregated with FECD in three pedigrees [7], the first proof beyond reports of familial clustering [4,8,9] that there was a genetic component to FECD risk. Variation in four genes has been identified in patients with FECD: collagen, type VIII, alpha 2 (*COL8A2*) [10-15]; solute carrier family 4, sodium borate transporter, member 11 (*SLC4A11*) [15-17]; zinc finger E-box binding homeobox 1 (*ZEB1* [also known as *TCF8*]) [18,19]; and lipoxygenase homology domains 1 (*LOXHD1*) [20]. Additionally, linkage [19,21-24] and association [25-29] studies have implicated several other loci in FECD risk, including the intronic single nucleotide polymorphism (SNP) rs613872 in transcription factor 4 (*TCF4*). However, FECD genetic studies have primarily focused on patients with European ancestry from the United

Correspondence to: Simon Gregory, 300 N. Duke St. Durham, NC 27701; Phone: (919) 684-0726; FAX: (919) 684-0924; email: simon.gregory@duke.edu

Kingdom, the United States, and Australia as well as a small number of Asian populations from China, India, Japan, and Korea. Other racial and ethnic groups of patients with FECD, including African Americans, have not been genetically examined.

The literature on African Americans with FECD is sparse with only two known articles describing guttae in this population. The first report describes a case study of a 53-year-old man treated by Whitham in 1924 [30], and the second study of 2,002 eyes in 1,016 individuals by Lorenzetti et al. [31] found no significant difference in the prevalence of central corneal guttae between African Americans and European Americans. In addition to these sparse reports, the experience of our ongoing FECD genetic study [27] is that we have observed fewer patients with African American ancestry in our cornea clinic than patients with European ancestry, indicating that there could be a difference in FECD prevalence between African Americans and European Americans. In this report, we detail the findings of the first genetic screen of African Americans with FECD.

METHODS

Participant enrollment: Participants were clinically examined as described previously [27]; briefly, study participants were recruited through the Duke University Eye Center (DUEC) after they underwent a clinical examination that included slit-lamp biomicroscopy observation for central corneal guttae. Severity of FECD was graded based on a modified Krachmer scale [3], and when tissue samples were available a histopathological confirmation of the clinical diagnosis was required. This study was performed in accordance with the tenets of the Declaration of Helsinki; was approved by the Duke University Medical Center Institutional Review Board for research on human subjects before initiating participant recruitment; and all participants gave written, informed consent.

We used 2,439 individuals recruited as part of the genetic study of primary open-angle glaucoma (POAG) at the DUEC [32], which included 1,455 African Americans, as controls for our variant screen. These participants were also recruited under the approval of the Duke University Medical Center Institutional Review Board, and consented to allow their biologic samples to be used by other research studies. Participants with POAG were examined for obvious ocular diseases at the time of POAG study enrollment by a glaucoma specialist. Although the participants were not specifically examined for FECD, the rate of FECD cases should be low as they would have likely been detected in the POAG enrollment screen.

Selection and demographics of genetic screen samples: Over an 8-year recruitment period, we ascertained 84 individuals who self-identified as having African American race; 65 of these were affected with FECD, and the other 19 were unaffected family members. The 65 cases represented 50 independent families. Three families were excluded based on the proband having atypical FECD (copresentation with keratoconus), an undetermined FECD status, or a low DNA yield during extraction, leaving 47 families. The probands of these 47 families were used in the genetic screen for coding variants in the *COL8A2*, *SLC4A11*, and *ZEB1* genes; sequencing in the other 18 non-proband FECD cases was performed only as needed to trace the inheritance of novel variants within a family.

The 47 probands represented 38 women (81%) and nine men (19%), an approximate gender ratio of 4:1 (women:men), slightly higher than the gender ratio we previously observed in European Americans (3:1; women:men) in our cornea clinic [1] and noted in other studies of patients with FECD [3-5,9,33,34]. The average age of our cases at the time of consent was 67 years and ranged from 41 to 90 years. We recruited at least one additional affected family member for ten of these probands; the other 37 may represent sporadic (not familial) forms of FECD.

DNA extraction, sequencing, and genotyping: The coding regions of the *COL8A2*, *SLC4A11*, and *ZEB1* genes were sequenced in 47 probands. Although variants in *LOXHD1* have recently been reported in one large family with Mendelian FECD [20], this report has yet to be replicated in an independent family or patient cohort so we opted not to screen this gene at this time. In addition, although strong evidence of linkage to and association with the *TCF4*/*FCD2* locus on chromosome 18 has been reported [22,25-29], efforts to identify causal variants within *TCF4* through sequencing have not yet been successful [28], so we also omitted the *TCF4* gene from our analysis.

Blood samples were obtained through venipuncture and stored at -80 °C in the Duke DNA Bank. DNA was extracted from peripheral blood using the PureGene system (Gentra Systems, Minneapolis, MN). Primers complementary to the coding regions of the *COL8A2* (NM_005202.2), *SLC4A11* (NM_032034.3), and *ZEB1* (NM_030751.5) genes were either taken from the literature [7,17,19] or were designed using the ExonPrimer and Primer3 [35] tools available online, and were checked for off-target sequence homology using the BLAT algorithm [36] in the University of California, Santa Cruz (UCSC) Genome Browser. Primer sequences and polymerase chain reaction (PCR) conditions are listed in Appendix 1, Appendix 2, Appendix 3, and Appendix 4;

all PCR amplifications used 30 ng input genomic DNA per primer pair. The suffixes ".2" or ".3" in primer names represent redesigned primers and were used to sequence samples only after the original pair of primers failed to generate clean sequencing data on those samples. The *ZEB1* 1a.3 primer pair was used in a nested PCR with the *ZEB1* 1a.2R primer used to sequence.

Sequencing was performed by either Eton Bioscience or GENEWIZ (both in Research Triangle Park, NC), or was performed using BigDye Terminator v3.1 Cycle Sequencing Kits (Life Technologies, formerly Applied Biosystems, Foster City, CA) and run on an ABI 3730 DNA analyzer (Applied Biosystems). Sequences were analyzed using the program Sequencher 5.0 (Gene Codes Corporation, Ann Arbor, MI). All suspected variants were confirmed with bidirectional sequencing. Novelty was then examined against the exomes sequenced as part of the National Heart, Lung, and Blood Institute (NHLBI) GO Exome Sequencing Project posted online on the Exome Variant Server (EVS) [37].

For nonsynonymous variants, the PolyPhen-2 [38] and SIFT [39] tools were used to predict the severity of the amino acid substitution. Additionally, novel nonsynonymous variants were screened in 2,439 POAG study individuals using a custom-designed Taqman assay. Taqman assays use unlabeled PCR primers and two allele-specific probes containing the TaqMan minor groove binding group (MGB) probe and the FAM and VIC dye labels in a 384-well plate format. PCR reactions were performed with Taqman Universal PCR Master Mix on the GeneAmp PCR System 9700 (Applied Biosystems), and the ABI7900HT Fast PCR System (Applied Biosystems) was used for reading allelic discrimination calls. Quality control samples, including two CEPH (Centre d'Etude du Polymorphisme Humain) pedigree individuals, one no-template sample, and two duplicate samples (one male, one female), were contained within each quadrant of each 384-well plate. If this assay detected a variant in any sample, its presence was confirmed with bidirectional sequencing.

The previously associated SNP rs613872 was genotyped as part of our ongoing FECD genetic studies; genotyping methods for this SNP have been described elsewhere [27] and as noted above. Genotypes for rs613872 are available for 39 of our 47 probands.

RESULTS

COL8A2 analysis: We detected six known variants that are listed in the database of single nucleotide polymorphisms (dbSNP) in our analysis of the *COL8A2* gene, as well as one coding variant not present in dbSNP (Table 1, Appendix 5). Two of these dbSNP coding variants have been previously reported in patients with FECD (Figure 1, red font). The first was a nonsynonymous p.R155Q substitution [7,12,14,15], which was present in 1/47 probands, a man with no known family history of FECD. This substitution is a known polymorphism (dbSNP: rs75864656), and EVS data show that this variant is present in 5/1,612 African Americans (minor allele frequency [MAF]=0.16%), and in 3/3,373 European Americans (MAF=0.04%). The second was a synonymous p.G495G variant (dbSNP: rs35495320) [7] that was present in 14/47 probands; EVS data show this variant is present in 193/1,535 African Americans (MAF=6.32%) and in 25/3,347 European Americans (MAF=0.37%).

Two coding variants (both synonymous) detected in our probands have not been previously reported in patients with FECD: p.A441A and p.Y648Y (Table 1; Figure 1, red font and asterisk). The first variant, p.A441A, was found in a female proband and her affected mother, suggesting that it may segregate with FECD in this family; however, no sample was available for the proband's father. The p.A441A variant is a known polymorphism (rs182708720), and EVS data show that this variant was present in 37/2,106 African Americans (MAF=0.88%) and in 1/4,151 European Americans (MAF=0.01%). The second variant, p.Y648Y, was identified in a single female patient with no known family history of FECD. There is no reference SNP (rs) ID for this variant, and in the EVS the minor allele has been detected in 1/4,300 European Americans (MAF=0.01%) but is absent from 2,203 African Americans. We did not detect the rare p.L450W or p.Q455K/V variants that have been linked to early-onset forms of FECD in European ancestry [7,11,13] and Asian [14] patients within our African American cohort.

SLC4A11 analysis: We detected 28 known dbSNP variants (nine coding) in our analysis of the *SLC4A11* gene, as well as one coding variant not present in dbSNP (Table 1, Appendix 5). Five of the dbSNP variants detected were synonymous variants that have been previously reported in Asian FECD cases and controls (Figure 2, red font): p.A135A (rs34460295) [15,17], 6/47 probands; p.R161R (rs3827075) [15,17], 29/47 probands; p.S213S (rs3803956) [15,17], 20/47 probands; p.N553N (rs41281860) [15], 6/47 probands; and p.T833T (rs58757394) [17], 16/47 probands. The minor allele frequencies of these variants in the EVS database are listed in Appendix 5.

Five coding variants were identified that have not been previously reported in patients with FECD (Table 1; Figure 2, red font and asterisk). Four of these are present in dbSNP: p.N150S (rs34520315), 5/47 probands; p.R158R (rs35262978), 1/47 probands; p.T463T (rs6084312), 6/47 probands; and p.D886D (rs76962118), 3/47 probands. All four were detected

Table 1. Coding variants present in 47 African American FECD probands that have not been previously reported in FECD patients.

Variant name	Amino acid change	rs ID	Location within gene	Physical location*	Number of cases with variant	Flanking sequence	MAF AA (%)	MAF EA (%)
COL8A2 c.1330T>C	p.A441A	rs182708720	Exon 2	Chr 1: 36,563,959	2	GCTCC**[T/C]**GCCAC	0.88	0.01
COL8A2 c.1951G>A	p.Y648Y	n/a	Exon 2	Chr 1: 36,563,338	1	TCATC**[G/A]**TAGGT	0	0.01
SLC4A11 c.497A>G	p.N150S	rs34520315	Exon 4	Chr 20: 3,214,851	5	GGATA**[A/G]**CGTGC	4.24	0.02
SLC4A11 c.522C>T	p.R158R	rs35262978	Exon 4	Chr 20: 3,214,826	1	CGCCG**[C/T]**TTCGC	1.18	0
SLC4A11 c.1437G>A	p.T463T	rs6084312	Exon 11	Chr 20: 3,211,235	6	TGGAC**[G/A]**GGCCT	3.95	11.71
SLC4A11 c.2232G>A	p.H728H	n/a	Exon 16	Chr 20: 3,209,540	2	CGCAC**[G/A]**TGCAG	0.02	0
SLC4A11 c.2706C>T	p.D886D	rs76962118	Exon 19	Chr 20: 3,208,451	3	ATGGA**[C/T]**GCTGA	2.84	0
ZEB1 c.666T>C	p.S201S	rs79134358	Exon 5	Chr 10: 31,799,722	4	TTTAG**[T/C]**TGCTC	5.76	0
ZEB1 c.852T>C	p.S263S	rs143232269	Exon 6	Chr 10: 31,803,635	1	CACAG**[T/C]**GGTAA	0.48	0
ZEB1 c.1721A>G	p.K553R	rs35753967	Exon 7	Chr 10: 31,809,921	3	CCTAA**[A/G]**GCAGC	4.95	0
ZEB1 c.1738C>T	p.P559S	n/a	Exon 7	Chr 10: 31,809,938	1	AGCCT**[C/T]**CTCCA	–	–
ZEB1 c.2037C>G	p.N658K	rs151205909	Exon 7	Chr 10: 31,810,237	1	AAGAA**[C/G]**AATGA	0.48	0
ZEB1 c.2124A>C	p.P687P	rs34846414	Exon 7	Chr 10: 31,810,324	6	TCCCC**[A/C]**GTTTT	5.65	0.047
ZEB1 c.2623C>A	p.Q854K	rs139581793	Exon 7	Chr 10: 31,810,823	4	CAGTC**[C/A]**AAGAA	1.59	0

Variants are named based nucleotide affected in NM_005202.2 (*COL8A2*), NM_032034.3 (*SLC4A11*), or NM_030751.5 (*ZEB1*). Some sequencing was performed on the reverse (negative) strand, but the sequences presented in this table are on the forward (positive) strand. *Physical coordinates obtained from the February 2009 (GRCh37/hg19) assembly of the UCSC Genome Browser. Minor allele frequencies (MAF) obtained from the Exome Variant Server (EVS). n/a, not available; Chr, chromosome; AA, African American; EA, European American; –, variant not found in EVS database.

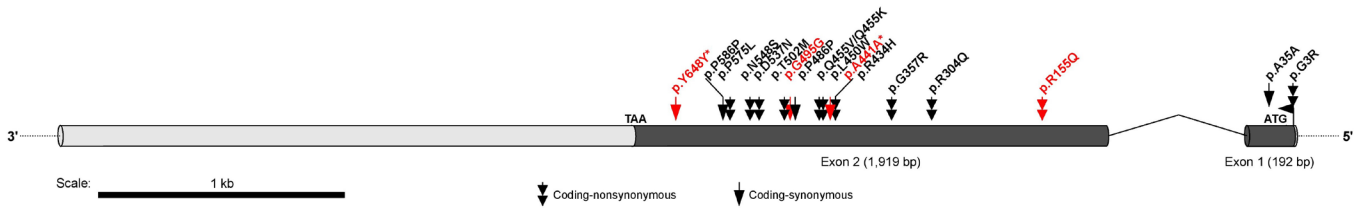

Figure 1. Variants in the collagen, type VIII, alpha 2 (*COL8A2*) gene on chromosome 1p34.3 that have been detected in patients with Fuchs endothelial corneal dystrophy (FECD). Solid cylinders represent coding portions of the gene, while light gray cylinders represent untranslated regions. The lines connecting each cylinder indicate splicing events, and the start and stop codons of the gene are indicated with bold font. The gene is drawn from 3′ to 5′ reflecting its physical orientation on the reverse strand of the reference genome. All variants in this figure are taken from previous FECD reports in the literature (black font) or are coding variants detected in African Americans with FECD in this report (red font). Variants are indicated with double-headed arrows: black arrows indicate coding-nonsynonymous variants (produce an amino acid change), while white arrows indicate coding-synonymous variants (do not produce an amino acid change). Variants marked with an asterisk (*) are newly identified variants in African Americans with FECD that have not been previously reported in patients with FECD.

in African Americans in the EVS database, and only p.R158R and p.D886D were not detected in European Americans (Appendix 5). The fifth novel FECD variant was absent from dbSNP: p.H728H, which was present in two probands, both of whom did not have a family history of FECD. There is no rs ID for this variant, and it was detected in 1/2,201 African Americans in the EVS (MAF=0.02%) but was absent from 4,300 European Americans.

ZEB1 analysis: We detected nine known dbSNP variants (seven coding) in our analysis of the *ZEB1* gene, as well as one coding variant not present in dbSNP (Table 1, Appendix 5). Only p.D64D (Figure 3, red font) has previously been detected in patients with FECD [18]; it was detected in 17/47 probands, and is present in 705/2,203 African Americans (MAF=17.50%) and in 64/4,300 European Americans (MAF=0.76%) in the EVS.

Seven coding variants were identified that have not been previously reported in patients with FECD (Table 1; Figure 3, red font and asterisk). Six of these are in dbSNP: p.S201S (rs79134358), 4/47 probands; p.S263S (rs143232269), 1/47 probands; p.K553R (rs35753967), 3/47 probands; p.N658K (rs151205909), 1/47 probands; p.P687P (rs34846414), 6/47 probands; and p.Q854K (rs139581793), 4/47 probands. All were detected in African Americans, but only p.P687P was additionally detected in European Americans in the EVS (Appendix 5). The seventh novel FECD variant was absent from dbSNP: p.P559S, which confers a nonsynonymous protein amino acid substitution. This variant was present in a singleton man who was a heterozygous carrier, lacks an rs ID, and was absent from the EVS database. PolyPhen-2 and SIFT predict this variant to be benign and tolerated, respectively: The PolyPhen-2 score was 0.006 on a scale of 0 (benign) to 1 (probably damaging), and the SIFT score was 0.25 on a scale of 0 to 1 (where a score ≤0.05 is predicted to be damaging).

To determine the prevalence of the potentially causal variant p.P559S in controls, we screened for the presence of p.P559S in POAG controls. We identified one African American and zero European Americans from this cohort

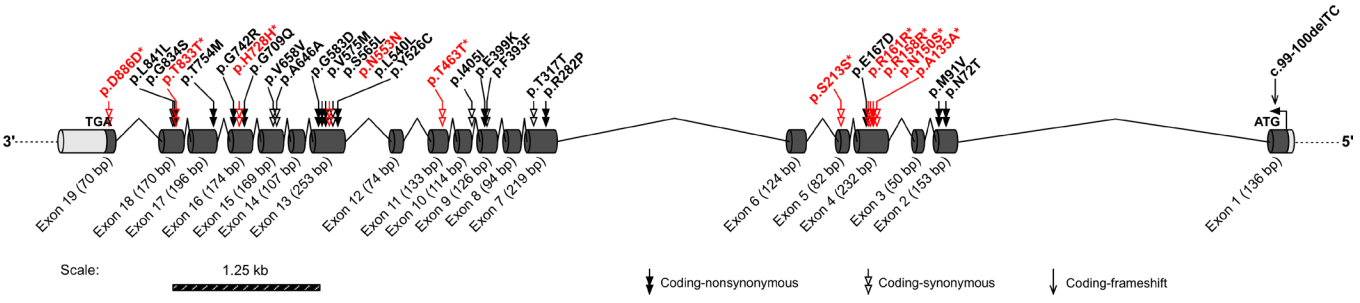

Figure 2. Variants in the solute carrier family 4, sodium borate transporter, member 11 (*SLC4A11*) gene on chromosome 20p13 that have been detected in patients with Fuchs endothelial corneal dystrophy (FECD). The gene is drawn from 3′ to 5′ reflecting its physical orientation on the reverse strand of the reference genome. Figure drawn as described in the caption for Figure 1, with the red font indicating variants identified in our African American FECD cases and variants marked with an asterisk (*) indicating newly identified variants in African Americans with FECD that have not been previously reported in patients with FECD.

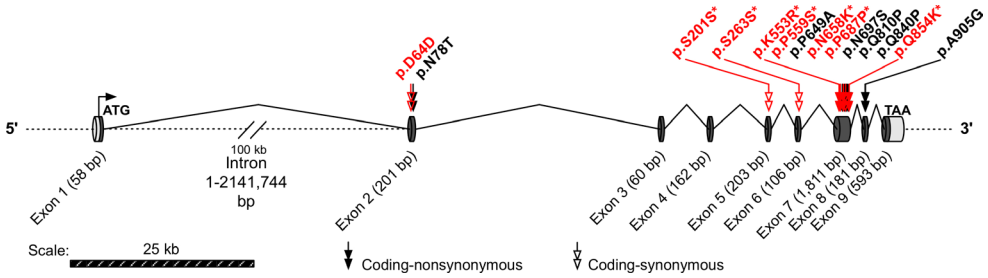

Figure 3. Variants in the zinc finger E-box binding homeobox 1 (*ZEB1*, also known as *TCF8*) gene on chromosome 10p11.22 that have been detected in patients with Fuchs endothelial corneal dystrophy (FECD). The gene is drawn from 5′ to 3′ reflecting its physical orientation on the forward strand of the reference genome. Drawn as described in the caption for Figure 1, with the red font indicating variants identified in our African American FECD cases and variants marked with an asterisk (*) indicating newly identified variants in African Americans with FECD that have not been previously reported in patients with FECD.

with this variant; the African American woman was a heterozygous carrier. Although she had been examined for ocular diseases at the time of POAG study enrollment by a glaucoma specialist and had been enrolled as a control for that study, she was not specifically examined for FECD and is now lost to follow-up.

rs613872 genotypes: Genotypes for the significant SNP rs613872 in *TCF4*, identified through genome-wide association studies of patients with FECD [25-29], are available for 39/47 probands. Seven individuals were heterozygous for the risk allele (G) while the remaining 32 were homozygous for the wild-type (T) allele. We did not observe any GG homozygotes. The carrier of the *ZEB1* p.P559S variant did not carry the rs613872 G risk allele.

DISCUSSION

This report is the first analysis of the genetic susceptibility of African Americans to FECD, and contains the largest cohort of African American patients with FECD collected to date. We sequenced the coding portions of three genes, *COL8A2*, *SLC4A11*, and *ZEB1*; variants in these genes have been reported by multiple groups in several FECD cohorts of European or Asian ancestry. We identified four coding variants in *COL8A2*, two of which have never been reported in patients with FECD; however, we failed to detect the rare p.L450W or p.Q455K/V variants that have been reported in early-onset FECD. In *SLC4A11* we detected ten coding variants, five of which have never been reported in patients with FECD. Finally, in *ZEB1* we identified eight coding variants, seven of which have never been reported in patients with FECD, including the novel nonsynonymous variant p.P559S, which was detected in 1/47 patients with FECD and 1/2,439 individuals enrolled in a POAG genetic study with unknown FECD status. Across the three genes, p.P559S in *ZEB1* was the only novel nonsynonymous variant detected. Sequencing a larger sample of African American FECD cases and controls is needed to determine the prevalence of the *ZEB1* p.P559S variant, and functional studies are needed to determine whether it is benign (as predicted by PolyPhen-2 and SIFT) or whether it may confer phenotypic consequences.

Across the three genes, we identified two novel synonymous variants: p.Y648Y in *COL8A2* (1/47 probands) and p.H728H in *SLC4A11* (2/47 probands). Both variants were present in a minute fraction of the EVS data set: p.Y648Y was present in 1/6,503 individuals (a European American), while p.H728H was present in 1/6,501 individuals (an African American). No ocular phenotype data from the EVS participants are available, making a genotype-phenotype correlation impossible for these individuals. According to the UCSC Genome Browser, the bases affected by these two variants are not well conserved through evolution and do not lie within any microRNA regulatory sites or transcription factor binding sites. However, in spite of not producing a change in the encoded amino acid sequence, synonymous variants still have the potential to induce phenotypic variation through mechanisms such as alteration of mRNA structure and stability by changing codon usage or inducing translational pausing [40,41], or by altering splicing efficiency by changing exonic splicing enhancer or silencer sequences [42]. Therefore, further studies are needed to determine what effect, if any, these synonymous variants may have on *COL8A2* and *SLC4A11* gene function and FECD pathogenesis.

The fact that we did not detect some of the previously reported FECD-associated variants in these genes in our probands is likely a combination of the fact that these genes carry a low genetic load in FECD and that we screened only 47 African American cases, a small sample that is underpowered for detecting variants that occur at such low frequencies. Clearly, additional studies on larger samples of African Americans with FECD are needed to draw any conclusions regarding whether these three genes influence FECD pathogenesis in this racial group. However, our data

indicate that any effect conferred by these genes is likely to be small, just as it seems to be in Europeans and Asians with FECD. Furthermore, we sequenced only the coding portions of the three FECD genes, and as such, additional variants in the intronic or regulatory regions of these genes may be present that could confer phenotypic change.

One of the risks of focusing genetic studies on patients of one particular ancestry is that it limits the ability to determine the relevance of genetic associations to patients from other ethnic/racial groups. There are several examples of variations in disease prevalence between racial and ethnic groups [43], and these differences may be linked to genetic risk alleles that differ in frequency between populations. The elevated incidence of some ocular conditions, such as POAG, has been noted to be more prevalent in people of African descent than in people of European descent [44-47]. The most consistently associated risk factor for sporadic, late-onset FECD is the G allele of rs613872 [25-29], the frequency of which varies significantly across racial and ethnic groups [27]. Data from the Human Genome Diversity Project indicate that the G risk allele is present at low frequencies or is nonexistent in the sampled African populations. Therefore, it is crucial that the underlying genetic predispositions of other racial and ethnic groups of patients with FECD beyond Europeans and Asians, such as African Americans, be examined more thoroughly than has been done to date. Although our study is the first to genetically examine African American FECD, a larger sample is needed to replicate previous linkage and association studies. In conclusion, racial or ethnic background does not appear to influence the prevalence of variations in the *COL8A2*, *SLC4A11*, and *ZEB1* genes in patients with FECD, and these three genes do not likely confer a sizeable effect on FECD risk in African Americans with FECD.

APPENDIX 1.

Collagen, type VIII, alpha 2 (*COL8A2*) primer sequences and reaction conditions. *Refer to Appendix 4 for details regarding the reaction conditions. To access the data, click or select the words "Appendix 1."

APPENDIX 2.

Solute carrier family 4, sodium borate transporter, member 11 (*SLC4A11*) primer sequences and reaction conditions. *Refer to Appendix 4 for details regarding the reaction conditions. To access the data, click or select the words "Appendix 2."

APPENDIX 3.

Zinc finger E-box binding homeobox 1 (*ZEB1* [also known as *TCF8*]) primer sequences and reaction conditions. *Refer to Appendix 4 for details regarding the reaction conditions. To access the data, click or select the words "Appendix 3."

APPENDIX 4.

PCR conditions for African American proband variant screening. * All reaction conditions use 3 µl of genomic DNA at 10 ng/µl; this brings all final reaction volumes up to 25 µl. To access the data, click or select the words "Appendix 4."

APPENDIX 5.

Variants from the database of single nucleotide polymorphisms (dbSNP) detected in African American FECD cases. Minor allele frequencies (MAF) taken from the exome variant server (EVS). All coding variants in this table are included in Figure 1, Figure 2, and Figure 3 (red font). UTR, untranslated region; n/a, not applicable; AA, African American; EA, European American; –, variant not found in EVS database. To access the data, click or select the words "Appendix 5."

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