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A Multi-Center Study to Map Genes for Fuchs' Endothelial Corneal Dystrophy: Baseline Characteristics and Heritability

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Abstract

Purpose—To describe the methods for family and case-control recruitment for a multi-center genetic and associated heritability analysis of Fuchs' Endothelial Corneal Dystrophy (FECD).

Methods—Twenty-nine enrolling sites with 62 trained investigators and coordinators gathered individual and family information, graded the phenotype, and collected blood and/or saliva for genetic analysis on all individuals with and without FECD. The degree of FECD was assessed in a 0–6 semi-quantitative scale using standardized clinical methods with pathologic verification of FECD on at least one member of each family. Central corneal thickness was measured by ultrasonic pachymetry.

Results—Three hundred twenty-two families with 330 affected sibling pairs with FECD were enrolled, and included a total of 650 sibling pairs of all disease grades. Using the entire 0–6 step FECD grading scale or a dichotomous definition of severe disease, heritability was assessed in families via sib-sib correlations. Both binary indicators of severe disease as well as semi-quantitative measures of disease severity were significantly heritable, with heritability estimates of 30% for severe disease, 37–39% for FECD score and 47% for central corneal thickness.

Conclusion—Genetic risk factors have a strong role in the severity of the FECD phenotype and corneal thickness. Genotyping this cohort with high-density genetic markers followed by appropriate statistical analyses should lead to novel loci for disease susceptibility.

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Keywords

heritability; Fuchs' dystrophy; corneal thickness; genetics

Introduction

Fuchs' Endothelial Corneal Dystrophy (FECD) is a common disease resulting in loss of vision associated with progressive corneal edema and loss of corneal transparency. FECD has been estimated to have a prevalence of approximately 4%[1] in the United States, where it is one of the most common indications for penetrating, and now, endothelial keratoplasty[2]. In the initial stages of the disease, excrescences of Descemet's membrane with the appearance of an abnormal posterior collagenous layer, result in the clinical and pathologic appearance of guttae[3]. Corneal edema ensues as endothelial function is compromised that may result in stromal edema, epithelial edema, and painful bullous keratopathy. Penetrating or endothelial keratoplasty is the only definitive treatment, with palliative care the only option prior to surgery. The pathophysiology underlying FECD, particularly in the common cases that affect individuals over 60 years of age, remains unknown[4,5]. Thus, the potential for developing new therapeutic approaches based on advances in the understanding of the disease process remains high.

The single major risk factor associated with FECD is genetic predisposition, with no consistent environmental correlates currently identified[6,7]. Linkage analyses in large multi-generational families affected by late-onset FECD have discovered loci on chromosomes 5, 9, 13 and 18[8,9], although the causal genes at these regions have not yet been identified. The largest FECD linkage to study to date examined 92 subjects in 22 families and implicated additional loci on chromosomes 1, 7, 15, 17 and X[10]. This study did not reidentify the other four FECD loci; however, the linkage peak on chromosome 1 did localize near collagen, type VIII, alpha 2 (*COL8A2*, MIM #136800), a gene identified as underlying early-onset FECD. The loci identified in linkage have not yet been extensively validated and the extent to which familial variants account for the genetic risk for FECD in the general population remains undetermined.

Candidate gene studies of *COL8A2*[11–13], solute carrier family 4, sodium borate transporter, member 11 (*SLC4A11*) (MIM #610206) (mutated in Congenital hereditary endothelial dystrophy 2, MIM # 217700)[14] and transcription factor 8 (*TCF8* [MIM #189909]) (implicated in posterior polymorphous corneal dystrophy)[15] have found mixed evidence for potentially causal variants. Current evidence indicates variants in these genes are likely to contribute to only a limited portion of FECD in a small subset of affected subjects. A genome-wide association analysis, conducted in a cohort assembled for a study of age-related macular degeneration and recoded for a FECD phenotype, implicated genetic variants in the transcription factor 4 gene (*TCF4* [MIM #602272]) as contributing to development of FECD[16]. Further characterization of this region will be necessary to determine which variations underlie the effect on FECD phenotype.

In light of these efforts, it is clear that the major genetic components underlying FECD are just beginning to be elucidated. To this end, we designed a study, the Fuchs' Endothelial Corneal Dystrophy (FECD) Genetics Multi-Center Study, to collect affected subjects, their families, and unaffected controls for use in genome-wide analyses for genetic risk factors. Through these efforts we believe novel insights into the genetic pathogenesis of FECD may be obtained, thus providing new targets for subsequent therapy development. Here we present the study design, enrollment process and demographics of our assembled cohort,

while examining heritability for the phenotype of FECD grade and central corneal thickness, a measure of corneal endothelial function.

Materials and Methods

Our study design combines two approaches: a familial sample and a case-control sample that will enable us to perform both linkage and association analyses. Recruitment efforts were focused on obtaining 500 sibling pairs and corresponding controls using a semi-quantitative measure of FECD severity. To maximize efficient collection of families a multi-site approach was chosen. Enrollment occurred over a three year period, from March 2006 to May 2009.

Recruitment and specimen acquisition

To assemble samples of this size, 29 clinical sites were established (See appendix 1 for Site Map), many of which are participating in the Cornea Donor Study (CDS)[17]. All sites had cornea fellowship-trained ophthalmologists who were capable of determining the FECD grading classification for the phenotype, who routinely performed keratoplasty for the disease, and who had a significant list of previous keratoplasty patients with available pathology to potentially collect families. All sites recruited families and the majority of sites recruited affected sibling pairs. The research conducted followed the Declaration of Helsinki. Institutional review board (IRB) approval of the study protocol and consent forms was obtained prior to the enrollment of any subjects. Written informed consent was obtained from all subjects prior to study examination. Those sites without IRB privileges were brought in under federal wide assurance, and added to a common consent form approved by the IRB located at University Hospitals Case Medical Center, Cleveland, Ohio. Family members living in remote locations were still encouraged to participate, since in such families pathology specimens could be obtained and subjects were able to provide saliva or blood sample. In one instance, to facilitate enrollment for families that could not be examined at an existing site(s), the Coordinating Center established a temporary site for the sole purpose of recruiting six different families. The Genetics Core Facility (GCF) at Case Western Reserve University received and processed all blood and saliva collected from subjects. Blood was drawn peripherally from the majority of study participants for DNA isolation. When blood samples were unavailable, participants provided saliva samples using Oragene kits.

Slit lamp biomicroscopic examination and determination of FECD Grading Scale

A detailed history was obtained regarding known onset of FECD, family history, ocular and systemic medications, prior history of glaucoma, contact lens wear, ocular trauma and ocular surgeries. All subjects underwent a slit lamp biomicroscopic examination, including determination of conjunctival inflammation, assessment of the cornea for abnormalities, anterior chamber inflammation, iris abnormalities and color, presence of cataract, measurement of intraocular pressure and central corneal thickness.

All investigators were certified on the FECD grading scale by provision and review of a detailed operations manual that included procedures for slit lamp biomicroscopy, a diagram of the grading scale, a photographic example of each grade, and a training video demonstration of the slit lamp biomicroscopic technique (see supplementary material). An investigator meeting at the outset of the study also provided live patient examples of grades on which each investigator submitted their assessment with a requirement that they be within one grading measure on the scale to be certified on the grading.

Every subject received a severity grade for both corneas. A previous classification scheme[1] was revised from a 5-step scale to a 6-step scale. The scale was as follows: Grade 0, no guttae; Grade 1, 1–12 central or paracentral non-confluent corneal guttae; Grade 2, more than 12 central/paracentral non-confluent corneal guttae; Grade 3, 1 to 2 mm of confluent central/paracentral corneal guttae at the widest diameter of the confluence after rotating the slit beam and measuring the diameter by narrowing the length of the beam and recording the length in mm; Grade 4, greater than 2 and up to 5 mm; Grade 5, greater than 5 mm of confluent central /paracentral guttae; Grade 6, over 5 mm of confluent central/paracentral guttae with clinically apparent stromal and/or epithelial edema (see Supplementary material for photographic grading guide and Appendix 2 for the grading scale). Subjects with stromal and/or epithelial edema overlying focally dense guttae less than 5 mm in diameter were graded according to the diameter of confluent guttae, with the edema noted separately. Grading was accomplished by scanning the cornea both horizontally and vertically from the center to the limbus by slit lamp biomicroscopy using a narrow slit beam. Eyes that had already had a keratoplasty (either penetrating or endothelial keratoplasty) for FECD were marked as such and assigned a grade of 5. Subjects who underwent penetrating keratoplasty were assigned a grade of 6 if corneal edema was visible on the subject's pathology specimen. As corneal edema was unable to be evaluated on DSEK pathology specimens, these subjects were assigned a grade of 5.

Grades 4 and higher were considered severe disease. Although grades 2 and 3 could possibly predispose to later progression, the rates and factors for progression to moderate/severe disease are not well defined in the literature[18]. Therefore for the purpose of this study, a grade 0 was considered unaffected, grades 1–3 were defined as intermediately affected, and 4–6 were considered affected.

Eligibility Criteria—Family Sample

All probands were 18 years of age or older and had at least one living sibling, grandparent, cousin or avuncular relative. Probands were required to have undergone a keratoplasty (penetrating or endothelial) with an available histopathology specimen of the endothelial/Descemet membrane layers, which was forwarded to the Pathology Core Facility (PCF) at CWRU. If no histopathology slides were available, a pathology report was requested for review by the PCF. In the event a proband had not yet undergone keratoplasty, the individual was still eligible for enrollment if FECD was confirmed on histopathologic examination of the excised corneal specimen from another family member. If no pathology was available from a family member, the proband remained eligible if the family had two affected sibling pairs (all members of the pairs scoring at least grade 4 or having had keratoplasty for FECD) or the grade of at least one of the affected siblings was confirmed by a separately certified study investigator. Sibling pairs were counted pairwise within larger sibships. For example, a nuclear family with three affected offspring contains three affected sib pairs (offspring 1 + 2, 1 + 3, and 2 + 3).

Family members were recruited only after confirming the eligibility of the proband. Affected family members were contacted through the proband and were required to meet specific inclusion and exclusion criteria prior to participation. If an affected sibling of a proband was already enrolled in the study, unaffected siblings and parents were subsequently recruited. Relatives necessary to connect affected family members together in the family tree were also collected even if the relative was graded as unaffected. All qualifying family members underwent the same history and clinical examination procedure as the probands. When pathology was obtained, examination of affected siblings performed, and demographic data and DNA samples collected, a family was considered closed; otherwise, it was considered open. Here, we report only the results from families considered closed, despite the recruitment of a number of additional subjects that did not meet the

criteria to close a family. The DNA from these closed families was stored for later use in genotyping.

Eligibility Criteria—Case-Control Sample

All control subjects were at least 60 years of age and matched according to age, gender, and ethnicity to the enrolled index cases. Controls were chosen to be five years older than their corresponding case to minimize the enrollment of controls who might later develop FECD and confound analysis. To qualify, each control subject was required to be grade 0 on the FECD grading scale, have no family history of a possibly inherited corneal disorder (e.g., FECD, keratoconus, stromal dystrophy), exhibit refractive astigmatism of less than 3.50D and have normal corneas with no abnormalities upon slit lamp examination other than the following noted exceptions: 1) peripheral degenerative changes related to age, including arcus senilis and limbal-girdle of Vogt, 2) corneal scar from infection or penetrating trauma, 3) pterygium or pterygium surgery, 4) inactive superficial vascularization of the epithelium and/or subepithelial layer, 5) previous glaucoma, or retina-vitreous surgery in one eye where the other eye had not had surgery and the endothelium was normal, 6) previous intraocular laser surgery. Bilateral pseudophakes who received an FECD grade of 0 and were FECD negative preoperatively also qualified. The limbal/peripheral corneal incisions related to cataract surgery were judged unlikely to affect the central cornea and, thus, the ability to assess the FECD phenotype. Subjects were excluded from participation as controls if they displayed any signs of corneal dystrophy or degeneration, had previous/active interstitial keratitis or anterior uveitis, or active/previous infectious keratitis or vascularization of the epithelium and/or stroma. Subjects were also excluded if they had previously undergone bilateral corneal surgery or had experienced perforating corneal trauma resulting in scarring.

Pathology Assessment

The PCF reviewed pathology slides of the majority of families for pathologic confirmation of FECD in the proband or a member of the family. Both an H&E and PAS stained slide were reviewed for those subjects whose pathology was received. Pathology slides were assessed by the Primary Reader (a fellowship-trained ophthalmic pathologist, DB) and classified in the following manner: 1) definite FECD, 2) consistent with FECD, 3) no pathologic diagnosis, 4) FECD with other pathology, or 5) other pathology. When the classification was not clear, slides were sent to the Secondary Reader, a second ophthalmic pathologist for an independent evaluation (Dr. Victor Elner MD PhD, University of Michigan, Ann Arbor, MI). If both reviewers gave the same classification, the classification was confirmed. In cases of disparity in the classification between the Readers, cases were resolved by joint review of the case with both pathologists present.

In cases with FECD together with other pathology (Group 4), the case was reviewed with a member of the Steering Committee (JL or SI) to determine if the other condition confounded the diagnosis of FECD. In cases where the pathology specimen was not available, clinical pathology reports generated following surgery were reviewed by the Primary Reader. Cases submitted by an experienced ophthalmic pathologist, a non-ophthalmic pathologist with great familiarity with FECD, or those whose reports specified pathologic features typical for the diagnosis of FECD, were accepted as Group 1 with definite FECD. If non-specific features were described and the report was not by an ophthalmic pathologist or pathologist familiar with FECD, the case was judged to be a consistent with FECD or Group 2. If other pathologic features were described in addition to FECD, the case was classified as FECD with secondary pathology or Group 4. If no features were specified and the interpretation was by a pathologist not familiar with FECD, the case was classified as possible FECD.

Computerized Database of FECD Families

After subjects were determined to be eligible for enrollment, subjects signed an informed consent and then provided a family history and underwent clinical examination at the enrolling site. Data forms from each subject were faxed for entry into a centralized web-based database, using double data entry to ensure accuracy. To maintain patient anonymity, each subject was assigned a unique identification number used in all data and sample collection at the time of examination. All clinical sites maintained limited access to the centralized database ensuring a mechanism for monitoring recruitment progress throughout the study.

FECD Sib-Sib Correlations

FECD grades for each eye from each participant were obtained from the FECD database and used to compute sib-sib correlations. We used four definitions of FECD: we classified individuals with grade 4–6 as affected and 0 as unaffected, discarding individuals with intermediate grades for a binary classification of disease; individuals with surgical intervention with a penetrating or endothelial keratoplasty, but without recorded preoperative stromal and/or epithelial edema were assigned to a grade of 5; and those recorded with stromal and/or epithelial edema were assigned to grade 6. We also examined the full range of FECD scores in the right eye, left eye and worse eye.

Central Corneal Thickness Sib-Sib Correlations

Central corneal thickness was measured at each site using a standard ultrasonic pachymeter, calibrated and operated as per the manufacturer's instructions. Up to three measurements were performed on each eye. The mean of all measurements was taken where more than one observation was available, except in one case in which one observation was clearly an outlier. A composite phenotype over both eyes was also generated, as the mean of all available measurements on both eyes. Phenotypes were adjusted for covariates by linear regression, and the residuals were used as adjusted phenotypes in heritability estimation. Initially, the corneal thickness phenotypes were adjusted by linear regression for sex, age, the square of age (age^2), FECD grade as defined above, intraocular pressure, and previous intraocular surgery. Effects for sex, age, age^2, and previous intraocular surgery were found to be non-significant ($p > 0.05$), and consequently, the final phenotypes were adjusted only for age (which was retained in the model even though non-significant), FECD grade and intraocular pressure.

Sibling correlations for FECD grades and central corneal thickness were estimated using the FCOR program in S.A.G.E., version 6.0[19]. FCOR estimates familial correlations and their standard errors by the product-moment estimation method of Keen and Elston[20]. Doubling the sibling-sibling correlation provides an estimate of the broad-sense heritability, i.e., the proportion of phenotype variability attributable to genetic factors[21].

Results

Family Sample

A total of 793 subjects were recruited, including 322 probands. In the familial sample, 82% of the subjects had FECD (grades 4–6) (Table 1). In addition to Caucasians (N = 780), recruitment included 4 African-Americans, 4 American Indian/Alaskan Natives, 1 Native Hawaiian/Pacific Islanders and 4 others of non-Caucasian ancestry enrolled. Based on self report, seventeen individuals were Hispanic or Latino in ethnicity, while the remaining 776 participants were Non-Hispanic or Latino. In the familial sample used for estimating heritability, we collected 330 affected-affected sibling pairs as defined by both siblings scoring a grade of 4 or higher on the FECD scale and 561 total sibling-pairs in 322 closed

families. The remaining pairs consisted of 67 discordant, affected-unaffected sibling pairs, 8 concordant unaffected sibling pairs and 156 pairs with one or both siblings of intermediate affection status. As indicated in the family sample eligibility criteria methods, these numbers reflect families which were considered closed, meaning the family met all study inclusion qualifications and that all necessary materials had been collected on the family and its members. Including all families, both open and closed, a total of 650 sibling pairs were recruited into the study.

Six hundred and forty-nine affected subjects had a prior diagnosis of FECD and could report approximately when the diagnosis was first made by their ophthalmologist. Of these, 42% had a diagnosis made within 10 years of enrollment in the study (Table 2), and a minority (1.2%) more than 20 years prior to enrollment. Probands and full siblings were similar in age distribution, although siblings were slightly younger than the probands on average (mean age 66.5 vs. 68.8 years respectively $p = 0.006$). Gender ratios were similar between the two groups (69% vs. 65% female). The average affection grade of siblings was lower than that observed in probands (4.1 vs. 5.6) ($p < .0001$). Eighty-one children and 20 parents of probands were also enrolled (Table 3). Children were on average 20 years younger than probands (mean age 48.8) and less severely affected (average FECD severity score 2.7). The average age of parents was 79.0 years with an average affection status of 4.0. Comparing all enrolled subjects from the familial study arm, subjects defined as affected had an average age of 67.6 years, older than the subjects defined as intermediate (60.3 years) or unaffected (55.8 years).

Histopathologic Findings

A total of 321 sets of pathology slides were collected and reviewed by our PCF. After initial review, 279 (87%) were assessed as definite FECD. Two were diagnosed as FECD with other pathology, and 14 were consistent with FECD. Thus, 92% carried the diagnosis of FECD. There were nine assessments of other pathology and five assessments of no pathologic diagnosis. Re-cuts were obtained for several sets of slides in order to make a diagnosis; however, re-cuts were unavailable for 12 sets of slides. With all specimens, ultimate agreement on classification was obtained. Eighteen cases were sent to the secondary reader, with diagnosis of FECD occurring in six cases.

Case Control Sample

The inclusion of a case-control sample enabled additional testing beyond that made possible by the family sample, including qualifying probands who lacked additional family members, preventing underutilization of available participants. Three hundred and four age, gender and ethnicity matched controls were recruited. Due to our matching paradigm, controls were older (70.3 years) than the unaffected participants (55.8 years) from the familial enrollment study sample (Table 1). Compared to affected probands, controls were slightly older, addressing the concern of enrolling younger controls who might yet develop disease and confound analysis.

Heritability

We determined sib-sib correlations and broad-sense heritability using product-moment estimation (Table 4). Our results show that both binary indicators of severe disease as well as semi-quantitative measures of disease severity are significantly heritable, with heritability estimates of 0.304 for severe disease and 0.368 for FECD score for the worse eye. FECD heritability estimates were very similar for the right (0.381) and left (0.392) eyes.

Central corneal thickness was found to be overall more strongly heritable than FECD, with an overall heritability of 0.466. However, because fewer sibling pairs with pachymetry

measurements were available than for FECD, this estimate is not as significant as the estimate for FECD score. The estimate for the left eye (0.572) was nearly twice that for the right (0.305), but these values are not significantly different ($p = 0.20$) for the difference, assuming normally distributed estimators.

Discussion

Sample

This study is the largest collection of FECD affected individuals and families gathered to our knowledge from a widely-distributed sample from the United States, with 793 individuals and 322 families. Previous studies of FECD have focused on a small number of large pedigrees containing numerous affected individuals. This type of sample is optimal for locating rare disease-causing genetic variants with large effect, and indeed, the previous studies had examined families in which FECD appears to segregate as a Mendelian dominant trait[8–10]. FECD is a common, complex disease, however, and the bulk of the heritable variability in FECD remains unexplained. In general, genetic factors for corneal diseases such as FECD and keratoconus are much less well characterized than for retinal diseases. Our large sample of cases and controls will offer greater power to detect genetic variants predisposing toward FECD present throughout the general population.

Phenotype

Our study employed a rigorous methodology to provide a standardized, reproducible, but simple grading system for the FECD phenotype across multiple centers utilizing fellowship-trained cornea specialists. Pathologic verification of the FECD diagnosis was an initial enrollment requirement and the inclusion of a clinical, family, and histopathologic definition (in the proband) of ‘affected’ in our study design has decreased the probability of misclassification of individuals. Utilizing a definition of affection status based only on clinical phenotyping without histopathologic confirmation, our study would have identified as many as 911 affected participants and nearly 600 affected-affected sibling pairs. Accurate phenotype classification is critical since small errors in inclusion can greatly weaken the results of genetic analysis. The identification in our cohort of cases with discrepancies between the clinical and pathologic diagnosis of FECD suggests that clinical diagnosis of FECD alone may not be sufficient. Examination findings from other corneal diseases related to aging, inflammation, and trauma, may be misconstrued as the guttae of FECD even by experienced clinicians[22]. Our study’s use of a standardized method for grading of the FECD phenotype and pathologic confirmation of the FECD phenotype will help support the most informative approach for future genetic analyses.

Heritability

This is the first study to show the clinical phenotype of guttae, as well as severe disease, are highly heritable in a general Caucasian population, particularly in a targeted, enriched sample. An exhaustive search of the literature revealed that no other groups had obtained heritability estimates, a key step in planning molecular strategies for gene mapping. Krachmer reported in 1978 that 38% of participants over the age of 40 were affected with FECD[1].

A limitation of our design is that we obtained cross-sectional data which will not allow us to assess if course and rate of progression are also heritable, although some reports suggests that this hypothesis is likely true[9,23]. When genes associated with the more common late onset form of FECD are identified, comparative analysis of progression in mutation-known individuals will provide better clinical insight to this issue. A second limitation of our heritability estimate is that it may not be fully representative of the contribution to

heritability of the documented group of individuals/families with progressive guttae development in the general population, since our sample was enriched for concordantly affected sibling pairs. Nonetheless, our initial analysis has shown clear evidence for strong heritability of late-onset FECD, and this result will drive our interest in identifying genes that contribute to this disease.

Our overall estimates of heritability for central corneal thickness fall at the low end of the range of previously reported values[24–28]. An early study of 86 Greenland Inuit families found corneal thickness to be highly heritable, with heritability estimates of 0.6 to 0.7[28]. Heritability was estimated from 33 Caucasian Australians selected for low or high corneal thickness and their first-degree relatives, and was found to be 0.68[26]. A recent report on 22 extended Caucasian pedigrees ascertained for primary open angle glaucoma estimated heritability for corneal thickness at 0.72[27]. Twin studies conducted on Caucasian twin pairs in the United Kingdom and Australia[25] and on Chinese twin pairs[24] reported even higher estimates of heritability: 0.95 and approximately 0.9 (0.88 in boys and 0.91 in girls), respectively. A meta-analysis conducted on these four studies found evidence of very high heterogeneity[29] in the heritability estimates[30]. The differences in estimates between our study and these previous ones may be explained by differences in study design, and family structure. Importantly, none of these studies ascertained their samples for FECD. Moreover, the mean age of participants in our sample is considerably greater than that in any of the previous studies. The important causes of variability in corneal thickness, including genetic factors, are likely to be different early and late in life. Although controlled statistically in our analysis, the influence of phenotype grade, contact lens wear, trauma, intraocular pressure, and prior ocular surgery, may have influenced the lower heritability estimate for corneal thickness in our study.

Corneal thickness is primarily influenced by the barrier and pumping function of the endothelium[31]. There have been limited studies regarding the relation of thickness to guttae and a deficit in endothelial function. Burns et al[32] found a positive correlation between corneal thickness and guttae in 21 individuals with early FECD, related to increased endothelial permeability despite a possible compensatory increase in pumping function. This group subsequently found in 26 individuals with advanced FECD with confluent guttae and increased corneal thickness that pump function was impaired compared to individuals with mild disease and normal controls[33]. On the other hand, Zoega et al.[6] found no significant correlation between density of guttae and corneal thickness. The relation of the severity of guttae development with corneal thickness as a measure of endothelial function warrants further study.

Analysis

We have designed our recruitment strategy to assemble a sample useful for both genomewide linkage and association analyses. The availability of 561 sibling pairs, including pairs concordant and discordant for FECD, along with numerical measures of FECD severity, will enable us to conduct genomewide linkage analysis using a model-free approach such as Haseman-Elston regression[34]. Because model-free linkage analysis requires no prior assumptions about the mode of inheritance, it is particularly well-suited to studies of complex traits. In addition, because the unit of analysis is often the sibling pair, model-free linkage approaches are well-suited for the study of late-onset traits for which multigenerational genetic data are very difficult to obtain. At the same time, we have collected the largest assembled FECD sample to date ($n = 1097$, with continuing recruitment of unrelated controls), making genomewide association analysis feasible. The presence of both families and unrelated individuals somewhat complicates association analysis, but several analytical methods are available that take into account correlations due to familial relationships (e.g., ASSOC[19] and GWAF[35]). A potential drawback of our sample, as with all

population-based samples, is the possibility of genetic heterogeneity: causal variants at the same locus may have arisen several times independently within a population, on different genetic backgrounds, thus attenuating the association between causal polymorphisms and nearby genetic markers. We expect this limitation to be offset by the ability to detect novel genetic loci affecting FECD that are widespread within Caucasians.

Study Challenges

There were several barriers encountered while trying to meet all goals associated with the study. The greatest challenge was our enrollment criteria emphasizing families with living affected sibling pairs and available pathologic material. Thus, while FECD is a very common indication for corneal transplantation, the number of qualified families was much smaller number than expected and sites with significant volumes of FECD patients were often unable to provide qualified families. The reasons for unavailability of specimens were multi-fold and included the remoteness of the surgery in location or time, pathology departments that discard or have inaccessible slides and blocks of older cases, and the increased use of endothelial keratoplasty with specimens not submitted for pathologic diagnosis of FECD. Additionally, FECD is a late onset disease, not manifesting until after 40 years of age in most cases[1,36]. This created problems where many potential families were unable to participate due to qualifying family members having died prior to study initiation. Lastly, our study recruited a little over twice as many female cases and relatives as males. Our nability to recruit more males may be due to recruitment bias, or more likely due to higher prevalence of disease in females. Women experience a higher occurrence of FECD and usually display a more severe form of the disease than men, making women more likely to be enrolled in our study[1,8,37,38]. We were able to address these recruitment limitations in part by adding additional sites to aid in recruitment efforts.

In summary, we expect that our large FECD sample will be highly beneficial for future research aimed at identifying novel genes associated with FECD, and ultimately, at discovering new therapeutic targets. The initial analyses in our assembled cohort provide continued evidence for the heritability of FECD and of central corneal thickness and, for the first time, determine heritability estimates of the FECD trait. Our recruitment of families with an emphasis on affected sibling pairs as well as unrelated controls uniquely positions our study to use both linkage and association analysis methods in identifying genetic factors that underlie FECD and the size of our sample will offer greater power in detecting common predisposing genetic variants. Through such efforts, we hope to expand the understanding of the genetic origins of corneal diseases such as FECD and open up new avenues of investigation into corneal pathology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Summary of FECD* affection status by age, sex, relationship and ethnicity

Summary of Status by Age, Sex, Relationship and Ethnicity		Status				
		Unaffected	Affected	Intermediate	Control	Total
Total		**49**	**649**	**95**	**304**	**1097**
Age	**Mean**	55.83	67.58	60.25	70.3	
	Median	54.24	68.47	59.62	68.9	
	Std	11.63	11.21	14.75	7.12	
	Min	36.87	20.37	22.5	60.04	
	Max	81.28	96.81	89.12	91.21	
Relationship	**Proband**	0	322	0	·	322
	Full Sibling	25	254	56	·	335
	Child	15	36	30	·	81
	Parent	1	13	6	·	20
	Cousin	2	16	2	·	20
	Aunt/Uncle	2	6	1	·	9
	Half Sibling	4	2	0	·	6
	Control	·	·	·	304	304
Sex	**Male**	17	199	31	131	378
	Female	32	450	64	173	719
Race	**White/Caucasian**	49	638	93	294	1074
	Black/African American	0	3	1	3	7
	American Indian/Alaskan Native	0	4	0	4	8
	Native Hawaiian or Other Pacific Islander	0	1	0	3	4
	Other	0	3	1	0	4

*Fuchs' Endothelial Corneal Dystrophy

Table 2

Time since diagnosis of symptomatic FECD*

		Time since Diagnosis of Symptomatic Disease						
		Never	Less than or Equal to 5 years ago	6–10 Years ago	11–20 Years ago	Over 20 years ago	Don't Know	Total
Status (N = 1097)	**Unaffected**	1	0	0	0	1	47	49
	Affected	210	156	118	97	8	60	649
	Intermediate	28	8	1	0	0	58	95
	Control	0	0	0	0	0	304	304
Relationship to Proband (N = 1097)	**Proband**	93	89	62	58	4	16	322
	Full Sibling	114	55	44	29	3	90	335
	Child	23	13	6	0	0	39	81
	Parent	2	3	3	6	0	6	20
	Cousin	3	4	3	3	1	6	20
	Aunt/Uncle	2	0	1	1	1	4	9
	Half Sibling	2	0	0	0	0	4	6
	Control	0	0	0	0	0	304	304

*Fuchs' Endothelial Corneal Dystrophy

Table 3

Clinical variables by participant relationship to proband

Summary of demographic data of probands and their relatives		Relationship to Proband							Control
		Proband	Full Sibling	Child	Parent	Cousin	Aunt/Uncle	Half Sibling	
Total (N = 1097)		**322**	**335**	**81**	**20**	**20**	**9**	**6**	**304**
Age	**Mean**	68.8	66.5	48.8	79.0	67.5	67.9	63.2	70.3
	Median	69.6	67.2	49.3	80.1	65.3	65.6	59.6	68.9
	Std	10.3	10.9	9.9	7.9	11.0	15.5	11.9	7.1
	Min	38.7	30.9	20.4	63.7	47.8	46.5	51.3	60.0
	Max	92.7	90.6	69.5	96.8	85.7	84.3	82.8	91.2
Worst FECD* Grade	**Mean**	5.6	4.1	2.7	4.0	4.4	3.7	1.8	0
	Median	6.0	5.0	3.0	5.0	5.0	4.0	0.0	0
	Std	0.6	1.8	2.0	2.0	2.0	2.3	2.9	0
	Min	4.0	0	0	0	0	0	0	0
	Max	6.0	6.0	6.0	6.0	6.0	6.0	6.0	0
Sex (N = 1097)	**Male**	101	114	18	6	3	3	2	131
	Female	221	221	63	14	17	6	4	173
Race (N = 1097)	**White/Caucasian**	316	329	80	20	20	9	6	294
	Black/African American	2	2	0	0	0	0	0	3
	American Indian/Alaskan Native	1	3	0	0	0	0	0	4
	Native Hawaiian or Other Pacific Islander	1	0	0	0	0	0	0	3
	Other	2	1	1	0	0	0	0	0

*Fuchs' Endothelial Corneal Dystrophy

Table 4

Sibling correlations and estimates of narrow-sense heritability

	Correlation		Heritability			
Trait	**Estimate**	**SE**	**Estimate**	**SE**	***p****	***n**†*
FECD∞ (binary)	0.152	0.052	0.304	0.105	0.0043	447
FECD score, worse eye	0.184	0.049	0.368	0.097	0.0002	625
FECD score, OD	0.191	0.050	0.381	0.099	0.0002	595
FECD score, OS	0.196	0.049	0.392	0.099	0.0001	604
Corneal Thickness, overall	0.233	0.068	0.466	0.136	0.0010	358
Corneal Thickness, OD	0.153	0.071	0.305	0.144	0.034	287
Corneal Thickness, OS	0.286	0.076	0.572	0.152	0.0004	305

** p*, two-tailed *p* value for test of H_0: correlation = 0;

† n, number of sibling pairs contributing to the calculation:

∞ Fuchs' Endothelial Corneal Dystrophy